Microbiology **Manual**

5 th Edition

CONDA pronadisa

knowledge. partnership. future.

pronadisa
Micro & Molecular Biology

LABORATORIOS CONDA S.A.

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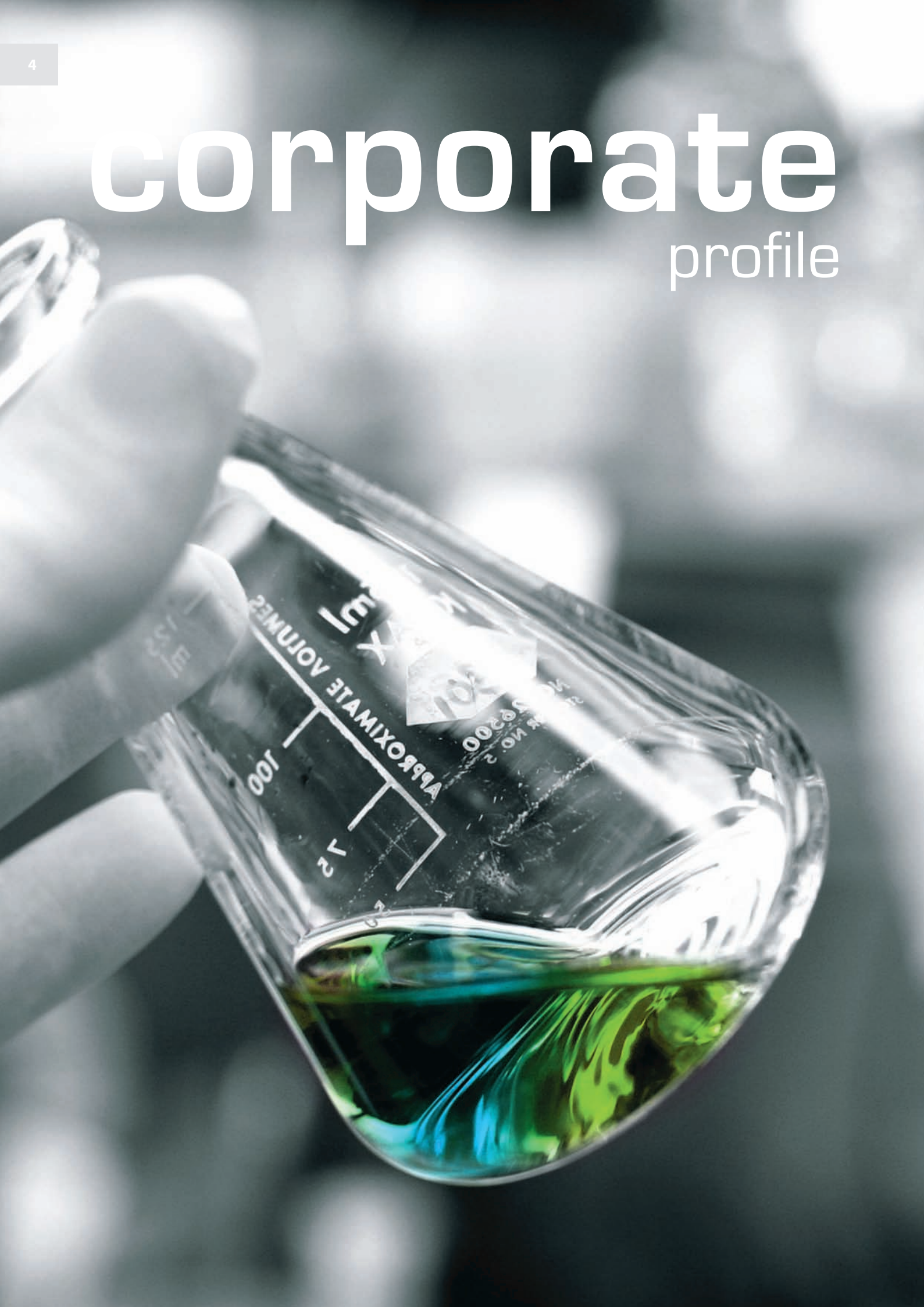
corporate profile



Laboratorios CONDA, S.A. was founded in 1960 as the first Spanish producer of Dehydrated Culture Media for Microbiology and Molecular Biology. The company is now internationally recognised as one of the leaders in the field and supplies key ingredients for use in research and testing, such as Agars, Peptones and Agaroses among other products.

CONDA's corporate mission is to be a major contributor to the field of Life Sciences through the design, production and provision of products and services of the highest quality and value.

CONDA's main aim is to provide World Class Customer Satisfaction, making us strive to be more efficient, more responsive and more competitive

From our factory located on the outskirts of Madrid, we export to more than 95 countries throughout the world, using our own branch offices and authorised distributors. Backed up by this great team of professionals, **CONDA** is confident in providing all of its customers with high quality products.

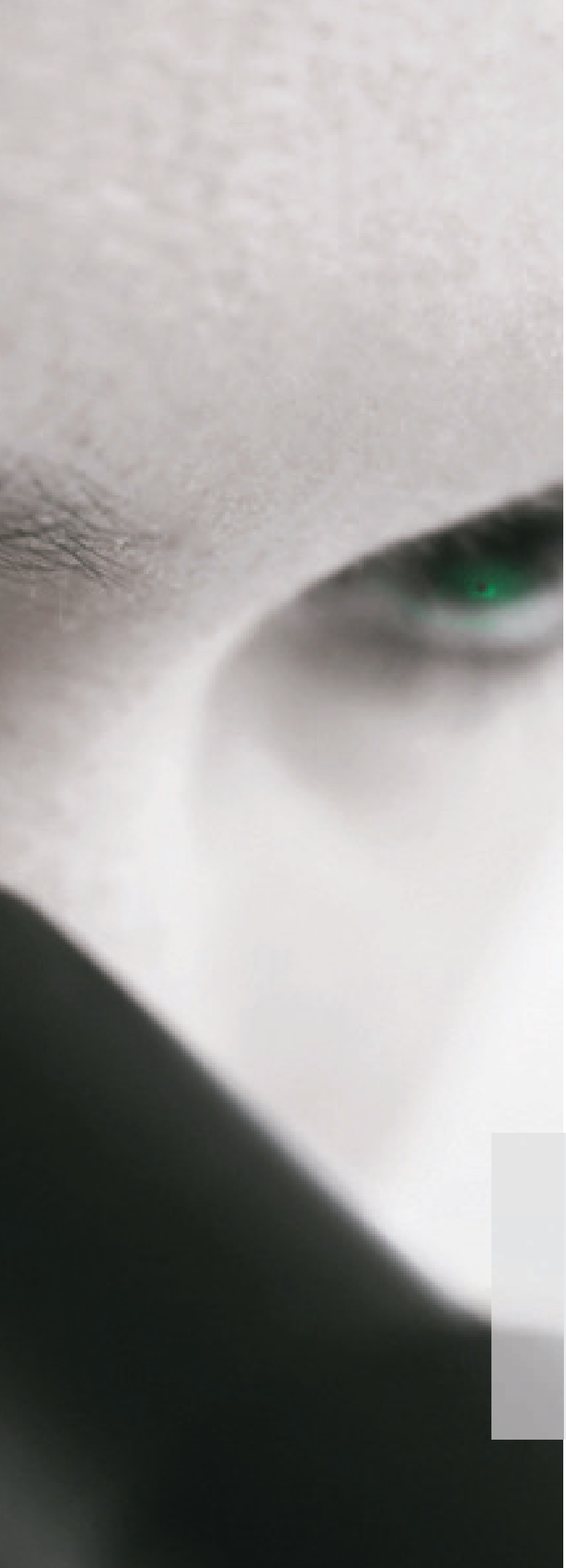
All our efforts are focused on becoming more efficient and more competitive in order to achieve our principal objective, which is to give full satisfaction to our clients.

Our growing customer list includes:

- **Public and private hospitals**
- **Universities and government institutions**
- **Clinical and veterinary diagnostic laboratories**
- **Pharmaceutical and cosmetic industries**
- **Food and agricultural industries**

worldwide
market

quality
commitment



CONDA continuously improves its production to meet the highest international quality standards.

The company complies with the **ISO 9001:2000 standard**, and was the first Spanish company in this sector to be granted the certificate in 1998.

In 2011 Conda has also obtained ISO 13485, specific for Medical devices.

We have endeavoured to continuously improve our implementation practices resulting in the highest quality services throughout the delivery chain and a growing product portfolio. Our objectives reflect our undertaking for constant progress and product innovation.

We also comply with ISO, the European Pharmacopoeia, FDA, APHA, USP, AOAC and CeNAN standards and with CE (European Community) mark.

Our reputation and success throughout the world is the result of the combination of uncompromising quality systems, technical expertise and innovative thinking

Strict Quality Control procedures are adopted by **CONDA** prior to and during the manufacturing process to ensure quality products and batch-to-batch consistency.

We also exert tight control over the selection and treatment of all raw materials and the various components (Peptones, Carbohydrates, Minerals & Chemicals, Agar and other additives) used in the manufacturing process.

CONDA extensively sources, selects and tests the best materials only for each application.

Physical-chemical characteristics are tested, such as appearance, colour, odour, moisture, solubility, clarity, gelling temperature and pH. The media also undergo additional microbiological tests that guarantee growth, differentiation, biochemical performance, recovery of small inocula, selectivity, etc.

A QUALITY CERTIFICATE IS ALWAYS SUPPLIED WITH EACH SHIPMENT OF THE FINAL PRODUCT

quality
procedures

GENERAL CULTURE MEDIA

COMPOSITION

Many of the studies in microbiology need a support on which to cultivate and maintain organisms in a laboratory. This is only possible if culture media are available. The culture media should provide a balanced mix of the nutrients required and the optimum concentration for the growth of microorganisms. Each medium varies its formulation due to the nutritional requirements of each organism. It should be mentioned that there is a great diversity of microorganisms and that is why there is also a large number of growth media.

GROWTH FACTORS

The correct growth of each microorganism depends on different factors:

- Energy source
- Nitrogen source
- Macronutrients
- Vitamins and other growth factors
- Minerals and traces
- Water

Nutrient	Ingredient of the culture medium
Energy source	Sugars, alcohol, amino acids
Nitrogen source	Peptones, infusions, extracts
Macronutrients and Micronutrients	Peptones, infusions, extracts, inorganic compounds
Vitamins and other growth factors	Peptones, infusions, extracts

ENERGY SOURCE

Heterotrophic organisms use organic compounds, such as sugars or amino acids, as a source of carbon and energy. Carbon is the main element in all macromolecules. Depending on the type of microorganism, this carbon and energy source varies. Some microorganisms are able to utilize complex carbohydrates like starch and cellulose, degrading these compounds into simple sugars. Although the carbon source is present in peptones, sugars such as glucose, lactose and other monosaccharides or disaccharides can be added.

NITROGEN SOURCE

The nitrogen source of microorganisms is derived from inorganic compounds such as ammonia or nitrates, or from organic compounds like amino acids, nitrogen bases of nucleotides and other compounds. Amino acids are usually the primary source of nitrogen. Later, microorganisms can use more complex compounds such as high molecular weight proteins. In a culture medium the nitrogen source is usually provided by peptones and derived extracts of meat, casein, yeasts and plants.

MACRO AND MICRONUTRIENT

In addition to carbon and nitrogen, microorganisms require macronutrients such as phosphorus, sulfur, potassium, magnesium, calcium, sodium and iron. Phosphorous forms part of nucleic acids and phospholipids. Sulfur is present in cysteine and methionine, and vitamins such as thiamine, biotin and coenzyme A. Most of the cell sulphur is obtained from inorganic sources such as sulfates and sulfides. Magnesium stabilizes the ribosomes, cells and nucleic acids. It also plays an important role in the activity of many enzymes. Calcium stabilizes the cell wall and plays an important role in the stability of the endospores. Iron is an essential compound for cytochromes and proteins involved in electron transport. The micronutrients or trace elements such as cobalt, nickel, copper or manganese play an important role in the functioning of enzymes and other processes.

OTHER GROWTH FACTORS

All compounds that an organism cannot synthesize must be provided as nutrients. These compounds may be vitamins, essential amino acids, purines or pyrimidines. These growth factors are needed in small quantities. For example NAD and hemin are growth factors for certain bacteria such as the *Haemophilus* species. Some microorganisms also need the addition of blood for an adequate growth.

WATER

Microorganisms require the presence of water, in an available form, to be able to grow and carry out their metabolic functions. The vast majority of microorganisms need high levels of activity values and water to be able to grow. When a microorganism is in a substrate with a water activity less than that required, it stops growing. This does not usually lead to the microorganism's death, rather it remains in resistance conditions during a period of time.

OTHER COMPOUNDS

In addition to the nutrients needed for bacterial growth, other compounds can be added to the medium:

SELECTIVE AGENTS

Selective agents are added to inhibit the accompanying microorganisms without affecting the growth of target microorganisms. The optimal dose varies depending on the microorganism and the medium used. Examples of selective agents are antibiotics, bile salts or crystal violet.

INDICATORS

pH indicators are used for identification or differentiation of organisms in terms of pH changes produced in the medium. This change in pH is due to basification or medium acidification caused by the use of carbohydrates. As a pH indicator phenol red, bromocresol purple or neutral red may be added.

SOLIDIFIERS

Solidifying agents are used for the preparation of solid media. Agar is the most used solidifying agent. It is extracted from various algae such as *Gelidium*, *Gracilaria* and *Pterocladia*. Other solidifying agents may be gelatin or albumen. The solidifying agent must be present in the medium at a concentration level of approximately 30%.

PROTECTIVE AGENTS

Protective agents are added to the medium to neutralize the toxic metabolites such as hydrogen peroxide or superoxide. Calcium carbonate, soluble starch, charcoal or polysorbate 80 are some examples of protective agents.

SODIUM CHLORIDE

Sodium chloride is added to media to maintain the osmotic balance of the medium.

BUFFER

Buffers are added to avoid drastic pH changes in the medium. The buffers most often used tend to be phosphates. It is not recommended to use buffers in media where pH indicators are added as this could lead to false results.

ENVIRONMENTAL CONDITIONS

In addition to nutrients and other compounds of the culture medium, environmental conditions are important for the proper development of the organism. The control of temperature, oxygen tension or pH is extremely important.

TEMPERATURE

Depending on the species, bacteria have a certain growth range where they can grow and reproduce. Mesophilic bacteria have a growth range between 20-50ºC. Pathogenic microorganisms are mainly mesophiles with an optimum temperature around 37ºC.

OXYGEN

Depending on each organism´s needs, it is necessary to modify incubation conditions in order to meet with the specific requirements of each bacterium. Strict aerobic microorganisms need oxygen at atmospheric concentration while strict anaerobes cannot grow in the presence of oxygen. You can also find microaerophilic and facultative anaerobic microorganisms.

PH

It is a critical parameter for the growth of microorganisms because each type of organism has a pH range in which it can live properly. To avoid drastic changes in media, pH buffers are added.

USER

GUIDELINES

The choice of media is essential for a good investigation or enumeration of the microorganism. Before carrying out laboratory tests, the medium or media required must be ascertained. The medium is chosen based on the nutritional needs of the organism, depending also on the degree of selectivity or differentiation desired. Knowledge of the normal habitat of an organism is useful for choosing the culture medium because the nutritional requirements reflect their natural environment.

In the case of microbiological tests on food, the legislation for each country must be ascertained for each particular food type. This will either require enumeration or investigation of the organism and, depending on the type of test, the culture media used will vary.

STORAGE

Dehydrated culture media are mixtures of hygroscopic substances sensitive to moisture, heat and light. Although the packaging of dehydrated media is protected from light and moisture, storage must be performed under appropriate conditions set out on the bottles so as to keep their initial properties. Sharp changes in temperature should be avoided as far as possible. Once the container has been opened, it should be kept tightly closed to protect it from hydration. A high moisture level in dehydrated culture media may reduce stability. With high moisture levels, chemical interactions will cause darkening of the product, falling pH and clumping.

The powder of dehydrated culture media should be uniform and free-flowing, and should be the indicated color for each medium. If there is any change in the physical appearance, the medium should be discarded.

The majority of media must be stored at room temperature between 2-25ºC. For certain culture media, storage must be in a refrigerator at a temperature range of 2-8ºC. If not described otherwise, the culture media in this manual will be stored at 2-25ºC.

Depending on the medium, and under suitable conditions, culture media have an expiry date of between 2 and 4 years, from the manufacturing date onwards. Dehydrated culture media should not be used after the expiry date. It is important that the inventory of powdered media is large enough to address all the necessary applications, but sufficiently small to assure constant rotation. Although many media can be kept at room conditions for long periods of time, not all, however, are stable indefinitely.

Before storing the culture media, the instructions on the label should be checked.

On the label, you will see different symbols explained below.

Keep away from moisture, dampness

Keep out of light

Indicates maximum and minimum temperature range

PREPARATION

Before preparing of the medium, it must be verified that the material being used is in a perfectly clean condition. All material should be rinsed with distilled or de-ionized water. It must also be checked that the media is not damaged or changed from its original state. The exact specified amount of each medium should be added to the amount of water, also specified. The water must be distilled or de-ionized and should not contain residues such as chlorine, copper, lead or detergents that may affect the medium.

The correct dissolution of the medium determines the clarity and effectiveness of the final product. It is essential to obtain a homogeneous solution with minimal exposure to heat.

The amount of powder required should be added to half the volume of water. After the powder and water are mixed completely, add the remaining water, taking care to remove any residual material from the wall of the container. Gently stir all together.

PREPARATION

On occasions, before adding the powder, it is advisable to heat the water to approximately 50°C to improve the dissolving process.

Allowing the medium to stand in water for 5 minutes before heating helps to obtain a uniform suspension. Many formulae that do not contain gelatine or agar dissolve without heat, but others require direct heat for complete dissolution, sometimes agitation as well. Apply heat evenly, boil as briefly as possible (normally a minute or two is sufficient). Follow the instructions for each specific medium as described on the label.

The media containing agar may boil suddenly and spill out of the containers in which they are being prepared. To avoid this, the media must be shaken frequently when they start to boil.

Some media show a slight cloudiness or precipitation due to their composition. When dispensed into the final container, it is essential for these types of media that the insoluble compounds are distributed as best as possible so that the nutrients are evenly distributed.

PH ADJUSTMENT

The media pH are set at the time of manufacture. Nevertheless, the quality of water used for hydration or the use of expired media can alter this parameter, so it is advisable to check and reset if necessary.

To check the pH, measure a sample extracted from the total volume of the medium prepared at 25 °C, both in liquid and solid media. In cases where the pH needs to be readjusted, a sterile solution of hydrochloric acid (to acidify the medium) or sodium hydroxide (to alkalise the medium) can be used.

STERILIZATION

The culture media contain different organisms which come from different raw materials and containers used. These organisms must be removed before inoculation to avoid false results. A great variety of sterilization methods exist, but sterilization by heat is the most used.

Vegetative cells are rapidly eliminated at temperatures around 60°C for 5-10 minutes. However, the elimination of spores needs a temperature of 121ºC for 15 minutes.

For sterilization of the medium, follow the preparation instructions on the label of each medium. In general, these instructions are for one liter of medium. For larger volumes, it is necessary to increase the sterilization time. Bear in mind that although the sterilization time increases, the temperature used should be maintained. In this case, validation studies must be made to determine what the cycle required for each volume is. More information should be obtained from the autoclave manufacturer.

The media containing carbohydrates should not be autoclaved at temperatures exceeding 116-118°C. In all cases overheating must be avoided.

In some liquid media, warming may lead to loss of activity of some compound. In these cases sterilization by filtration must be performed. The sample is passed through a filter and the microorganisms are retained. The efficiency of these systems depends on the filter pore size or its electrical charge: at an optimum pH most bacteria have a negative surface charge and therefore the most effective filtration will take place when the filter charge is more positive.

ADDING SUPPLEMENTS

Since most supplements have some thermal sensitive compound, the medium must be cooled to a temperature between 45-50ºC. For solid media, it is important not to cool too much because, if it starts to solidify, the supplement may not mix thoroughly in the medium.

In general, supplements are freeze-dried. Their restoration should be carried out with the substance indicated in each case, always in sterile conditions. The reconstituted supplement should be added when the suspension is at room temperature (25ºC). If it is at a lower temperature, the addition of the supplement can produce lumps due to the temperature difference between the medium and the supplement, resulting in poor homogenization of the supplement. After the addition of the supplement, it is essential to carry out a good homogenization of the final medium.

DISPENSING MEDIA

Tubes

The liquid or solid media should be dispensed in adequate tubes or flask for the intended use. Follow label instructions to know if Durham tubes must be added in case of liquid media or if agar must solidify in a slanted position.

Pouring on to plates

Before pouring the agar on to plates, the medium must have reached a temperature of 45-50ºC. If the temperature is higher, disponsable plate deformation or excessive formation of condensed water on the plates can occur.

The medium must be correctly homogenized before pouring it on to plates. The volume on 90 mm plates can vary between 15-20 ml, to produce a 2-3 mm layer of agar, unless a specific amount is indicated in the preparation instructions. The plates should be left to cool to room temperature on a horizontal surface with the plate lid on. Plates must not be moved until solidification has occurred. Care must be taken to avoid the formation of bubbles on the surface of the agar. Once solidified, keep upside down.

CONSERVING THE PREPARED MEDIA

The best practice is to prepare the media when it is about to be used. If this is not possible, it is essential that the medium is kept refrigerated at 2-8°C, away from light. Refrigeration promotes dehydration of the medium, so evidence of dehydration should be sought before use of the medium. Avoid condensation as the deposit of water drops can cause the medium to alter.

REMELTING

The remelting of the medium should be performed by placing the bottle or tube with the lid closed in a water bath, microwave oven or autoclave in strong steam. In media, the warming should be the minimum to avoid loss of quality of the medium. Remelted media have a tendency to darkening or precipitation, which increases when left melted for long periods of time. Overheating should be avoided. Never remelt the agar more than once.

QUALITY CONTROLS

RAW MATERIALS AND MANUFACTURE

The controls of raw materials are extremely important in order to obtain a quality dehydrated medium. That is why compatibility tests with other ingredients, performance tests and physical-chemical tests are carried out. Some of the basic constituents of culture media are natural products and, therefore, there is a possibility that there are small variations in their characteristics from batch to batch. In addition to performing the same controls as for other raw materials, a rigorous study on natural origin raw materials is carried out to ensure that the culture media have batch-to-batch reproducibility.

All production phases are carried out according to internal quality controls among which are maintenance, cleanliness and calibration of equipment, stock control and storage.

After manufacturing, the appearance, uniformity and moisture are controlled and the media are prepared to verify that all features such as appearance and pH, as well as chemical, physical and growth characteristics are correct. All controls are performed in parallel with a reference batch previously approved. These strict procedures ensure the uniformity and reproducibility of all lots.

CLIENT QUALITY CONTROLS

If they have been properly prepared, culture media should maintain the properties listed on the label and quality control. To ensure quality, checks should be made from each batch to verify that all parameters are correct.

Also verify:

- All dehydrated culture media are released within the pH range indicated on the label. However, after sterilization verify that the pH of the medium is consistent with the indicated values. The pH measurement must be performed at a **temperature of 25ºC.** If necessary, the pH can be adjusted before the medium solidifies. Avoid excessive pH adjustment, as this may alter the chemical composition of the medium
- **Sterility**. Check that the sterility of the medium is indeed sterile, incubating a significant sample of the prepared plates and leaving at the temperature specified for each
- **Physical-chemical properties**. The color of the medium or the transparency and absence of foreign particles should be verified
- **Growth/inhibition** of key organisms

THINGS TO CONSIDER

CHANGE IN PH

- The culture medium is expired or deteriorated
- Inadequate storage
- Impure water
- Incorrect weighing
- Residues in containers or use of alkaline glass
- Lack of homogenization
- Overheating or prolonged sterilization
- pH measured at a temperature above 25ºC
- Repeated remelting

SOLUBILITY

Precipitation (this can sometimes be an essential part of the medium, e.g.Bismuth Sulphit Agar)

- The culture medium is expired or deteriorated
- Inadequate storage
- Impure water
- Incorrect weighing
- Residues in containers or use of alkaline glass
- Insufficient shaking/agitation
- Lack of homogenization

Turbidity

- The culture medium is expired or deteriorated
- Inadequate storage
- The appropriate of water has not been used
- Lack of homogenization
- Incorrect pH
- Incomplete dissolution
- Overheating
- Loss of water in the prepared medium due to evaporation

BLACKENING

- The culture medium is expired or deteriorated
- Incorrect weighing
- Insufficient shaking/agitation
- Overheating
- Incorrect pH
- Repeated remelting
- Soft Gel
- The culture medium is expired or deteriorated
- Incorrect weighing
- Insufficient shaking/agitation
- Lack of homogenization
- Overheating
- Acid hydrolysis of the agar
- Excess of inoculum
- Repeated remelting

ABNORMAL MEDIUM COLOR

- The culture medium is expired or deteriorated
- Impure water
- Residues in the containers used
- Overheating
- Incorrect pH in the case where the medium has pH indicators

CONTAMINATION

- Inappropriate or inadequate sterilization
- Mistakes when adding supplements or additives

LOSS OF GROWTH PROMOTING OR DIFFERENTIATING PROPERTIES

Due to the culture media

- The culture medium is expired or deteriorated
- Incorrect weighing
- Incomplete dissolving
- Overheating
- Repeated remelting
- Excess of inoculum

Due to the supplements

- The supplement is expired or deteriorated
- Poor reconstitution of the supplement
- Adding a concentration lower than required
- Poor homogenization of the supplement with the culture medium

Other factors

- The organisms used for the medium control were damaged
- Poor culture conditions

TOXICITY

- Residues in the containers used
- Impure water
- Burning or scorching of medium

OTHER POSSIBLE ERRORS IN THE PREPARATION OF MEDIA

- The cooling process following sterilization must be a slow one as a sharp cooling could lead a loss of the media characteristics
- The media containing phosphate buffers and glucose or another carbohydrate may hide if there is overheating. A precipitate may also appear in these media when using low quality water
- A common phenomenon of overheating is the Maillard-type browning reaction which causes discoloration and loss of nutritional quality of the medium. This reaction is normally caused by a decrease of sugars which interact with amino acids originated from proteins. In these cases the carbohydrates should be sterilized

SAFETY

These products are exclusively for professional use and should be used only by trained and qualified personnel. They should not be used for self-administrated analysis by patients preparation guidelines should be read and strictly adhered to.

Dehydrated culture media may contain hazardous or toxic substances. An excessive concentration of dust in the workplace can reduce visibility and can get in the eyes, ears or respiratory tract. The inhalation of these substances in high concentrations can be harmful, causing irritation to the nose, throat and respiratory tract. Therefore, it is advisable to wear a mask and eye protection when using dehydrated media. The use of gloves is also recommended.

Some of the **hazardous substances** used in the culture media are: *Acetamide, Acriflavin, Bile salts, Brilliant green, Cetrimide, Chloramphenicol, Cycloheximide, Dicloran, Lithium chloride, Malachite green, Rose bengal, Sodium azide* and *Tergitol.*

Never heat a completely closed container since it may explode. When heating an open container the opening should face away from the person so that if there is a splash or spray, it does not cause injury to oneself or someone nearby.

If during cultivation a gas burner is used (e.g. a Bunsen burner), ensure that there is no flammable material nearby. Never use an open flame to heat.

Once cultivated, the medium must be autoclaved at 121ºC for 30 minutes before final disposal. The same should be done with laboratory materials and equipment before washing and reuse.

Hazardous wastes produced must be deposited in special containers which are intended for collection, the same applies to glass products. Solid products such as agar should not be flushed down the drain because they can clog pipes.

Please consult specific biohazard legislation in your country.

For more information about safety, MSDS are available.

QUICK GUIDE FOR MANUAL USE

ABBREVIATIONS

APHA American Public Health Association

AOAC Official Methods of Analysis of AOAC International

ATCC American Type Culture Collection

BAM Bacteriological Analytical Manual

CECT Colección Española de Cultivos Tipo (Spanish Type Culture Collection)

DEV Deutsches Einheitverfahren zur Wasser-, Abwasser-, und Schlammuntersuchung (German Methods for the examinations of Water, Wastewater and Sludge)

EP European Pharmacopeia

ISO International Organization for Standardization

NCCLS National Committee of Clinical Laboratory Standards

NCTC National Collection of Type Cultures

USP United States Pharmacopeia

WHO World Health Organization

Additional information for users

- Decisions and interpretation of the diagnosis must be carried out by trained and qualified personnel
- Do not use prepared media if they present evidence of microbial contamination, discoloration, dehydration, cracks, or any other signs of deterioration
- The standard protocols for extraction, transport and processing samples must be followed

DEHYDRATED CULTURE MEDIA

PRODUCTS

Microbiology Products

DEHYDRATED CULTURE MEDIA

A1 MEDIUM

CAT. 1252

For detection of coliforms in water and foods

FORMULA IN g/l

Tryptone	**20.00**	Lactose	**5.00**
Sodium Chloride	**5.00**	Salicin	**0.50**
Triton X-100	**1.00 ml**		
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 31.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

Note: for 10 ml water samples, prepare a double-strength medium to ensure the ingredient concentrations are not reduced below those of the standard medium.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Escherichia coli ATCC 25923 | Unnoculated Tube

USES

A1 MEDIUM is also known as A1 Broth. It is used for purity analysis (source water, marine water, treated wastewater) and for foods, particularly seafood, using the detection of coliforms and in particular *Escherichia coli*. This medium was created to reduce the timing of recovery and also to be used without a need of a pre-enrichment step. Furthermore, the use of this medium improves reliability as it reduces the presence of false positives.

Tryptone provides the nitrogen, vitamins, minerals and aminoacids for growth. Lactose is the fermentable carbohydrate providing carbon and, together with Salicin, provides energy for growth. Triton X-100 is a surfactant and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Inoculate sample into tubes and incubate at 35°C for three hours. Transfer tubes to a 44.5°C water bath and incubate for an additional 21 ± 2 hours.

Gas production is a positive reaction indicating the presence of coliforms. Gas may be produced in the inverted vial or may appear as dissolved gas that forms gas bubbles when slightly agitated. Calculate densities using MPN standard methods.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C for three hours, and at 44.5°C for 21 ± 2 hours.

Microorganisms	Growth	Gas Production	
		35 ± 2°C	44.5°C
Enterobacter aerogenes ATCC 13048	Good	+/-	-
Escherichia coli ATCC 25922	Good	+	+
Bacillus cereus ATCC 6633	Inhibited	-	-
Enterococcus faecalis ATCC 19433	Inhibited	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

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ACETAMIDE AGAR

CAT. 1391

For the differentiation of non-fermentative gram-negative bacteria, in particular *Pseudomonas aeruginosa*

FORMULA IN g/l

Sodium Chloride	**5.00**	Dextrose	**0.20**
Acetamide	**3.00**	Phenol red	**0.03**
Monopotassium Phosphate	**1.00**	Bacteriological Agar	**15.00**
Yeast Extract	**0.50**		
Final pH 6.3 ± 0.2 at 25°C			

PREPARATION

Suspend 24.7 grams of the medium in one liter of distilled water Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5 - 2.0 cm of depth. The prepared medium should be stored at 2 - 8°C. The color is yellow-orange.

The dehydrated medium should be homogeneous, free-flowing and pink-orange in color. If there are any physical changes, discard the medium.

CAUTION: This medium contains acetamide. It is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

ACETAMIDE AGAR is used to determine the ability of non-fermenting Gram-negative bacteria to deaminate the acetamide. The deamination of the acetamide produces ammonia which increases the pH of the medium. The resulting alkalinisation is shown by a color change of the Phenol red from yellow-orange to purple-red.

Acetamide deamination is accomplished by *Pseudomonas aeruginosa*, *Pseudomonas acidovorans*, Group III (*Achromobacter xylosoxidans*), and *Alcaligenes odorans*.

Acetamide is a carbon source. Dextrose is a fermentable carbohydrate providing carbon and energy, the Potassium salts have a high buffering capacity. Sodium chloride supplies essential electrolytes for transport and osmotic balance, Phenol red is a pH indicator and Bacteriological agar is the solidifying agent.

A positive reaction turns the medium an intense purple-red. *P. aeruginosa* is confirmed by positive asparagine and acetamide tests.

Pseudomonas aeruginosa is an opportunist pathogen for humans, capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water are *Pseudomonas aeruginosa* free at the time of their commercialization. This microorganism can also be found in swimming pool water.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Purple - Red
Escherichia coli ATCC 25922	Inhibited	-
Proteus mirabilis ATCC 29906	Inhibited	-
Pseudomonas aeruginosa ATCC 9027	Good	+
Pseudomonas aeruginosa ATCC 25668	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

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ACETAMIDE BROTH

CAT. 1211

For the confirmation of *Pseudomonas aeruginosa* in bottle water

FORMULA IN g/l

Acetamide	**10.00**	Monopotassium Phosphate	**0.73**
Sodium Chloride	**5.00**	Phenol red	**0.012**
Dipotassium Phosphate	**1.39**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 17.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution Sterilize by filtration.

AVOID OVERHEATING. DO NOT AUTOCLAVE. Dispense into appropriate containers. The prepared medium should be stored at 2 - 8°C. The color is pink-orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

Pseudomonas aeruginosa
ATCC 9027

CAUTION: This medium contains acetamide. It is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

ACETAMIDE BROTH contains acetamide which, as a sole source of carbon in the medium, is used for the confirmation and identification of *Pseudomonas aeruginosa*. It uses the ability of non fermenting Gram-negative bacteria to deaminate the acetamide, the deamination of the acetamide produces ammonia which increases the pH of the medium, the resulting alkalinisation is shown by a color change of the Phenol red from orange-red to purple-red.

Acetamide deamination is accomplished by *P. aeruginosa*, *P. acidovorans*, Group III *Achromobacter xylosoxidans*, and *Alcaligenes odorans*.

Acetamide is the single carbon source. Potassium salts have a high buffering capacity. Sodium chloride supplies essential electrolytes for transport and osmotic balance and Phenol red is a pH indicator.

Inoculate with one or two loopfuls from a tube of presumptive fresh medium (Asparagine Broth, **Cat. 1207**) and incubate at 35 ± 2°C for 2 - 4 days.

A positive reaction turns the medium an intense purple-red. *P. aeruginosa* is confirmed by a positive asparagine test and a positive acetamide test.

Pseudomonas aeruginosa is an opportunist pathogen for humans which is capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water are *Pseudomonas aeruginosa* free at the time of their commercialization. This microorganism can also be found in swimming pool water.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Change to purple -red
Escherichia coli ATCC 25922	Inhibited	-
Proteus mirabilis ATCC 29906	Inhibited	-
Pseudomonas aeruginosa ATCC 9027	Good	+
Pseudomonas aeruginosa ATCC 25668	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Kelly, N.M., C.T. Keans (1.983) Acetamide Broth for Isolation of Pseudomonas aeruginosa from patients with cystic fibrosis. J. Clin. Microbiol 17:159-163.

CeNAN (1.982) Técnicas para el Examen microbiológico de Alimentos y Bebidas. Madrid.

ACETAMIDE BROTH
UNE-EN 12780, EN ISO 16266

CAT. 1155

For confirmation of *Pseudomonas aeruginosa* by membrane filtration

FORMULA IN g/l

Acetamide	**2.00**	Sodium Chloride	**0.20**
Monopotassium Phosphate	**1.00**	Magnesium Sulfate	**0.20**
Final pH 7.0 ± 0.5 at 25°C			

PREPARATION

Suspend 3.4 grams of the medium in 900 ml of distilled water. Adjust the pH to 7.0 ± 0.5 at 25°C. Add one ml of recently prepared *Solution B**. While agitating, add water to obtain a final volume of one liter. Distribute into tubes in 5 ml aliquots, close and sterilize in autoclave at 121°C for 15 minutes. The prepared

medium should be stored at 2 - 8°C. Prepared tubes must be stored in a dark place. The color is colorless.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

SOLUTION B*

Sodium Molibdate..... 0.50 g

Iron Sulfate Heptahydrate ($FeSO_4$, $7H_2O$).....0.05 g

Distilled Water ..... 100 ml

CAUTION: This medium contains acetamide. It is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

ACETAMIDE BROTH contains acetamide which is the sole source of carbon. It is used for the confirmation and identification of *Pseudomonas aeruginosa*, as specified by the UNE-EN12780 and EN ISO 16266. It uses the ability of non-fermenting Gram-negative bacteria to deaminate the acetamide. The deamination of the acetamide produces ammonia which increases the pH of the medium, acetamide deamination is accomplished by *P.aeruginosa*, *P. acidovorans*, Group III (*Achromobacter xylosoxidans*), and *Alcaligenes odorans*.

Acetamide is the single carbon source. The Potassium salt has a high buffering capacity and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

The colonies from Nutrient Agar (**Cat. 1156**) that need to be confirmed are inoculated in this medium and incubated for 22 ± 2 hours at 36 ± 2°C.

Add 1 or 2 drops of Nessler reagent and examine the production of Ammonia in the tubes, characterized by a color ranging between yellow and tile red, depending on the concentration.

This medium is prepared according to UNE-EN 12780 and EN ISO 16266.

Pseudomonas aeruginosa is an opportunist pathogen for humans, capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water are *Pseudomonas aeruginosa* free at the time of their commercialization, This microorganism can also be found in swimming pool water.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, at a temperature of 36 ± 2°C and observed after 22± 2 hours.

Microorganisms	Growth	Ammonium Production
Pseudomonas aeruginosa ATCC 9027	Good	+
Pseudomonas aeruginosa ATCC 10145	Good	+
Pseudomonas aeruginosa ATCC 25783	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

UNE-EN 12780, Quality of water. Identification and enumeration of Pseudomonas aeruginosa by membrane filtration.

EN ISO 16266 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration

ACETATE DIFFERENTIAL AGAR

CAT. 1192

For the differentiation of *Shigella* from *E. coli* and non fermentative gram-negative bacilli

FORMULA IN g/l

Sodium Chloride	**5.00**	Magnesium Sulfate	**0.10**
Sodium Acetate	**2.00**	Bromothymol Blue	**0.08**
Dipotassium Phosphate	**1.00**	Bacteriological Agar	**20.00**
Monoammonium Phosphate	**1.00**		
Final pH 6.7 ± 0.2 at 25°C			

PREPARATION

Suspend 29 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes Cool to 50°C, mix well and dispense into appropriate containers. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium is green.

The dehydrated medium should be homogeneous, free-flowing and beige with a green tint in color. If there are any physical changes, discard the medium.

USES

ACETATE DIFFERENTIAL AGAR is used to test the ability of an organism to use acetate as the sole source of carbon.

Most bacteria can use citrate and acetate with organic nitrogen present. Simmons Citrate Agar was elaborated by Simmons to measure citrate use without the presence of organic nitrogen. Trabulsi and Ewing replaced sodium citrate with sodium acetate in their formulation of Acetate Differential Agar.

The medium contains a mixture of salts and Sodium acetate, as a sole source of carbon, which results in the production of alkaline products. The increment in pH creates a blue color in the medium due to the presence of Bromothymol blue. Dipotassium phosphates act as a buffer system. Bacteriological agar is the solidifying agent.

Typical cultures of *Shigella* are unable to use acetate and fail to grow; therefore, the medium remains unchanged. The majority of *Escherichia coli* grow well within 24 - 48 hours, but some strains grow more slowly and may give a false-negative reaction if results are observed at 24 - 48 hours only. The growth is indicative of the use of Acetate.

Incubate at 35°C ± 2°C and observe periodically for 7 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed periodically for 7 days.

Microorganisms	Growth
Shigella sonnei ATCC 25931	Inhibited
Escherichia coli ATCC 25922	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Simmons, J.S.1926 J.Infect. Dis.39209

Trabulsi, L.R. and W.H. Ewing 1962 Public Health Lab.20.137

Edwards, P.R. and W.H. Ewing 1972. Identification of Enterobacteriaceae

AEROMONAS AGAR BASE (RYAN)

CAT. 1370

For the selective isolation of *Aeromonas hydrophila* from clinical and environmental samples when Ampicillin is used as a selective supplement

FORMULA IN g/l

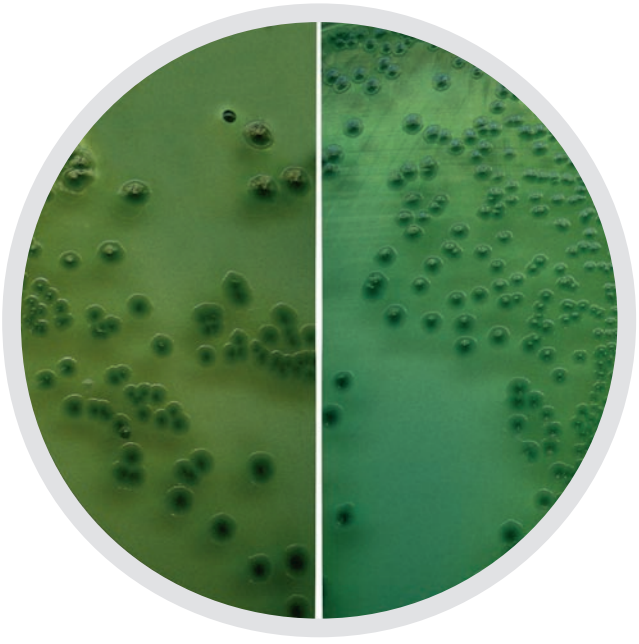
Sodium Thiosulfate	**10.67**	Inositol	**2.50**
Sodium Chloride	**5.00**	L-Arginine Hydrochloride	**2.00**
Proteose Peptone	**5.00**	Lactose	**1.50**
Xylose	**3.75**	Ferric Ammonium Citrate	**0.80**
L-Lysine Hydrochloride	**3.50**	Bromothymol Blue	**0.04**
Sorbitol	**3.00**	Thymol Blue	**0.04**
Bile Salts N°3	**3.00**	Bacteriological Agar	**12.50**
Yeast Extract	**3.00**		

Final pH 8.0 ± 0.2 at 25°C

PREPARATION

Suspend 28.1 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C and aseptically add one vial of Ampicillin Supplement (**Cat. 6052**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is greenish-blue.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.



Aeromonas hydrophila ATCC 7966 | *Pseudomonas aeruginosa* ATCC 27853

AMPICILLIN SUPPLEMENT (CAT. 6052)
(1 vial for 500 ml of the medium)

Sodium Ampicillin ..... 2.5 mg

USES

AEROMONAS AGAR BASE (RYAN) is based on the formulation of Ryan, it is a modification of the XLD Medium (**Cat. 1080**) that has been designed to improve the count and isolation of *Aeromonas* in clinical and environmental samples. The medium is better than other medium in the detection of *Aeromonas* in water, bottled water and foods stuffs (meat, fish, etc).

Proteose peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. L-lysine and L-arginine provides nitrogen, sulfur and trace elements. Yeast extract is source of vitamins, particularly the B-group. Inositol, Lactose, Sorbitol and Xylose are the carbohydrate substrates, Sodium thiosulfate provides Sulphur and Ferric ammonium citrate is the indicator for H_2S production. H_2S positive colonies have a black center. The mixed indicators Bromothymol blue and Thymol blue change their color to yellow, when acid is formed. Sodium chloride supplies essential electrolytes for transport and osmotic balance and Bile Salts are inhibitors of Gram-positive organisms.

Aeromonas are aquatic, fresh water bacteria that were identified during the last century as responsible of infectious processes in aquatic animals: amphibians, reptiles, fish, snails and others. *Aeromonas* also cause intestinal tract diseases. Infections are contracted frequently due to direct exposure to the water in which these microorganisms live. This medium is also useful for clinical diagnoses.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the supplement added, after incubation at a temperature of 30 - 35°C and observed after 24 hours.

Microorganisms	Growth	Colony Color
Aeromonas hydrophila ATCC 7966	Good	Green with a black center
Pseudomonas aeruginosa ATCC 27853	Good	Blue-Green
Pseudomonas aeruginosa ATCC 9270	Good	Blue-Green
Escherichia coli ATCC 25922	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ryan N. (1985) Personal communication.

Rogol M., Sechter I., Grinberg L., Gerichter Ch. B. (1992) J. Med. Microbiol. 12. 229-331
Atkinson M. (1986) Culture Vol. 7, No.2.

ALKALINE PEPTONE WATER

CAT. 1407

For the enrichment of *Vibrio* species from food, water and clinical samples

FORMULA IN g/l

Proteose Peptone	**10.00**	Sodium Chloride	**5.00**
Peptone	**10.00**		
Final pH 8.6 ± 0.2 at 25°C			

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. To make a 10x-strength base suspend 250 grams instead of 25 grams. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ALKALINE PEPTONE WATER is used for the enrichment of *Vibrio cholerae* and *Vibrio* species from food, water, feces and clinical studies.

This medium has been recommended by various authors to increase the recovery of *Vibrio* species in fecal materials and other samples.

Peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance and encourages the growth of *Vibrio cholerae*.

It is claimed that raising the medium's pH leads the medium's alkalinity to inhibit most of the unwanted flora background, leaving the viability of the *Vibrio* species intact.

Growth in tubes is indicated by turbidity compared to an uninoculated control. Additional steps are recommended, like plating onto a selective and non-selective media for isolation and morphology, and biochemical and serological studies for identification.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C ± 2°C and observed after 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	-
Streptococcus pneumoniae ATCC 6301	-
Vibrio cholerae EITOR ATCC 14033	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Colwell, R.R. 1996. Global climate and infectious disease: the cholera paradigm. Science 274. Kelly, M,T, F.W. Hickman-Bremer, and J.J. Framer III

AMIES TRANSPORT MEDIUM WITH CHARCOAL

CAT. 1535

For transport and maintenance of microbiological samples

FORMULA IN g/l

Activated Charcoal	**10.00**	Monopotassium Phosphate	**0.20**
Sodium Chloride	**3.00**	Calcium Chloride	**0.10**
Disodium Phospate	**1.10**	Magnesium Chloride	**0.10**
Sodium Thioglicollate	**1.00**	Agar N°2	**7.50**
Potassium Chloride	**0.20**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 23.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Maintain a homogeneous mixture of the charcoal throughout the medium by inverting the tubes as they cool. The prepared medium should be stored at 2 - 8°C. The color is black.

The dehydrated medium should be homogeneous, free-flowing and black in color. If there are any physical changes, discard the medium.

USES

AMIES TRANSPORT MEDIUM WITH CHARCOAL is used for collecting, transporting and preserving microbiological specimens. It is formulated to maintain the viability of microorganisms without significant increase in growth, being non-nutritive, phosphate buffered and semi-solid.

Amies developed his formula (1967) with charcoal upon proving that *Neisseria gonorrhoeae* increased its survival rate when charcoal swabs were used. Amies solved the problem of charcoal removal from the swabs by incorporating charcoal into the formulation. Amies Transport Medium is recommended for throat, vaginal, and wound samples.

In the formulation, Charcoal neutralizes fatty acids that are toxic to microorganisms. The Chloride salts supply essential electrolytes for transport and osmotic balance. Phosphates act as a buffer system. Sodium thioglycollate suppresses oxidative changes and provides a reduced environment.

Insert inoculated sterile swabs into the upper third of the transport medium within the transport container; break off the protruding portion of the swab stick and tightly screw shut. Send to laboratory within 24 hours for culture analysis. Specimens may be refrigerated until ready for shipment.

The survival of bacteria in a transport medium depends on various factors such as bacteria type and concentration in the specimen, transport medium formulation, the temperature and duration of transport, and inoculation to appropriate culture media within 24 hours. Optimal growth and typical morphology can only be expected if direct inoculation and appropriate cultivation are followed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures kept at different temperatures (4°C and room temperature) for up to 72 hours.

Microorganisms	Recovery at 4°C	Recovery at 25°C
Neisseria gonorrhoeae ATCC 19424	≥ 50%	≥ 50%
Brucella abortus ATCC 4315	≥ 50%	≥ 50%
Streptococcus pneumoniae ATCC 6303	≥ 50%	≥ 50%
Shigella flexneri ATCC 12022	≥ 50%	≥ 50%
Salmonella typhi ATCC 6539	≥ 50%	≥ 50%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Amies C.R. (1967) "A Modified Formula for the Preparation of Stuart's Transport Medium". Can. J. Public Health 58: 296-300.

AMIES TRANSPORT MEDIUM WITHOUT CHARCOAL

CAT. 1530

For transport and maintenance of microbiological samples

FORMULA IN g/l

Sodium Chloride	**3.00**	Monopotassium Phosphate	**0.20**
Disodium Phosphate	**1.10**	Calcium Chloride	**0.10**
Sodium Thioglicollate	**1.00**	Magnesium Chloride	**0.10**
Potassium Chloride	**0.20**	Agar N°2	**7.50**

Final pH 7.3 ± 0.2 at 25°C

PREPARATION

Suspend 13.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

AMIES TRANSPORT MEDIUM WITHOUT CHARCOAL is used for collecting, transporting and preserving microbiological specimens. It is formulated to maintain the viability of microorganisms without significant increase in growth, being non-nutritive, phosphate buffered and semi-solid. Amies Transport Medium is recommended for throat, vaginal, and wound samples.

The Chloride salts supply essential electrolytes for transport and osmotic balance. Phosphates act as a buffer system. Sodium thioglycollate suppresses oxidative changes and provides a reduced environment.

Insert inoculated sterile swabs into the upper third of the transport medium within the transport container; break off the protruding portion of the swab stick and tightly screw shut. Send to laboratory within 24 hours for culture analysis. Specimens may be refrigerated until ready for shipment.

The survival of bacteria in a transport medium depends on various factors such as bacteria type and concentration in the specimen, transport medium formulation, the temperature and duration of transport, and inoculation to appropriate culture media within 24 hours. Optimal growth and typical morphology can only be expected if direct inoculation and appropriate cultivation are followed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures kept at different temperatures (4°C and room temperature) for up to 72 hours.

Microorganisms	Recovery at 4°C	Recovery at 25°C
Neisseria gonorrhoeae ATCC 19424	≥ 50%	≥ 50%
Brucella abortus ATCC 4315	≥ 50%	≥ 50%
Streptococcus pneumoniae ATCC 6303	≥ 50%	≥ 50%
Shigella flexneri ATCC 12022	≥ 50%	≥ 50%
Salmonella typhi ATCC 6539	≥ 50%	≥ 50%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Amies C.R. (1967) "A Modified Formula for the Preparation of Stuart's Transport Medium". Can. J. Public Health 58: 296-300.

ANAEROBIC AGAR

CAT. 1000

For the cultivation of anaerobes, especially *Clostridium*

FORMULA IN g/l

Casein Peptone	**17.50**	Sodium Formaldehyde Sulfoxyde	**1.00**
Dextrose	**10.00**	L-Cystine	**0.40**
Soy Peptone	**2.50**	Methylene Blue	**0.002**
Sodium Chloride	**2.50**	Bacteriological Agar	**15.00**
Sodium Thioglycollate	**2.00**		

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 51 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. The prepared medium should be stored

at 8 - 15ºC. The color is white with a blue tint. The medium can be incubated in an anaerobic jar or with Brewer lids for anaerobiosis.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

ANAEROBIC AGAR is used for cultivation of anaerobic microorganisms. Anaerobic bacteria are unable to use oxygen as a terminal electron acceptor.

Casein peptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium thioglycollate and Sodium formaldehyde sulfoxylate act as reducing agents generating a low oxidation-reduction potential, thus securing good anaerobic conditions. Methylene blue acts as the redox indicator, the blue color indicates the presence of oxygen.

The testing procedures can be carried out using standard Petri dishes or Brewer's Anaerobic Agar Plates, both with the medium cooled to 45 - 50°C.

The seeding of the sample (clinical or food) can be performed by surface inoculation or by pour plate method. Normally the sample should never be heated to destroy the vegetative forms of the aerobe, as the anaerobic non-spore formers will also be destroyed. Nevertheless, sometimes it could be useful to heat the sample when spore formers such as *Clostridium* are sought, except *C. perfringens*, which rarely forms spores. When heating is indicated, warm the sample suspended in a liquid diluent (peptone water, buffering phosphate solution, etc.) to 70 - 80°C for 10 minutes. Incubate at 35 ± 2°C for 18 - 48 hours.

The plates of Anaerobic Agar can also be incubated in a normal atmosphere covering the surface of the plates with a Brewer lid. When growth is observed, open the plate and pick the desired colonies. Incubate longer if necessary. If the medium has not been prepared fresh before use, it is necessary to heat and remelt to expel the dissolved oxygen.

Thioglycollate Medium (**Cat. 1508**) without Indicator is an excellent enrichment broth and, frequently using it previously, yields better results than direct seeding.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours under anaerobic conditions.

Microorganisms	Growth
Clostridium butyricum ATCC 9690	Good
Clostridium perfringens ATCC 12919	Good
Clostridium sporogenes ATCC 11437	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Standard Methods for the Examination of Water and Wastewater 1 5th Ed. American Public Health Association, Inc, Washington, D.C. 1980. Andrew, W.H.C.D. Diggs, and C.R. Wilson, 1975.

Evaluation of medium for the rapid recovery of Escherichia coli from shellfish. App. Microbiol. 29: 130-131

ANTIBIOTIC MEDIUM Nº1 (SEED AGAR) USP, EUROPEAN PHARMACOPOEIA

CAT. 1520

Standard medium used for the preparation of the seed layer in antibiotic assays

FORMULA IN g/l

Peptone	**6.00**	Beef Extract	**1.50**
Pancreatic Digest of Casein	**4.00**	Glucose Monohydrate	**1.00**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 6.6 ± 0.2 at 25°C			

*This medium has the same formula as Antibiotic Medium N° 11 (**Cat. 1528**), with the difference that the pH of the medium has been adjusted to 6.6.

PREPARATION

Suspend 30.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 1 is used for testing the potency of antibiotics through their inhibitory effects on microorganisms. The cylinder assay plate is based on the diffusions of the antibiotics under test, measuring the size of the inhibition zones of microorganisms.

Gelatin peptone, Casein peptone, Yeast extract and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological Agar is the solidifying agent.

ASSAY PLATES

Seed Agar is used as an inoculum substrate. It is cooled to 48°C and inoculated accordingly with the specific antibiotics under test. Use 2 ml of the liquid culture to inoculate 100 ml of the Seed Agar. Agitate the mixture gently to produce a homogeneous distribution and pour 4 ml on each plate of solidified Base Agar (21 ml).

It is very important that the seed layer is evenly distributed over the entire surface of the Base Agar. Once the seed layer is solid, the cylinders are placed evenly spaced on a 2.5 - 3 mm radius. The standard sample is added as described above. This method is used for testing the potency of bacitracin and penicillin preparations.

Seed Agar is used for the basic layer as well as the seed layer for the assay of chloramphenicol in plates. A medium with a higher pH and the same formula is used for the assay of erythromycin, carbomycin and neomycin: Neomycin Test Agar (Antibiotic Medium N° 11, **Cat. 1528**).

ENUMERATION OF MICROORGANISMS

Seed Agar can be used to determine the number of microorganisms in many antibiotic preparations.

DETERMINATION OF ANTIBIOTICS IN MILK

Disk diffusion methods are utilized to detect the presence of residual antibiotics. The milk used to manufacture fermented products is tested for inhibitory substances, such as residual antibiotics for the treatment of mastitis of the animal, which can interfere with the normal activity of the initial culture.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Inhibition zones
Staphylococcus aureus ATCC 6538	Good	Cephalothin, Chloramphenicol, Penicillin
Micrococcus luteus ATCC 9341	Good	Cephalothin, Chloramphenicol, Penicillin

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP EP

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696. The United States Pharmacopoeia Convention, Rockville, Md.

European Pharmacopoeia 7.0

ANTIBIOTIC MEDIUM N°2 (BASE AGAR) USP

CAT. 1002

Standard medium used for the preparation of the base layer in the antibiotic assays

FORMULA IN g/l

Gelatin Peptone	**6.00**	Beef Extract	**1.50**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 6.6 ± 0.1 at 25°C			

* This medium has the same formula as Antibiotic Medium N° 5 (**Cat. 1524**) and Antibiotic Medium N° 8 (**Cat. 1004**), with the difference that the pH of the medium has been adjusted to 6.6.

PREPARATION

Suspend 25.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 2 is the standard medium used to prepare the base layer in the microbiological assay of antibiotics. This medium is prepared in accordance with the FDA and USP guidelines. It is used to prepare the base layer in the microbiological assay of antibiotics such as bacitracin, chloramphenicol and penicillin. The potency of an antibiotic can be demonstrated under appropriate conditions by its inhibitory effect on microorganisms.

Gelatin peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Bacteriological Agar is the solidifying agent.

To carry out the antibiotic test, the Base Agar should be prepared on the same day as the test is done. The sample can be tested by the two methods of dilution and assay plate diffusion.

The assay plate diffusion method is the most common and can be performed using various techniques: cylinders, punched-hole or paper disc tests.

For the cylinder method, pour 21 ml of the medium into a Petri dish (20 x 100 mm) and cover to avoid dehydration.

Once the medium has solidified, add 4 ml of the seed layer inoculated with the standardized culture to be used for the particular antibiotic to be tested. Be sure to obtain an even distribution of this layer. The layer is allowed to solidify and the cylinders are placed on the surface. The dilutions of the antibiotic will be added to these cylinders.

The plate is incubated for 24 hours at 35 ± 2°C. The zones of inhibition are observed, measured and compared with the calibration curve determined by adding known amounts of the same antibiotic under the same experimental conditions.

The use of standardized culture media and strict control of all test conditions are essential requirements in the microbiological assay of antibiotics in order to obtain satisfactory test results.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Inhibition zones
Staphylococcus aureus ATCC 6538	Good	Methicillin, Dicloxacillin
Micrococcus luteus ATCC 10240	Good	Bacitracin
Staphylococcus epidermidis ATCC 12228	Good	Novobiocin

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696. The United States Pharmacopoeia Convention, Rockville, Md.

ANTIBIOTIC MEDIUM N° 3 USP

CAT. 1534

Standard medium for use in antibiotic assays

FORMULA IN g/l

Gelatin Peptone	**5.00**	Beef Extract	**1.50**
Dipotassium Phosphate	**3.68**	Monopotassium Phosphate	**1.32**
Sodium Chloride	**3.50**	Dextrose	**1.00**
Yeast Extract	**1.50**		
Final pH 7.0± 0.2 at 25°C			

PREPARATION

Suspend 17.5 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8ºC. The color is clear amber.

The dehydrated medium should be homogeneous, free flowing and beige in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 3 is prepared according to the formula specified by the Food and Drug Administration (FDA) and the United States Pharmacopoeia USP.

Gelatin peptone, Yeast extract and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Potassium phosphates act as a buffer system. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological Agar is the solidifying agent.

The potency of an antibiotic can be demonstrated under appropriate conditions by its inhibitory effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.

Antibiotic Medium N° 3 can be used with the following microbiological methods for Antibiotic Assays:

1. Cylinder method in plates.
2. Serial dilution method.
3. Turbidimetric method.

In the cylinder method in plates, Antibiotic Medium N° 3 is used to resuspend the inoculum in the potency assay for penicillin, erythromycin, neomycin, chlortetracycline and chloramphenicol.

The serial dilution method is used for penicillin assay.

Lastly, this medium can also be used in the turbidimetric determination of the potency of bacitracin, streptomycin and terramycin. The turbidimetric method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform solution of an antibiotic. Use of this method is appropriate only when test samples are clear.

Plates are prepared and incubated following the FDA and the USP guidelines. The use of standardized culture media and strict control of all test conditions are essential requirements in the microbiological assay of antibiotics in order to obtain satisfactory test results.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Inhibition zones
Micrococcus luteus ATCC 9431	Good	Erythromycin
Staphylococcus aureus ATCC 6538	Good	Kanamycin, Neomycin
Klebsiella pneumoniae ATCC 10031	Good	Streptomycin

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696. The United States Pharmacopoeia Convention, Rockville, Md.

ANTIBIOTIC MEDIUM N° 5 (STREPTOMYCIN ASSAY AGAR) USP

CAT. 1524

For use in the potency assay of streptomycin

FORMULA IN g/l

Gelatin Peptone	**6.00**	Beef Extract	**1.50**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**

Final pH 7.9 ± 0.2 at 25°C

*This medium has the same formula as Antibiotic Medium N° 2 (**Cat. 1002**) and Antibiotic Medium N° 8 (**Cat. 1004**), with the difference that the pH of the medium has been adjusted to 7.9.

PREPARATION

Suspend 25.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 5 can be used in the cylinder plate method for the assay of streptomycin, generally with *Bacillus subtilis* as the organism tested.

Gelatin peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Bacteriological Agar is the solidifying agent.

This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. The diameter of an inhibition zone after incubation depends, in part, on the concentration or activity of the antibiotic. This method is used in the assay of commercial preparations of antibiotics, as well as in the quantitative determination of antibiotics in body fluids, animal feeds and other materials.

Plates are prepared and incubated following the FDA and the USP guidelines. The use of standardized culture media and strict control of all test conditions are essential requirements in the microbiological assay of antibiotics in order to obtain satisfactory test results.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Inhibition zones
Bacillus subtilis ATCC 6633	Good	Gentamicin, Streptomycin

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1 696. The United States Pharmacopoeia Convention, Rockville, Md.

ANTIBIOTIC MEDIUM N°8 (BASE AGAR WITH LOW pH) USP

CAT. 1004

For use in the plate assay of tetracycline and other antibiotics

FORMULA IN g/l

Gelatin Peptone	**6.00**	Beef Extract	**1.50**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 5.7 ± 0.1 at 25°C			

*This medium has the same formula as Antibiotic Medium N° 2 (**Cat. 1002**) and Antibiotic Medium N° 5 (**Cat. 1524**), with the difference that the pH of the final medium has been adjusted to 5.7.

PREPARATION

Suspend 25.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize at 121°C for 15 minutes, cool at 45 - 50°C and dispense into sterile Petri dishes. Prepare the inoculum for assay by washing growth from a fresh 24 - 48 hours agar slant, using sterile distilled water or saline water. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 8 is used to prepare the base layer for the assay of tetracycline's and other antibiotics.

Gelatin peptone, Yeast extract and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological Agar is the solidifying agent.

The potency of an antibiotic can be demonstrated under appropriate conditions by its inhibitory effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.

Plates are prepared and incubated following the FDA and the USP guidelines. The use of standardized culture media and strict control of all test conditions are essential requirements in the microbiological assay of antibiotics in order to obtain satisfactory test results.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Inhibition zones
Bacillus cereus ATCC 11778	Good	Tetracycline
Micrococcus luteus ATCC 10240	Good	Tetracycline, Chlortetracycline

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696.

ANTIBIOTIC MEDIUM N° 11 (NEOMYCIN ASSAY AGAR) USP

CAT. 1528

For use in the plate assay of neomycin and other antibiotics

FORMULA IN g/l

Gelatin Peptone	**6.00**	Dextrose	**1.00**
Casein Peptone	**4.00**	Beef Extract	**1.50**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 7.9± 0.2 at 25°C			

*This medium has the same formula as Antibiotic Medium N° 1 (Seed Agar, **Cat. 1520**), with the difference that the pH of the medium has been adjusted to 7.9.

PREPARATION

Suspend 30.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 11 is a medium especially prepared to analyze the neomycin content in pharmaceutical preparations according to the FDA and the USP. It can also be used to test other antibiotics, including erythromycin and carbomycin. Neomycin Assay Agar is used in the cylinder plate method for the assay of neomycin.

Peptones and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological Agar is the solidifying agent.

The potency of an antibiotic can be demonstrated under appropriate conditions by its inhibitory effect on microorganisms. This agar can be used in plates as either the base or seed layer, or to prepare the *Staphylococcus aureus* PCJ 209-P inoculum. It can also be used to prepare the *Klebsiella pneumoniae* PCL 602 or ATCC 10031 inoculum, used in the turbidimetric assay for neomycin. The inoculum for the erythromycin assay is *Sarcina lutea* ATCC 9314.

The use of standardized culture media and strict control of all test conditions are essential requirements in the microbiological assay of antibiotics in order to obtain satisfactory test results.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Inhibition zones
Micrococcus luteus ATCC 9431	Good	Ampicillin, Erythromycin
Staphylococcus aureus ATCC 6538	Good	Kanamycin, Neomycin

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

United States Pharmacopoeia Convention. 1995. The United States Pharmacopoeia, 23rd ed. Biological Tests and Assays, p. 1690- 1696. The United States Pharmacopoeia Convention, Rockville, M.D.

Federal Register. 1992. Tests and methods of assay of Antibiotics and Antibiotic-Containing Drugs. Fed. Regist. 21:436.100-436-106

ANTIBIOTIC MEDIUM N° 12

CAT. 1525

Medium used to assay Amphotericin B

FORMULA IN g/l

Peptone	**10.00**	Yeast Extract	**5.00**
Dextrose	**10.00**	Beef Extract	**2.50**
Sodium Chloride	**10.00**	Bacteriological Agar	**25.00**
Final pH 6.1± 0.1 at 25°C			

PREPARATION

Suspend 62.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ANTIBIOTIC MEDIUM N° 12 is recommended for preparing test plates for the cylinder plate assay of the antifungal agents nystatin and anisomycin, using only *Saccharomyces cerevisiae* as the test organism. It is used for the assay of amphotericin B.

Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological Agar is the solidifying agent.

The potency of an antibiotic can be demonstrated under appropriate conditions by its inhibitory effect on microorganisms. This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. The diameter of an inhibition zone after incubation depends, in part, on the concentration or activity of the antibiotic.

Incubate at 30°C ± 2°C and observe for 40 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 2°C and observed after 40 - 48 hours.

Microorganisms	Growth
Saccharomyces cerevisiae ATCC 2601	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

United States Pharmacopeial Convention , Inc. 2001. The United States pharmacopeia 25/ The national formulary 20-2002. United Ststes Pharmacopeial Convention, Inc., Rockville.Md.

Abraham, Chain Fletcher, Florey, Gardner, Hestley and Jennings. 1941. Lancett ii 177.

ASPARAGINE BROTH

CAT. 1207

For the presumptive identification and enumeration (MPN) of *Pseudomonas aeruginosa*

FORMULA IN g/l

Monopotassium Phosphate	**10.00**	Dipotassium Phosphate	**1.00**
DL- Asparagine	**2.00**	Magnesium Sulfate	**0.50**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 13.5 grams of the medium in one liter of distilled water. Add 8 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. To obtain a double-strength broth, dissolve 27 grams of the medium and add 16 ml of glycerol. The prepared medium should be stored at 2 - 8°C. The color is colorless.

The dehydrated medium should be homogeneous, free-flowing and white in color. If there are any physical changes, discard the medium.

USES

ASPARAGINE BROTH is an excellent enrichment broth for *Pseudomonas aeruginosa*.

The formula contains a strictly mineral base with Asparagine as the sole source of nitrogen and Glycerol as the carbon source. The Potassium salts act as a buffer system and Magnesium sulfate is a magnesium ion required in a large variety of enzymatic reactions, including DNA replication and also acts as a buffer.

Asparagine Broth is recommended for enumeration by the MPN method with series of 5 tubes inoculating 10 ml, 1 ml and 0.1 ml. All tubes are incubated at 35 ± 2°C for 48 hours.

P. aeruginosa hydrolyze asparagine to aspartic acid. The appearance of growth with or without fluorescent pigmentation is considered a presumptive test for the presence of *P. aeruginosa* and counts are determined using the MPN tubes.

Confirmation is made by subculturing a loopful from each turbid tube into Acetamide Broth (**Cat. 1211**).

Pseudomonas aeruginosa is an opportunist pathogen for humans, capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water are *Pseudomonas aeruginosa* free at the time of their commercialization. This microorganism can also be found in swimming pool water.

Incubate at 30°C ± 2°C and observe for 40 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Pseudomonas aeruginosa ATCC 27853	Good
Pseudomonas aeruginosa ATCC 10145	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

APHA. Standard Methods for Examination of Water and wastewater, 1 4th ea. 1975.

AZIDE BLOOD AGAR BASE

CAT. 1113

For the isolation of streptococci and staphylococci. With blood, for researching hemolytic reactions

FORMULA IN g/l

Peptone Mixture	**10.00**	Sodium Azide	**0.20**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Beef Extract	**3.00**		
Final pH 7.2 ± 0.2 at 25°C			

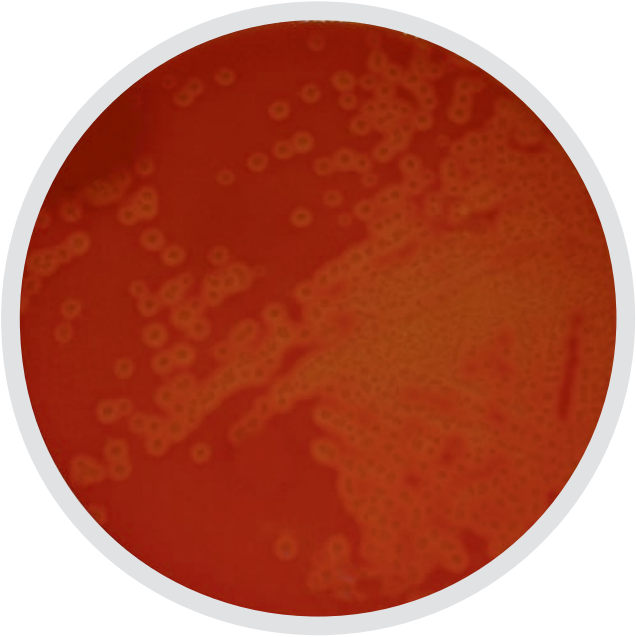
PREPARATION

Suspend 33.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes.

To prepare blood agar, cool to 45 - 50°C and aseptically add 5% sterile defibrinated blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent. The color of the prepared medium with blood is opaque cherry red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.



Streptococcus pyogenes
ATCC 19615

USES

AZIDE BLOOD AGAR BASE contains Sodium azide which has been proved to have a bacteriostatic effect on Gram-negative bacteria, thus, this medium is used for the isolation of streptococci and staphylococci in clinical specimens, water, foods, etc.

Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. 0.02% Sodium Azide in blood agar was reported to prevent the swarming of *Proteus* and allows the selective isolation from mixed bacterial populations. Gram-negative organisms are inhibited by Sodium azide. The medium can be supplemented with 5% sheep blood that allows for the investigation of hemolytic reactions of fastidious pathogens. Hemolytic patterns may vary with the type of blood or base medium used. For instance, defibrinated sheep blood allows the recovery of *Thermophilus* species and gives best results for Group A streptococci. Bacteriological agar is the solidifying agent.

Inoculate sample onto the surface of the medium, streak for isolation with an inoculating loop. Incubate plates aerobically, anaerobically or under CO_2 (5 - 10%) in accordance to standard procedures.

Examine plates for growth and hemolytic reactions after 18 - 24 and 40 - 48 hours incubation at 35 ± 2°C.

Results:

1. Alpha-hemolysis: greenish discoloration of medium
2. Beta-hemolysis: clear zone surrounding colony
3. Gamma-hemolysis: no change

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with 5% sheep blood, after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Hemolysis
Enterococcus faecalis ATCC 19433	Good	Alpha/gamma
Staphylococcus epidermidis ATCC 12228	Good	Gamma
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta
Escherichia coli ATCC 25922	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Edwards, S. J. 1933 The diagnosis of Streptococcus mastitis by cultural methods. J. Comp. Pathol. Ther. 46:211.

Lichstein, H. C., and M.L. Snyder. 1941. The inhibition of the spreading growth of Proteus and other bacteria to permit the isolation of associated streptococci. J. Bacteriol. 42:653

AZIDE DEXTROSE BROTH

CAT. 1422

For enterococci preliminary test and selective enrichment

FORMULA IN g/l

Casein Peptone	**15.00**	Beef Extract	**4.50**
Dextrose	**7.50**	Sodium Azide	**0.20**
Sodium Chloride	**7.50**		

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 34.7 grams of the medium in one liter of distilled water (69.4 grams if double concentration is desired). Mix well and dissolve by heating with frequent agitation until boiling point. DO NOT OVERHEAT. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is yellowish brown.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

AZIDE DEXTROSE BROTH is used for the detection and enumeration of enterococci/streptococci in water, sewage, foods and other materials. The medium contains Sodium azide that inhibits the growth of the accompanying flora in Gram-negative bacteria and permits the growth of enterococci.

The presence of enterococci is an indicator for faecal contamination, especially when occurred a long time ago and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

Beef Extract and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The use of Sodium azide to selectively inhibit Gram-negative bacteria first appeared in the studies of EDWARDS (1938) on the isolation of *Streptococcus agalactiae*, later was showed that Sodium azide can also be used for the isolation of enterococci from water.

Incubate inoculated tubes at 35°C ± 2°C and observe after 24 - 48 hours. Turbidity in tubes indicates presence of enterococci however, it should be further confirmed in EVA Broth (**Cat.1230**).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Enterococcus faecalis ATCC 11700	Good
Enterococcus faecalis ATCC 19433	Good
Streptococcus bovis dsm 20065	Scarce
Escherichia coli ATCC 25922	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

Litsky, W., Mallmann, W.L., a. Fifield, C.W.: A new medium for the detection of enterococci in water. - Amer. J. Publ. Hlth., 43; 873-879 (1953).

Verordnung Trinkwasser und über Wasser für Lebensmittelbetriebe (Trinkwasserver-ordnung) vom 22. Mai 1986. - Bundesgesetzblatt, Teil I, 760-773 (1986).

BACILLUS CEREUS SELECTIVE AGAR BASE (MYP)

CAT. 1124

For the enumeration and isolation of *Bacillus cereus* in food, according to MOSSEL

FORMULA IN g/l

Meat Peptone	**10.00**	Beef Extract	**1.00**
Sodium Chloride	**10.00**	Phenol red	**0.025**
D-Mannitol	**10.00**	Bacteriological Agar	**12.00**

Final pH 7.1 ± 0.2 at 25°C

PREPARATION

Suspend 43 grams of the medium in 900 ml of distilled water. Mix well and dissolve by heating with frequent agitation.

Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 100 ml of Egg Yolk Emulsion (**Cat. 5152**) and, if desired, aseptically add 2 vials of Bacillus Cereus Supplement (**Cat. 6021**) reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into appropriate

containers. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is pink.

The dehydrated medium should be homogeneous, free-flowing and pink-cream in color. If there are any physical changes, discard the medium.

Bacillus Cereus Supplement (Cat. 6021)
(1 vial for 500 ml of the medium)

Polymixin B ..... 50.000 IU

USES

BACILLUS CEREUS SELECTIVE AGAR BASE (MYP) (Mannitol-Egg Yolk- Polymyxin) has been adapted to meet the nutritional needs of *Bacillus cereus*, and was proposed by Mossel et al. (1967) for the enumeration, detection and isolation of *Bacillus cereus* in food.

Beef Extract and Peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Mannitol is the fermentable carbohydrate providing carbon and energy, *Bacillus cereus* is mannitol-negative. The Mannitol content allows the identification of the accompanying mannitol positive flora, which are characterized by a yellow color. Phenol red is the pH indicator. Bacteriological agar is the solidifying agent.

Bacillus cereus is resistant to certain concentrations of Polymyxin, which inhibits the accompanying flora, and is effective mainly against gram-negative organisms.

Bacillus cereus produces lecithinases. The insoluble degradation products of the lecithin of egg yolk accumulate around the *Bacillus cereus* colonies, forming a white precipitate. Inoculated plates should be incubated for 24 - 40 hours at 35 ± 2ºC. The colonies of *Bacillus cereus* will appear red in color and surrounded by a ring of precipitation.

Bacillus cereus
ATCC 11778

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with supplement and egg yolk added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 40 hours.

Microorganisms	Growth	Colony color	Precipitation
Bacillus cereus ATCC 11778	Good	Red	+
Bacillus subtilis ATCC 6051	Good	Yellow	-
Proteus mirabilis ATCC 29906	Inhibited	Colorless	-
Staphylococcus aureus ATCC 6538	Inhibited	Yellow	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ADonovan, K.O.: A Selective Medium for Bacillus cereus in Milk, J. appl. Bact., 21; 100:103 (1958)

Mossel. D.A.A. Koopman, M.J. a Jongerius, E.: Enumeration of Bacillus cereus in Foods. Appl. Microbiol., 1 5; 650:653 (1967)

BAIRD PARKER AGAR BASE EUROPEAN PHARMACOPOEIA

CAT. 1100

For the selective isolation of staphylococci

FORMULA IN g/l

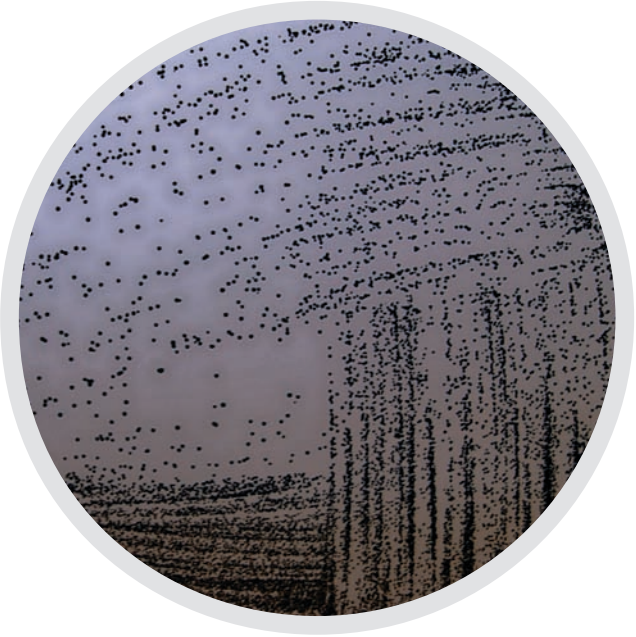
Glycine	**12.00**	Lithium Chloride	**5.00**
Pancreatic Digest of Casein	**10.00**	Yeast Extract	**1.00**
Sodium Piruvate	**10.00**	Bacteriological Agar	**20.00**
Beef Extract	**5.00**		
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 63 grams of the medium in 950 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 50 ml of Tellurite Egg Yolk Emulsion (**Cat. 5129**). Homogenize gently and dispense into Petri dishes. The base without additive can be kept for long periods of time and can be melted as needed.

The prepared plates of the complete medium should be used within 24 hours. The color of the prepared medium without the egg yolk emulsion added is clear amber slightly opalescent. The color with it is yellow opalescent.

The dehydrated medium should be homogeneous, free-flowing and light toasted in color. If there are any physical changes, discard the medium.



Staphylococcus aureus
ATCC 25923

USES

BAIRD PARKER AGAR BASE is used for the selective isolation and enumeration of staphylococci. This medium is widely used and is included in many standard method procedures for testing foods, dairy products, etc.

Pancreatic digest of casein, Beef extract and Yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Lithium chloride and Potassium tellurite inhibit the accompanying flora, and Glycine and Sodium pyruvate facilitate staphylococci growth. Staphylococci that contain lecithinase break down the egg yolk and form clear zones around the colonies. Black colonies are formed due to the reduction of the Potassium tellurite to tellurium. Bacteriological agar is the solidifying agent.

The plates should be dry before inoculation (the drying can be done by incubating at 35 ± 2°C for approximately 10 minutes before use). Prepare the sample in an adequate solution, dilute it and place from 0.1 ml to 1.0 ml of the appropriate dilution in the plates. Spread the inoculum over the entire surface. Incubate at 35 ± 2°C for 24 - 48 hours. Typical *S. aureus* colonies are black, shiny, convex and surrounded by a clear zone of approximately 2 - 5 mm in diameter.

Some other microorganisms, which occasionally grow on this medium, are micrococci that form small dark or black colonies, yeasts that form white colonies and some species of *Bacillus* that form dark brown matte colonies.

The European Pharmacopoeia recommends this medium in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products":

A test for specified microorganisms after inoculation and incubation in Trypticasein Soy Broth (**Cat. 1224**) at 35-37°C 18 - 72 hours. Subculture in this medium and incubate 35-37°C for 18-72 hr. Black colonies of Gram-positive cocci surrounded by a clear zone indicate the presence of *S. aureus*. Confirmation may be effected by suitable biochemical test such as the coagulase test and the deoxyribonuclease test. The product passes the test if colonies of the type described do not appear on Baird-Parker Agar Base or if the confirmatory biochemical tests are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with Egg Yolk + Potassium Tellurite added, from type cultures after incubation at a temperature of 35 ±2 °C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony color	Lecithinase (Transparency around the colonies)	Inoculum (cfu/ml)	Recovery Rate (%)
Bacillus subtilis ATCC 6633	Slight-inhibited	Brown	-	> 10^5	≤ 0.01
Staphylococcus epidermidis ATCC 12228	Slight-Good	Black	-	> 10^3–10^5	≥ 30
**Staphyloccus aureus* ATCC 6538	Good	Black	+	> 10^3–10^5	≥ 70
Staphylococcus aureus ATCC 25923	Good	Black	+	> 10^3–10^5	≥ 70
Proteus mirabilis ATCC 25933	Good	Brown	-	> 10^3–10^5	≥ 30

*According European Pharmacopoeia incubate at 35 - 37 °C for 18 - 72 h.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

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J. AOAC. 54:728, 1971.

European Pharmacopoeia 6th Ed. 2007

BAIRD PARKER AGAR BASE (RPF) ISO-FDIS 6888-2

CAT. 1319

For the selective isolation of coagulase-positive staphylococci in foods

FORMULA IN g/l

Glycine	**12.00**	Lithium Chloride	**5.00**
Casein Pancreatic Digest	**10.00**	Yeast Extract	**1.00**
Sodium Pyruvate	**10.00**	Bacteriological Agar	**13.00**
Beef Extract	**5.00**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 5.6 grams of the medium in 90 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of the RPF Supplement ISO-FDIS 6888:2 (**Cat. 6024**) reconstituted in 10 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15ºC, protected from light and for one month maximum. The color of the prepared medium is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light toasted in color. If there are any physical changes, discard the medium.

RPF Supplement (ISO-FDIS 6888-2) (Cat. 6024)
(1 vial to prepare 100 ml of medium)

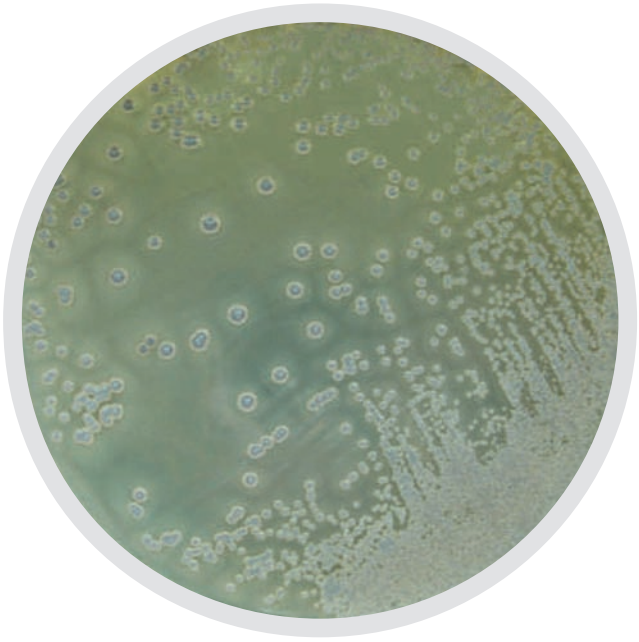
Bovine Fibrinogen ..... 380 mg
Rabbit Plasma ..... 2.5 ml
Trypsin Inhibitor ..... 2.5 mg
Potassium Tellurite ..... 2.5 mg

USES

BAIRD PARKER AGAR BASE (RPF) is used for the isolation and enumeration of coagulase-positive staphylococci in food and other materials. Supplement RPF (**Cat. 6024**) is used with Baird Parker Agar Base instead of Tellurite Egg Yolk Emulsion, saving 24 hours on the traditional method.

Casein pancreatic digest, Beef extract and Yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Lithium chloride, Trypsin inhibitor and Potassium tellurite inhibit the accompanying flora, and Glycine and Sodium pyruvate facilitate the staphylococci growth. Bacteriological agar is the solidifying agent.

Baird Parker Agar Base formula is described in ISO- FDIS 6888-1 normative. ISO 6888.2 recommends this medium for the count of coagulase-positive *Staphylococcus aureus*. RPF supplement allows the isolation of coagulase-positive *Staphylococcus aureus*.



Staphylococcus aureus
ATCC 25923

Inoculate and incubate at 35 ± 2°C and observe after 18 - 24 hours but, if needed, re-incubate for a further 18 - 24 hours. Coagulase-positive *S. aureus* colonies are ringed with a precipitation halo.

At the start of incubation, *Proteus* colonies can present similar physical characteristics to coagulase-positive *Staphylococcus aureus* colonies. However, after 24 or 48 hours of incubation, they can acquire a brown color that expands and invades the plate, and which allows them to be distinguished from *Staphylococcus*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with supplement added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color	Coagulase. Transparence aroundthe colonies
Escherichia coli ATCC 25922	Inhibited	-	-
Proteus mirabilis ATCC 25933i	Good	Brown	-
Staphylococcus aureus ATCC 25923	Good	Black	+
Staphylococcus epidermidis ATCC 12228	Good	Black	-
Staphylococcus aureus ATCC 6538	Good	Black	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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BCP AGAR

CAT. 1051

For the isolation of coliforms

FORMULA IN g/l

Lactose	**10.00**	Bromocresol Purple	**0.025**
Peptone mixture	**5.00**	Bacteriological Agar	**10.00**
Beef Extract	**3.00**		
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 28 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC.The color of the prepared medium is purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BCP AGAR is a non-inhibitory medium used for the detection and isolation of coliforms and in differential studies based on lactose fermentation. BCP stands for Bromocresol Purple.

Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Bromocresol purple is a pH indicator. Bacteriological agar is the solidifying agent.

All coliforms ferment lactose with acid and gas production. This group includes the genera *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella*.

When lactose is fermented it produces acid that changes the color of the medium from blue-purple (alkaline) to yellow (acid). Blue colonies are lactose-negative and yellow colonies are lactose-positive. Inoculate and incubate at 35 ± 2°C. Reading must be carried out after 18 - 24 hours as longer incubation times may cause the diffusion of the acid in the medium and result in an error.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Color
Escherichia coli ATCC 25922	Good	Yellow
Klebsiella pneumoniae ATCC 13883	Good	Yellow
Salmonella typhimurium ATCC 14028	Good	Blue
Shigella sonnei ATCC 58931	Good	Blue

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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BCP GLUCOSE AGAR ENTEROBACTERIACEAE CONFIRMATORY AGAR ISO 21528:2

CAT. 1320

For the differentiation and enumeration of Enterobacteriaceae

FORMULA IN g/l

Tryptone	**10.00**	Yeast Extract	**1.50**
D-glucose	**10.00**	Bromocresol Purple	**0.015**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 41.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BCP GLUCOSE AGAR, also known as Enterobacteriaceae Confirmatory Agar ISO 21528:2, is used for the differentiation of Enterobacteriaceae in urine, water and food. It differentiates species on the basis of dextrose fermentation.

Tryptone and Yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. D-glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bromocresol purple is a pH indicator. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours. The glucose-fermenting microorganisms produce yellow colonies (acid) and the non-fermenting ones, purple colonies.

ISO 21528:2 recommends this medium for glucose fermentation testing. Inoculate oxidase-negative colonies in tubes containing BCP Glucose Agar and incubate at 37°C for 24 ± 2 hours. A yellow color indicates a positive reaction. Colonies that are oxidase negative and glucose-positive are confirmed as Enterobacteriaceae.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Acid production (Color changes to yellow)
**Escherichia coli* ATCC 25922	Good	+
**Salmonella typhimurium* ATCC 14028	Good	+
Staphylococcus aureus ATCC 25923	Inhibited	-

*Inoculate and incubate at 37°C for 24 ± 2 hr.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 21528:2 Microbiology of food and animal feeding stoffs- Horizontal methods for the dectention and enumeration of

Enterobacteriaceae Part 2: Colony-couny method

Drigalsky, C. (1902) Uber ein Verfahren zum Nachweis der typhusbacillen.

BIGGY AGAR

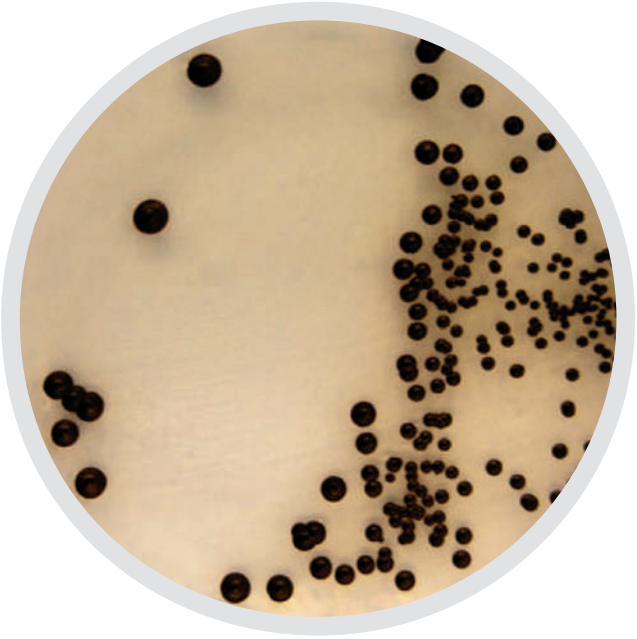
CAT. 1006

For the isolation and presumptive identification of *Candida* species

FORMULA IN g/l

Dextrose	**10.00**	Sodium Sulfite	**3.00**
Glycine	**10.00**	Yeast Extract	**1.00**
Bismuth Ammonium Citrate	**5.00**	Bacteriological Agar	**16.00**

Final pH 6.8 ± 0.2 at 25°C



SCandida albicans
ATCC 10231

PREPARATION

Suspend 45 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is white-opaque.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BIGGY AGAR is the abbreviation for Bismuth Glucose Glycine Yeast Agar. It is used to isolate and differentiate *Candida albicans* and *Candida tropicalis*, and to differentiate the species according to the Nickerson method. Nickerson discovered that *Candida albicans* can be differentiated from other *Candida spp.* on this medium based on colony morphology.

Yeast extract is a source of vitamins, particularly of the B-group essential for growth. Glycine stimulates growth. Dextrose is the fermentable carbohydrate providing carbon and energy. *Candida spp.* reduce bismuth sulfite to bismuth sulfide forming brown to black colonies. Bismuth ammonium citrate and Sodium sulfite inhibit bacterial growth without affecting the growth of *Candida* especies.

Inoculate and incubate at 25 ± 2°C for 18 - 72 hours, and up to 5 days if required. Freshly poured plates should only be used. Inoculation onto slanted surfaces is not generally satisfactory.

The different species of *Candida* produce different kinds of infections. Candidiasis, the most commonly encountered opportunistic fungal infection, is mostly caused by *Candida albicans*. *Candida tropicalis* and *Candida glabrata* infections occur less often. *Candida spp.* are present in clinical specimens resulting from environmental contamination, colonization, or a disease process.

CHARACTERISTICS OF THE COLONIES

Differentiation is based on colony morphology:

C. albicans: Brown to black, smooth, circular or hemispherical colonies with a slight mycelial fringe.	*C. tropicalis*: Dark brown, discrete colonies, with black center prominence and a slight mycelial fringe. Diffuse blackening of medium, with this species only, after about 72 hours of incubation.
C. parakrusei: Flat, frequently wrinkled, medium-sized colonies, glistening dark reddish-brown grading to light reddish-brown and an extensive yellowish mycelial fringe.	*C. krusei*: Large, flat, wrinkled colonies with silvery black-brown grading into a brown periphery and yellow halo.
C. pseudotropicalis: Dark reddish-brown, glistening, large, flat colonies with a slight mycelial fringe.	*C. stellatoidea*: Very dark brown, flat medium-sized colonies with almost no mycelial development.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 ± 2°C and observed after 18 – 72 hours, and up to 5 days if required.

Microorganisms	Growth	Colony Color
Candida albicans ATCC 10231	Good	Brown-to-black
Candida pseudotropicalis ATCC 14245	Good	Brown-to-red
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 28923	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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IN P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington D.C.

BILE ESCULIN AGAR ISO 10273

CAT. 1031

For the isolation and presumptive identification of enterococci and for studies of fermentation of esculin by *Yersinia* according to ISO 10273

FORMULA IN g/l

Bile Salts	**40.00**	Esculin	**1.00**
Meat Peptone	**5.00**	Ferric Citrate	**0.50**
Meat Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 6.6 ± 0.2 at 25°C			

PREPARATION

Suspend 64.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is tournasol.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

BILE ESCULIN AGAR is ideal for the isolation and differentiation of intestinal enterococci, based on Esculin hydrolysis in the presence of bile.

Organisms positive for Esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric citrate to form a dark brown or black colony. Bile Salts do not inhibit enterococci while other Gram-positive bacteria are inhibited. Beef extract and Peptone supply the nutrients essential for growth. Bacteriological agar is the solidifying agent.

Tolerance to bile and the ability to hydrolyze esculin constitutes a reliable presumptive test for the identification of enterococci. The brown color (positive reaction) around the colonies appears after 18 - 24 hours of incubation at a temperature of 35 ± 2°C. Positive cultures are confirmed on KAA Confirmatory Agar (**Cat. 1027**) or KF Streptococcal Agar (**Cat. 1034**).

The presence of intestinal enterococci, is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

ISO 10273 recommends this medium for fermentation studies of esculin by *Yersinia*. An esculin test shall be carried out to determine presumed pathogenicity since pathogenic *Yersinia enterocolitica* strains are esculin negative. Streak the slant surface of the agar. Inoculate 30°C for 24 hours. A black halo around the colonies indicates a positive reaction. This test for fermentation of esculin is equivalent to the test for fermentation of salicin.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Esculin Hydrolysis
Enterococcus faecalis ATCC 11700	Good	+
Enterococcus faecalis ATCC 19433	Good	+
Enterococcus faecium ATCC 8043	Good	+
Streptococcus pyogenes ATCC 12344	Null	-
Streptococcus pneumoniae ATCC 6301	Null	-
Staphylococcus aureus ATCC 25923	Good	+ light
Escherichia coli ATCC 25922	Light	-
**Yersinia enterocolitica* ATCC 27729	Good	-

*ISO 10273 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of presumptive pathogenic. *Yersinia enterocolitica*

*Inoculate at 30°C for 24 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

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BILE ESCULIN AZIDE AGAR ISO 7899-2

CAT. 1005

Selective medium for the isolation and presumptive identification of intestinal enterococci by Membrane filtration method

FORMULA IN g/l

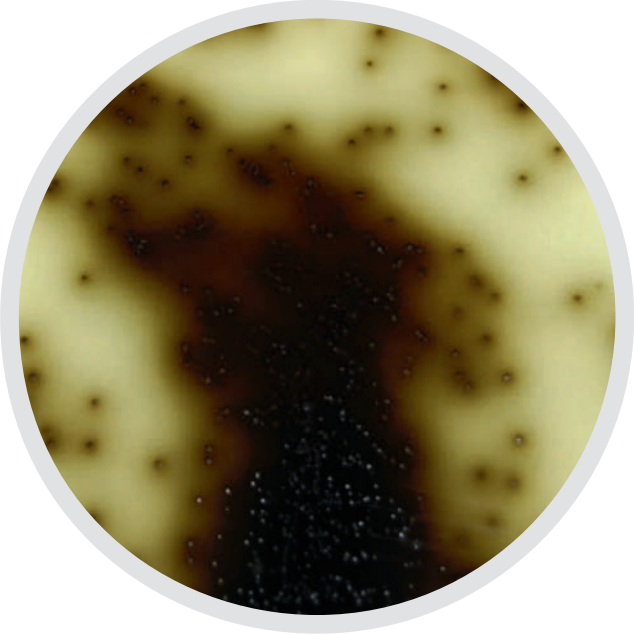
Tryptone	**17.00**	Esculin	**1.00**
Ox Bile	**10.00**	Ferric Ammonium Citrate	**0.50**
Yeast Extract	**5.00**	Sodium Azide	**0.15**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Peptone	**3.00**		
Final pH 7.1 ± 0.1 at 25°C			

PREPARATION

Suspend 56.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is tournasol.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.



Enterococcus faecalis
ATCC 11700

USES

BILE ESCULIN AZIDE AGAR is a modification of Bile Esculin Agar with the addition of sodium azide as an inhibitor and with the reduction of the bile concentration. The resulting medium is more selective but still provides rapid growth and efficient recovery of enterococci. The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci. Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric citrate to form a dark brown or black colony. Ox bile does not inhibit enterococci while other Gram-positive bacteria are inhibited. Sodium azide inhibits Gram-negative bacteria. Tryptone, Peptone and Yeast extract supply the nutrients essential for growth. Sodium chloride provides the osmotic balance. Bacteriological agar is the solidifying agent.

The presence of intestinal enterococci, also known as faecal streptococci, is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

Tolerance to bile and the ability to hydrolyze esculin constitutes a reliable presumptive test for the identification of enterococci. The brown color (positive reaction) around the colonies appears after 18 - 24 hours of incubation at a temperature of 35 ± 2°C.

For the confirmation of enterococci, according to ISO 7899-2, transfer the membrane with colonies, without inverting them, to a plate with Bile Esculin Azide Agar, pre-heated to 44°C, and incubate at 44 ± 0.5°C for 2 hours. The plate should be read immediately.

It is considered that the typical colonies that show a brown-black color in the surrounding medium give positive reactions and are recounted as intestinal *Enterococcus*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Esculin
**Enterococcus faecalis* ATCC 11700	Good	+
**Enterococcus faecium* ATCC 8043	Good	+
Streptococcus pyogenes ATCC 12344	Null	-
Escherichia coli ATCC 25922	Null	-

* Incubate at 44 ± 0.5°C for 2 hours (ISO 7899-2)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

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BILE ESCULIN AZIDE BROTH

CAT. 1359

Selective medium recommended for the isolation and presumptive identification of intestinal *Enterococcus*

FORMULA IN g/l

Tryptone	**17.00**	Peptone	**3.00**
Ox Bile	**10.00**	Esculin	**1.00**
Yeast Extract	**5.00**	Ferric Ammonium Citrate	**0.50**
Sodium Chloride	**5.00**	Sodium Azide	**0.15**

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 41.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is tournasol.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.

USES

BILE ESCULIN AZIDE BROTH is a selective medium for the differentiation, isolation and presumptive identification of enterococci.

The ability to hydrolyze esculin is a characteristic of enterococci. Organisms positive for esculin hydrolysis, hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric citrate to form a dark brown or black colony. Ox bile does not inhibit enterococci while other Gram-positive bacteria are inhibited. Sodium azide inhibits Gram-negative bacteria. Tryptone, Peptone and Yeast extract supply the nutrients essential for growth. Sodium chloride provides the osmotic balance. Bacteriological agar is the solidifying agent.

The presence of intestinal enterococci, is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

Incubate at 35°C ± 2°C and observe after 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Esculin
Enterococcus faecalis ATCC 11700	Good	+
Enterococcus faecium ATCC 8043	Good	+
Streptococcus pyogenes ATCC 12344	Null	-
Escherichia coli ATCC 25922	Null	-

* Incubate at 44 ± 0.5°C for 2 hours (ISO 7899-2)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

FACKLAM, R.R.: Recognification of group D streptococcal species of human origin by biochemical and physiological test. – Appl. Microbiol., 23; 1131-1139 (1972)

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SWAN, A.: The use of bile-esculin medium and of Maxted's technique of LANCEFIELD grouping in the identification of enterococci. (Group D streptococci) J. Clin. Pathol., 7: 160-163 (1954) .

BISMUTH SULFITE AGAR (WILSON BLAIR) USP

CAT. 1011

Highly selective medium for the isolation of *Salmonella spp.*, particularly *Salmonella thyphi*, from clinical specimens and food

FORMULA IN g/l

Bacteriological Peptone	**10.00**	Disodium Phosphate	**4.00**
Bismuth Sulfite Indicator	**8.00**	Ferrous Sulfate	**0.30**
Beef Extract	**5.00**	Brilliant Green	**0.025**
Dextrose	**5.00**	Bacteriological Agar	**20.00**
Final pH 7.5 ± 0.2 at 25°C			

PREPARATION

Suspend 52.3 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45°C (very important), mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is opaque white with a green tint.

The dehydrated medium should be homogeneous, free-flowing and light green in color. If there are any physical changes, discard the medium.

USES

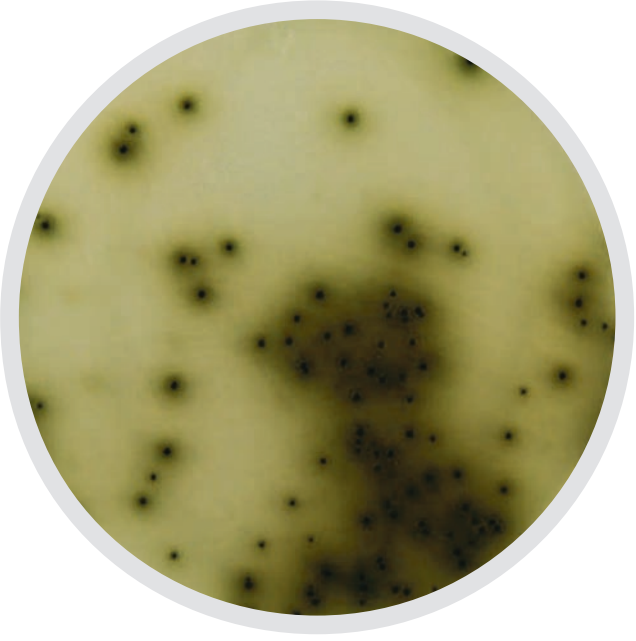
BISMUTH SULFITE AGAR is a modification of the Wilson Blair Medium, and generally accepted as routine for the detection of most *Salmonella*, in particular *Salmonella typhi*.

Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy, Bismuth sulfite indicator and Brilliant green are inhibitors of Gram-positive bacteria and members of the coliform group. Disodium phosphate acts as a buffer system and Bacteriological agar is the solidifying agent.

In the presence of H_2S, *Salmonella spp.* reduce the iron salts to iron sulfate, which produces a black colony and turns the bismuth indicator to metallic bismuth, surrounding the area of the colonies with a bright sheen.

Generally, Bismuth Sulfite Agar is inoculated by streaking the surface to obtain isolated colonies but the pour plate inoculation method can be also used, mixing the sample with the liquid medium and allowing the plate to solidify. All plates are incubated 40 - 48 hours at 35 ± 2°C. The solidified plates should have a uniform, opaque, cream to pale green appearance. If kept in refrigeration, the medium will slowly oxidize. It is recommended to keep the plates refrigerated for 4 days before use to reduce inhibition and thus to be able to isolate *Salmonella* in less heavily contaminated samples.

The colonies of *S. typhi* are black surrounded by a black or brownish zone, with a metallic sheen. In heavy growth areas, these may appear as light green colonies. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium. *Shigella spp.*, other than *Shigella flexneri* and *Shigella sonnei*, do not grow. Those colonies that do grow are brown to green, raised with a crater-like appearance. *E. coli* is partially inhibited, occasionally growing with brown or greenish glistening colonies. A few *Enterobacter* strains may grow with raised, mucoid colonies, having a silvery sheen lighter than *S. typhi*. Colonies of coliforms that produce H_2S form colonies similar in appearance to *S. typhi*. These may be readily differentiated as they produce gas with lactose media, e.g. TSI Agar (**Cat.1046**) or Kligler Iron Agar (**Cat. 1042**). The hydrolysis of urea in Urea Broth (**Cat. 1226**) or Urea Agar Base (**Cat. 1110**) may be used to identify *Proteus spp.*



Salmonella typhi
ATCC 19430

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 40 - 48 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Partial Inhibition	Brown-Green
Salmonella enteritidis ATCC 13076	Good	Black with bright metallic
Salmonella typhi ATCC 19430	Good	Black with bright metallic
Shigella flexneri ATCC 12022	Partial Inhibition	Brown
Enterococcus faecalis ATCC 29212	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Wilson, W.J., and E.M. Blair 1.926 A combination of Bismuth and Sodium Sulfite affording an enrichment and selective medium for the typhoid-paratyphoid groups of bacteria. J. Pathol. Bactend 29:310.

United States Pharmacopoeia Convention 1.995. The United States Pharmacopoeia 23rd ed.

BLOOD AGAR BASE

CAT. 1108

For the isolation, cultivation and detection of hemolytic activity of fastidious microorganisms

FORMULA IN g/l

Heart Infusion	**10.00**	Sodium Chloride	**5.00**
Meat Peptone	**10.00**	Bacteriological Agar	**15.00**
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 5 - 10% of sterile defibrinated blood, homogenize and pour intro Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. If desired, Polyenrichment Supplement (**Cat. 6011**) may be added to increase growth. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent. The color of the prepared medium with blood is opaque cherry red.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

BLOOD AGAR BASE is used for the isolation, cultivation and detection of hemolytic reaction of fastidious microorganisms.

It is suitable for isolating and cultivating a wide range of microorganisms with difficult growth characteristics. Upon adding blood, it can be utilized for determining hemolytic reactions.

The Heart infusion and Meat peptone are rich sources of nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The addition of blood provides extra growth factors for fastidious microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the type of blood or base medium used. For example, defibrinated sheep blood allows the recovery of *Thermophilus* species and gives best results for Group A streptococci.

Use standard procedures to obtain isolated colonies from specimens. Incubate at 35 ± 2°C for 24 - 48 hours. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5 - 10% CO_2.

Results:

1. Alpha-hemolysis: greenish discoloration of medium
2. Beta-hemolysis: clear zone surrounding colony
3. Gamma-hemolysis: no change

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with 5% of defibrinated sheep blood, after incubation at temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Hemolysis
Neisseria meningitidis ATCC 13090	Good	-
Staphylococcus aureus ATCC 25923	Good	Beta
Staphylococcus epidermidis ATCC 12228	Good	-
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Snavely and BrahierA. J. Clin. Path. 33:511. 1 960.Hosty, Freeman and Irwin, Public, Health. Lab., 1953.

Schubert, Edwards and Ramsey J. Bact. 77:648, 1959. APHA Diagnostic Procedures and Reagents 3.a edition, 1951. Tharshis and Frish AM. J. Clin. Path. 21:101. 1951

BLOOD AGAR BASE N°2 ISO 7932

CAT. 1328

For the cultivation and detection of hemolytic activity of fastidious microorganisms, confirmation of *Bacillus cereus* (ISO 7932) and *Listeria monocytogenes* (ISO 11290-1)

FORMULA IN g/l

Proteose peptone	**15.00**	Liver Extract	**2.50**
Yeast Extract	**5.00**	Bacteriological Agar	**12.00**
Sodium Chloride	**5.00**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 39.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 5 - 7% of sterile defibrinated blood, homogenize and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is opaque red without hemolysis.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BLOOD AGAR BASE N° 2 is a base medium rich in nutritional properties, used for the preparation of blood agar plates. It is used for the isolation, cultivation and recovery of fastidious microorganisms to study hemolysis activity.

Liver extract and the Yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride maintains the osmotic equilibrium. The blood is an additional

source that provides growth factors for the microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the type of blood or base medium used. For example, defibrinated sheep blood allows the recovery of *Thermophilus* species and gives best results for Group A streptococci. Bacteriological agar is the solidifying agent.

This medium can be used to prepare a selective medium for *Brucella spp.* or *Campylobacter spp.* by adding an antibiotic supplement. It may also be used for the primary isolation of *Haemophilus spp.* Add horse blood to enrich the medium.

Incubate at 35 ± 2°C and observe after 24 - 48 hours.

This medium has been recommended by ISO normative 7932 for the confirmation of *Bacillus cereus*. Incubate at 30°C for 24 ± 2 hr and interpret the hemolysis reaction. The *Bacillus cereus* has positive reaction of ß -hemolysis. The width of the hemolysis zone may vary.

It is also a medium recommended by ISO normative 11290-1 for the confirmation of *Listeria monocytogenes*. The normative recommends incubation at 35°C or 37°C for 18 - 24 hours. A zone of ß- hemolysis is considered a positive reaction.

Results:

1. Alpha-hemolysis: greenish discoloration of medium
2. Beta-hemolysis: clear zone surrounding colony
3. Gamma-hemolysis: no change

MICROBIOLOGICAL TEST

The following results were obtained adding 5% of defibrinated sheep blood in the performance of the medium from type culture after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Hemolysis
Neisseria meningitidis ATCC 13090	Good	-
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta
**Bacillus cereus* ATCC 11778	Good	Beta
***Listeria monocytogenes* ATCC 11778	Good	Beta

* Incubate at 30°C for 24 ± 2 hours according to ISO 7932

** Incubate at 35 or 37°C for 18 - 24 hours according ISO 11290-1

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD ISO

WATERWORTH, P.M.: BRIT. J. Exp. Pathol., 36(02); 186-194 (1955)

ISO 7932 Horizontal Method for the enumeration of Bacillus cereus

ISO NORMATIVE 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

BLOOD AGAR BASE + NALIDIXIC ACID

CAT. 1128

For the differentiation of the hemolytic activity of streptococci

FORMULA IN g/l

Heart Infusion	**10.00**	Nalidixic Acid	**0.04**
Meat Peptone	**10.00**	Bacteriological Agar	**15.00**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 5% of sterile defibrinated blood. Homogenize and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent. The color of the prepared medium with blood is opaque cherry red.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

BLOOD AGAR BASE + NALIDIXIC ACID is a modification of Blood Agar Base with the addition of nalidixic acid as an inhibitor of the accompanying flora. Nalidixic acid blocks the DNA replication of susceptible bacteria and acts against many Gram-negative bacteria.

The Heart infusion and Meat peptone are rich sources of nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The blood is an additional source that provides growth factors for the microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the type of blood or base medium used. For example, defibrinated sheep blood allows the recovery of *Thermophilus* species and gives best results for Group A streptococci. Bacteriological agar is the solidifying agent.

Use standard procedures to obtain isolated colonies from specimens. Incubate at 35 ± 2°C for 24 - 48 hours. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5 - 10% CO_2.

Streptococcal colonies will be 2 - 3 mm of diameter; colorless or smooth, round, white and will produce α-hemolysis (*Streptococcus pneumoniae*), ß (*Streptococcus pyogenes*) alpha or negative (*Streptococcus bovis*).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with 5% sheep blood, after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Hemolysis
Staphylococcus aureus ATCC 25923	Partially inhibited	Beta
Staphylococcus epidermidis ATCC 12228	Good	-
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta
Escherichia coli ATCC 25922	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Cruikshank, R. (1 972) Medical Microbiology. 11th Edition. Livingstone. London.

BORDET-GENGOU AGAR BASE

CAT. 1107

For the detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis* from clinical samples

FORMULA IN g/l

Proteose Peptone	**10.00**	Potato Infusion	**4.50**
Sodium Chloride	**5.50**	Bacteriological Agar	**16.00**
Final pH 6.7 ± 0.2 at 25°C			

PREPARATION

Suspend 36 grams of the medium in one liter of distilled water with 10 ml of glycerol. Allow to stand for 5 minutes and mix well until a uniform suspension is obtained. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 10 - 15% of sterile defibrinated blood, homogenize and pour into Petri dishes. The medium can be made more selective by aseptically adding 2 vials of Bordetella Supplement (**Cat. 6015**), previously reconstituted in 5 ml of sterile distilled water. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is clear to opalescent amber, and may have a precipitate. The color of the prepared medium with blood is opaque cherry red.

The dehydrated medium should be homogeneous, free flowing and beige in color. If there are any physical changes, discard the medium.

Bordetella Supplement (Cat.6015)
(1 vial for 500 ml of the medium)

Cephalexin ..... 20 mg

USES

BORDET-GENGOU AGAR BASE is used with the addition of blood for isolating *Bordetella pertussis* and other *Bordetella* species.

The genus *Bordetella* consists of 4 species, all being respiratory pathogens: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*.

Potato infusion and Proteose peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Glycerol provides carbon. Sodium chloride supplies essential electrolytes for transport and osmotic balance, and Bacteriological agar is the solidifying agent. The addition of blood provides extra growth nutrients for *Bordetella* species. Starch from the potato infusion absorbs fatty acids, from nasal secretions on cotton swabs, which inhibit growth of *B. pertussis*. Inoculate and incubate the plates at 35 ± 2°C for 48 - 72 hours in a humid environment. Use 2 plates per sample: one with supplement, one without it.

After 48 - 72 hours, colonies of *B. pertussis* are small, white, opaque with an unclear edge as the hemolysis zone merges into medium, smooth, slightly elevated, shiny and less than 1 mm in diameter. They are surrounded by hazy zone of hemolysis.

Colonies of *B. parapertussis* grow faster and at 48 hours are well developed with a similar appearance to *B. pertussis*, giving a green-black tint to the medium. Colonies of Gram-positive cocci are usually opaque and darker.

All suspect colonies should be identified by serological methods.

After 24 - 48 hours, colonies of *B. bronchiseptica*, grow similar to *B. pertussis* colonies but they are larger with a rough, pitted surface.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with 5% sheep blood added, after incubation at a temperature of 35 ± 2°C and observed after 48 - 72 hours.

Microorganisms	Growth	Hemolysis
Bordetella bronchiseptica ATCC 4617	Good	Gamma
Bordetella pertussis ATCC 8467	Good	Beta
Bordetella parapertussis ATCC 15311	Good	Gamma

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bordet, J. y Gengou, O. Ann.Inst. Pasteur 20. 731-741 American Public Health Association (1963) "Diagnostic Procedures and Reagents"

4th Ed. APHA Inc., New York p. 150. 294-5.

BPRM BROTH BASE (Bacteorides Phage Recovery Medium) ISO 10705:4

CAT. 1451

For the cultivation of *Bacteroides fragilis* and for phage recovery from environmental samples

FORMULA IN g/l

Peptone	**10.00**	Glucose	**1.80**
Tryptone	**10.00**	L-Cysteine	**0.50**
Sodium Chloride	**5.00**	Magnesium Sulfate Heptahydrate	**0.12**
Yeast Extract	**2.00**		
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 29.42 grams of the medium in one liter of distilled water. Add 1ml of Calcium Chloride Dihydrate $CaCl_2$ X H_2O 5%. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize at 121°C for 15 minutes. Cool to 45 - 50°C. Aseptically add 1ml/liter of 1% of hemin sterile solution prepared in 0.02% NaOH. Mix well. Just before use add 25 ml/liter of a sterile solution of 10.6% (w/v) of Disodium Carbonate. Adjust the pH to 7.0 with HCl. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BPRM Broth Base (Bacteroides Phage Recovery Medium) is a medium recommended by the ISO normative 10705-4 for the cultivation of *Bacteroides fragilis* and for the recuperation of phage from human faecal samples and environmental samples. Bacteroides Phage Recovery Medium in the culture of phages affecting *B. fragilis* allows faster bacterial growth and produces higher phage yields.

With the adequate concentration of agar you can prepare a semi-solid or solid medium. To inhibit the accompanying flora, add 100 mg/ml of Kanamycin and 7.5 mg/ml of Vancomycin sterilized by filtration.

Incubate at 35 - 37°C and observe after 48 hours under 5 - 10% CO_2 conditions.

Bacteroides fragilis is a Gram-negative bacteria, an obligate anaerobe and one of the most abundant bacteria in the human colon. It causes 90% of the anaerobic peritoneum infections.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 - 37°C under 5 - 10% CO_2 conditions and observed after 48 hours.

Microorganisms	Growth
Bacteroides fragilis ATCC 25285	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 10705-4 Water quality -Detection and enumeration of bacteriophages - Part 4: Enumeration of bacteriophages infecting Bacteroides fragilis

Donia D., Divizia M., Pana' A. Analysis of concentration methods for bacteriophages. Moderna, 1998, 109: 1.

Tartera C., Jofre J. Bacteriophages active against Bacteroides fragilis in sewage-polluted waters. Applied and Environmental Microbiology, 1987, 53, 1632

BRAIN HEART INFUSION AGAR (BHI AGAR)

CAT. 1048

Recommended for the development of fastidious microorganims

FORMULA IN g/l

Peptone Mixture	**10.00**	Disodium Phosphate	**2.50**
Beef Heart Infusion	**10.00**	Sodium Chloride	**5.00**
Calf Brain Infusion	**7.50**	Bacteriological Agar	**15.00**
Dextrose	**2.00**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 52 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

To prepare a selective medium for fungi, the sterilized and melted medium should be cooled to 45 - 50°C before adding the appropriate antibiotics.

Occasionally a small amount of sediment may appear which should be resuspended with a gentle swirl before dispensing.

USES

BRAIN HEART INFUSION AGAR (BHIA) is a solid medium rich in nutrients, suitable for the cultivation of several fastidious strains of bacteria, fungi, and yeasts.

Brain Heart Infusion Agar is used for the cultivation of a wide variety of fastidious microorganisms such as streptococci, meningococci and pneumococci. BHIA is recommended in Standard Methods for water testing and in antimicrobial susceptibility tests. The nutritionally rich base of Beef heart and Calf brain infusions and Peptone mixture provide nitrogen, vitamins, minerals and amino acids that supports the growth of a variety of microorganisms. Disodium phosphate acts as a buffer. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride maintains the osmotic balance. Bacteriological agar is the solidifying agent.

Inoculate and incubate anaerobically at 35 ± 2°C for 24 - 72 hours. If 10% sterile defibrinated blood is added, the medium can be used for the cultivation and isolation of *Histoplasma capsulatum*. With the addition of antibiotics the medium can be used for the isolation of fungi. Brain Heart Infusion Agar with cycloheximide and chloramphenicol restrict growth of bacteria and saprophytic fungi, and is recommended for the isolation of fungi difficult to grow such as *H. capsulatum* and *Blastomyces dermatiditis*. Adding polysorbate to BHIA allows for identification of *Mycobacterium avium*-intracellulare complex organisms and *M. tuberculosis* from blood cultures.

Occasionally BHIA plates are used for general sensitivity tests. However, it is not suitable to determine hemolytic reactions as this medium has a high dextrose concentration and it may give atypical readings.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2, and observed after 24 - 72 hours. (It is recommended to grow *Aspergillus brasiliensis* and *Saccharomyces cerevisiae* aerobically at 30 ± 2°C).

Microorganisms	Growth without blood	Growth with 5% sheep blood
Aspergillus brasiliensis ATCC 16404	Good	Good
Neisseria meningitidis ATCC 13090	Moderate	Good
Saccharomyces cerevisiae ATCC 9763	Good	Good
Streptococcus pneumoniae ATCC 6303	Moderate	Good
Streptococcus pyogenes ATCC 19615	Moderate	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Creitz and Pucket A.J. Clin. Path 24:1318, 1954. Golding and Davidson Modern, Hospital, 92:April 1954

BRAIN HEART INFUSION BROTH (BHI BROTH)

CAT. 1400

For the growth of pathogenic cocci and other microorganisms including aerobic and anaerobic bacteria from a variety of clinical and non-clinical materials

FORMULA IN g/l

Gelatin Peptone	**10.00**	Sodium Chloride	**5.00**
Beef Heart Infusion	**10.00**	Disodium Phosphate	**2.50**
Calf Brain Infusion	**7.50**	Dextrose	**2.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 37 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8ºC. The color of the prepared medium is amber. For best results, the medium should be used on the same day or, if not, heated in a boiling water bed to expel the dissolved oxygen and left to cool before using.

The dehydrated medium should be homogeneous, free-flowing and light toasted in color. If there are any physical changes, discard the medium.

USES

BRAIN HEART INFUSION BROTH (BHIB) is a liquid medium rich in nutrients, suitable for the cultivation of several fastidious strains of bacteria, such as streptococci, meningococci and pneumococci, fungi and yeasts. BHIB is recommended in Standard Methods for water testing and in antimicrobial susceptibility tests.

Tubes of 0.5 ml BHI broth are used to cultivate bacteria used in the preparation of inocula for use in microdilution minimal inhibitory concentration (MIC) and identification (ID) test panels.

The nutritionally rich base of Beef heart and Calf brain infusions and Peptone mixture provide nitrogen, vitamins, minerals and amino acids essential for growth of a variety of microorganisms. Dextrose is the carbon energy source and Sodium chloride maintains the osmotic balance.

This medium is very versatile and supports the growth of many fastidious organisms. With the addition of 0.1% agar, the medium is used for the cultivation of anaerobes. Adding 0.1% agar reduces the flow of oxygen convection currents and encourages the development of anaerobes and microorganisms.

BHI Broth is recommended for the preparation of the culture of *S.aureus* for use in coagulase tests.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good with turbidity
Streptococcus pneumoniae ATCC 6303	Good with turbidity
Streptococcus pyogenes ATCC 19615	Good with turbidity
Brucella abortus ATCC 4315	Moderate

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Chapman. Trans. N.Y. Acad. Science. 9:52. 1946. Newman. J. Milk and Food Technol. 13:226. 1950.

Roseburg, Epps, and Clark. J. Infection Diseases, 74:131. 1944. APHA Diagnostic Procedures and Reagents. 3rd Edition, 1951.

BRAIN HEART INFUSION BROTH ISO 6888-1

CAT. 1331

For the confirmation of coagulase-positive staphylococci

FORMULA IN g/l

Calf Brain Infusion	**12.50**	Sodium Chloride	**5.00**
Enzymatic Digest of Animal Tissue	**10.00**	Disodium Phosphate	**2.50**
Beef Heart Infusion	**5.00**	Glucose	**2.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 37 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BRAIN HEART INFUSION BROTH (BHI Broth) is a general purpose medium recommended by the ISO normative 6888-1 for the confirmation of coagulase-positive staphylococci.

Calf brain infusion, Enzymatic digest of animal tissue and Beef heart infusion provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Disodium phosphate is a buffer. Glucose is the fermentable carbohydrate providing carbon and energy.

Collect the inoculum from the surface of each selected colony using a sterile wire and introduce the inoculum in a tube or bottle that contains the Brain-heart infusion. Incubate at 35 - 37°C for 24 ± 2 hours. Under aseptic conditions, add 0.1 ml of each culture to 0.3 ml of rabbit plasma in sterile hemodialysis tubes or bottles. Incubate at 35 - 37°C. Examine the coagulase plasma by inclining the tube after incubating for 4 to 6 hours. If the test is negative, re-examine after 24 hours of incubation or after the incubating period recommended by the manufacturer is over.

The coagulase test is considered positive if the clot volume is more than half of the original liquid volume.

As a negative control for each plasma lot, add 0.11 ml of Brain-heart infusion to the quantity of rabbit plasma indicated by the manufacturer and incubate without inoculation. For the test to be valid, the plasma control should not show any signs of coagulation.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 - 37°C and observed after 24 ± 2 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 25923	Good
Proteus mirabilis ATCC 25933	Good
Staphylococcus epidermidis ATCC 12228	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

International Standard ISO 6888-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -- Part 1: Technique using Baird-Parker agar medium.

BRILLIANT GREEN AGAR EUROPEAN PHARMACOPEIA, USP

CAT. 1078

Highly selective medium for the isolation of *Salmonella*, other than *Salmonella typhi*, from foods, faeces and dairy products

FORMULA IN g/l

Peptone (Meat and Casein)	**10.00**	Yeast Extract	**3.00**
Lactose Monohydrate	**10.00**	Phenol red	**0.08**
Sucrose	**10.00**	Brilliant Green	**0.0125**
Sodium Chloride	**5.00**	Bacteriological Agar	**20.00**
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 58.1 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121° C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. If necessary, allow to dry about for approximately 2 hours with the covers partially removed. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is dusky green.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

BRILLIANT GREEN AGAR, as recommended by the European Pharmacopoeia, is used for the selective isolation of *Salmonella spp.* other than *S. typhi* in foods and clinical specimens, via Lactose/Sucrose fermentation.

The Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly the B-group. Sucrose and Lactose are fermentable carbohydrates providing carbon and energy. Phenol red is the pH indicator, turning the medium a yellow color with the formation of acid as a result of Lactose/Sucrose fermentation. Brilliant green inhibits Gram-positive bacteria and most Gram-negative bacilli other than *Salmonella spp.* Lactose/Sucrose fermenters are usually inhibited. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

When there is a suspicion that the material under study contains low concentrations of Salmonellae, it is necessary to initially inoculate the sample in Brilliant Green Tetrathionate Broth (**Cat. 1253**) or Selenite Cystine Broth (**Cat. 1220**) as a preenrichment step.

As this medium is very inhibitory it allows for moderately heavy inocula, which should be evenly distributed on the surface of the medium.

Inoculate and incubate at 35 ± 2 °C for 18 - 24 hours. At the same time, inoculate other selective media that are less inhibitory, such as Desoxycholate Agar (**Cat. 1020**), Salmonella Shigella Agar (**Cat. 1064**), XLD Agar (**Cat. 1274**), MacConkey Agar (**Cat. 1052**), EMB Agar (**Cat. 1050**) or Hektoen Enteric Agar (**Cat. 1030**).

The medium, which has a coffee color at the beginning, changes to red during incubation at 35 - 37°C. A probable presence of Salmonellae is indicated by small, transparent, either colorless or pink or opaque-white colonies, often surrounded by a pink or red zone. Some of the uninhibited Gram-negative, Lactose/Sucrose fermenting organisms present opaque green-yellow colonies, surrounded by a yellow halo. Other lactose negative microorganisms, such as *Proteus spp.*, form colonies of a pale pink or red color, transparent and surrounded by a brilliant red halo.

Confirmation of suspect samples is carried out by transferring a few of the suspect colonies to TSI (**Cat. 1046**).

The European Pharmacopoeia recommends in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products":

Subculture in this medium after incubation in Brilliant Green Tetrathionate Bile Broth (**Cat. 1253**), at 41 - 43°C for 18 - 24 hours and incubate at 35 - 37 °C for 18 - 72 hr. The probable presence of Salmonellae is indicated by the growth of cultures having the following appearance in this medium: small, transparent, colourless or pink or opaque-white colonies often surrounded by pink or red zone. Precise confirmation may be carried out by appropriate biochemical and serological test. The product passes the test if colonies of the type described do not appear or if the confirmatory biochemical and serological test are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2 °C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony color
Escherichia coli ATCC 25922	Inhibited-Moderate	Yellow-green
Salmonella enteritidis ATCC 13076	Good	Pink-white
Staphylococcus aureus ATCC 25923	Inhibited	-
Salmonella typhi ATCC 19430	Inhibited-Moderate	Red
**Salmonella typhimurium* ATCC 14028	Good	Pink-white

*According European Pharmacopeia Incubate at 35 - 37°C for 18 - 72 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP USP

European Pharmacopoeia. 6th Ed. 2007.

American Public Health Association. Standard Methods for the Examination of Water and Waster water, 11th Edition APHA, New York, 1960. American Public Health Association. Recommended Methods for the Microbiological Examination of Foods, APHA, Inc. New York, 1958.

BRILLIANT GREEN AGAR (MODIFIED) ISO 6579

CAT. 1143

For the selective isolation of *Salmonella*

FORMULA IN g/l

Lactose	**10.00**	Disodium Phosphate	**1.00**
Sucrose	**10.00**	Monosodium Phosphate	**0.6**
Meat Peptone	**5.00**	Phenol red	**0.09**
Beef Extract	**5.00**	Brilliant Green	**0.005**
Casein Peptone	**5.00**	Bacteriological Agar	**15.00**

Yeast Extract	**3.00**
Final pH 6.9 ± 0.2 at 25°C	

PREPARATION

Suspend 54.7 grams of the dehydrated medium in one liter of distilled water and leave for 15 minutes. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Dispense into appropriate containers. The prepared medium should be stored at 8 - 15°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and red in color. If there are any physical changes, discard the medium.

USES

BRILLIANT GREEN AGAR (Modified) is a selective medium for the isolation of *Salmonella*, except for *S. typhi*, from water, food and animal feed stuffs. It is prepared according to the formulation of Edel and Kampelmacher.

This medium is recommended by ISO normative 6579:1998 as the first selective medium used for the isolation and identification of *Salmonella*. The ISO normative 6579:2002 recommends as first selective medium the XLD Agar instead of the Brilliant Green Agar however it informs that Brilliant Green Agar can be used as a second selective medium.

Brilliant Green Agar Modified inhibits the growth of *Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus spp.* which may be similar in appearance to *Salmonella*.

Beef extract, Casein peptone and Meat peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Lactose and Sucrose are the fermentable carbohydrate providing carbon and energy. Phenol red is the pH indicator. Brilliant green inhibits Gram-positive and most Gram-negative bacteria, except *Salmonella*. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 37°C ± 1°C for 24 ± 3 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C ± 1°C and observed after 24 ± 3 hours.

Microorganisms	Growth	Colony color
Salmonella enteritidis ATCC 13076	Good	Red colonies, surrounded by a diffused red halo
Salmonella typhimurium ATCC 14028	Good	Red colonies, surrounded by a diffused red halo
Escherichia coli ATCC 25922	Inhibited-Moderate	Yellow-Green
Salmonella typhi ATCC 19430	Inhibited-Moderate	Red
Staphylococcus aureus ATCC 25923	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

UNE-EN-ISO 6579 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Salmonella spp.

BRILLIANT GREEN BILE AGAR

CAT. 1010

For the determination of the degree of contamination by coliforms in drinking water and wastewater

FORMULA IN g/l

Gelatin Peptone	**8.25**	Ferric Chloride	**0.0295**
Lactose	**1.90**	Monopotassium Phosphate	**0.0153**
Sodium sulfite	**0.205**	Ox Bile	**0.00295**
Basic Fuchsin	**0.0776**	Brilliant Green	**0.0295 mg**
Erioglaucine	**0.0649**	Bacteriological Agar	**10.15**
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 20.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is blue.

The dehydrated medium should be homogeneous, free-flowing and light purple in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.

USES

BRILLIANT GREEN BILE AGAR can be used to assess the degree of contamination of water samples, diverse foods and other products. It uses basic fuchsin to differentiate between lactose-fermenting and lactose non fermenting bacteria. Acid production by lactose fermenting organisms, such as *Escherichia coli*, produce characteristic red colonies with a pink surrounding area. Lactose non-fermenters form colorless and transparent colonies.

The Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria except coliforms. Erioglaucine and Basic fuchsin together indicate pH of the medium. Monopotassium Phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

For the enumeration of coliform bacteria employ sample dilutions, which yield between 10 - 50 colonies per plate using the pour plate method. Incubate at 35 ± 2°C for 18 - 24 hours. The coliform colonies have an intensely red center zone surrounded by a pink halo which is sharply outlined against the uniformly blue background of the medium. *Salmonella spp.*, which do not ferment lactose, produce colorless to pale pink colonies.

The medium is sensitive to light, which reduces its effectiveness and changes its color from strong blue to purple or pink. The medium should be prepared immediately before use and, if necessary, stored in the dark for the least time possible.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony color
Escherichia coli ATCC 25022	Good	Deep red with bile precipitate
Salmonella enteritidis ATCC 13076	Good	Colorless to pale pink
Enterobacter aerogenes ATCC 13048	Good	Pink
Staphylococcus aureus ATCC 25923	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Methods for the Examination of Water and Wastewater, 10th Ed APHA, Inc. New York, 1958.

Recommended Methods for the Microbiological Examination of Foods, APHA, Inc. New York, 1958.

BRILLIANT GREEN BILE BROTH 2% ISO 4831, ISO 4832

CAT. 1228

For the detection of coliforms in water and foods

FORMULA IN g/l

Ox bile	**20.00**	Lactose	**10.00**
Gelatin Peptone	**10.00**	Brilliant Green	**0.0133**

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection and sterilize in autoclave at 121°C for 15 minutes. AVOID OVERHEATING. The prepared medium should be stored at 2 - 8ºC. The color of the prepared medium is brilliant green.

- When the sample has 1 ml or less volume, dispense medium in volumes of 10 ml.

- To analyze samples of 10 ml, dissolve 80 grams of the medium in a liter of distilled water. Distribute in the same manner.

The dehydrated medium should be homogeneous, free-flowing and beige-greenish in color. If there are any physical changes, discard the medium.

Uninoculated Tube | *Escherichia coli* ATCC 25922 | *Enterobacter aerogenes* ATCC 13048

USES

BRILLIANT GREEN BILE BROTH 2% is a selective medium recommended by APHA for the cultivation of coliforms in drinking water, wastewater, foods and dairy products, and other products of sanitary concern. It is used as a confirmation test in procedures where presumptive tests for presence of coliforms are positive. The production of gas both at 37°C and 44.5°C confirms the presence of coliforms.

The Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria except coliforms. They also prevent the growth of the anaerobic lactose-fermenters such as *Clostridium perfringens*, which could give false positive reactions at 44.5°C.

Inoculate the medium and incubate at 37°C and 44.5°C for 24 - 48 hours. For milk analysis incubation at 32°C is recommended. The presence of gas is considered a positive test for the coli-enterobacter group.

This medium is recommended by ISO 4831 and ISO 4832 normatives for the confirmation of coliforms. From an incubated tube of Lauryl Sulfate Broth – Lauryl Tryptose Broth (**Cat.1310**) inoculate a tube of confirmation medium (Brilliant green bile broth 2 %). Incubate at 30 °C or 37 °C for 24 hr ± 2 hr or, if gas formation is not carried out, for 48 hr ± 2 hr. A tube in which gas formation is observed after 24 hr ± 2 hr or 48 hr ± 2 hr is considered as a positive tube.

To indicate the presence of *Escherichia coli*, incubate Brilliant Green Bile Broth 2% at 44 ± 1°C for 48 hours. Turbidity in the broth and gas production in the inverted tube are positive signs. An indole production test at 44.5°C is also carried out in Peptone-Tryptone Water (**Cat.1403**) to confirm *Escherichia coli*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C and 44.5°C and observed after 24 - 48 hours.

Microorganisms	Growth	Gas Production	
		37°C	44.5°C
**Escherichia coli* ATCC 25922	Good	+	+
Enterobacter aerogenes ATCC 13048	Good	+	-
Staphylococcus aureus ATCC 25923	Inhibited	-	-
Enterococcus faecalis ATCC 19433	Inhibited	-	-

*According ISO incubate at 30-37°C for 24 ± 2 hours and 48 ± 2 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Standard Methods for the Examination of Water and Sewage, 9th. Edition 195. 1946.
Standard Methods for the Examination of Dairy Products, 9th. Edition 152. 1948.

ISO 4832. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique

BRILLIANT GREEN SELENITE BROTH

CAT. 1221

For the selective enrichment of *Salmonella* species

FORMULA IN g/l

Gelatin Peptone	**5.00**	Monopotassium Phosphate	**1.02**
D-Mannitol	**5.00**	Sodium Taurocholate	**1.00**
Yeast Extract	**5.00**	Sodium Sulfapyridine	**0.50**
Sodium Selenite	**4.00**	Briliant Green	**0.005**
Dipotassium Phosphate	**2.65**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 24.2 grams of the medium in one liter of distilled water. Mix well and dissolve heating with frequent agitation. Dispense into sterile containers. AVOID OVERHEATING. DO NOT AUTOCLAVE. The prepared medium should be stored at 2 - 8°C in the dark. It is not recommended to store longer than 8 days. Once prepared, use as soon as possible. The color is green- blue.

The dehydrated medium should be homogeneous, free-flowing and cream with a green tint in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.

USES

BRILLIANT GREEN SELENITE BROTH is a selective enrichment for *Salmonella spp.*, generally following a pre-enrichment step.

The Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Mannitol is the fermentable carbohydrate providing carbon and energy. Brilliant green, Sodium sulfapyridine and Sodium selenite inhibit Gram-positive bacteria and most Gram-negative bacteria except for *Salmonella spp.* Sodium taurocholate acts as a selective agent inhibiting Gram-positive organisms. The Potassium phosphates act as a buffer system.

After the pre-enrichment of the sample in a suitable medium, pass 10 ml of the sample to Brilliant Green Selenite Broth. Incubate at 35 ± 2°C for 48 hours. After 24 hours subculture to plated media such as Brilliant Green Agar (**Cat. 1078**), Desoxycholate Citrate Agar (**Cat. 1067**) and Hektoen Enteric Agar (**Cat. 1030**) to obtain isolated colonies. Incubate these plates at 35 ± 2°C for 48 hours.

Repeat the subculture to selective plated selective media after 48 hours of incubation of the enrichment broth. Observe the plated media after 24 and 48 hours, noting the appearance and color of colonies on in each medium.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 6 and 24 hours.

Microorganisms	Growth		Concentration of the inoculum
	6 hours	24 hours	
Salmonella typhimurium ATCC 14028	>70%	>95%	approx. 1%
Escherichia coli ATCC 25928	<30%	<5%	approx. 99%

After 24 hours subculture to following plated media to obtain isolated colonies. Incubate these plates at 35 ± 2°C for 48 hours.

	Brilliant Green Agar	Desoxycholate Citrate Agar	Hektoen Enteric Agar
Salmonella	Pink to red with a red halo	Colorless to pale pink at 18 hours. When incubation time increases, they grow larger, opaque with gray to black center	Blue-green. Centers may or not be black
Shigella	Null	Initially colorless, then pale pink	Greenish, moist, convex

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

International standard. ISO 3565. (1975).

Meal and meat products-detection of Salmonella (reference method). ISO 3565 (1975).

BRILLIANT GREEN SELENITE BROTH II

CAT. 1219

For the selective enrichment of *Salmonella* species

FORMULA IN g/l

Peptone	**5.00**	Dipotassium Phosphate	**2.65**
D-Mannitol	**5.00**	Monopotassium Phosphate	**1.02**
Yeast Extract	**5.00**	Briliant Green	**0.005**
Sodium Selenite	**4.00**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 22.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Dispense into sterile containers. AVOID OVERHEATING. DO NOT AUTOCLAVE. The prepared medium should be stored at 2 - 8°C in the dark. It is not recommended to store longer than 8 days. Once prepared, use as soon as possible. The color is green.

The dehydrated medium should be homogeneous, free-flowing and cream with a green tint in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.

USES

BRILLIANT GREEN SELENITE BROTH II, like Brilliant Green Selenite Broth (**Cat. 1221**) is a selective enrichment for *Salmonella spp.*, generally following a pre-enrichment step.

This medium is not as inhibitory since it has neither Sodium taurocholate nor Sodium sulfapyridine. The Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Mannitol is the fermentable carbohydrate providing carbon and energy. Sodium selenite inhibits Gram-positive bacteria and most Gram-negative bacteria except for *Salmonella spp.* The Potassium phosphates act as a buffer system.

After the pre-enrichment of the sample in a suitable medium, pass 10 ml to Brilliant Green Selenite Broth II. Incubate at 35 ± 2°C for 48 hours. After 24 hours subculture to plated media such as Brilliant Green Agar (**Cat. 1078**), Desoxycholate Citrate Agar (**Cat. 1067**) and Hektoen Enteric Agar (**Cat. 1030**) to obtain isolated colonies. Incubate these plates at 35 ± 2°C for 48 hours.

Repeat the subculture to selective plated media after 48 hours of incubation of the enrichment broth. Observe the plated media after 24 and 48 hours, noting the appearance and color of colonies on each medium.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 6 and 24 hours.

Microorganisms	Growth		Concentration of the inoculum
	6 hours	24 hours	
Salmonella typhimurium ATCC 14028	>70%	>95%	approx. 1%
Escherichia coli ATCC 25928	<30%	<5%	approx. 99%

After 24 hours subculture to the following plated media to obtain isolated colonies. Incubate these plates at 35 ± 2°C for 48 hours.

	Brilliant Green Agar	Desoxycholate Citrate Agar	Hektoen Enteric Agar
Salmonella	Pink to red with a red halo	Colorless to pale pink at 18 hours. When incubation time increases, they grow larger, opaque with gray to black center	Blue-green. Centers may or not be black
Shigella	Null	Initially colorless, then pale pink	Greenish, moist, convex

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

International standard. ISO 3565. (1975).

Meal and meat products-detection of salmonella (reference method). ISO 3565 (1975).

BRILLIANT GREEN TETRATHIONATE BILE BROTH EUROPEAN PHARMACOPEIA

CAT. 1253

For the enrichment of *Salmonella spp.* in foods, water and faeces

FORMULA IN g/l

Calcium Carbonate	**20.00**	Dry Ox Bile	**8.00**
Potassium Tetrathionate	**20.00**	Sodium Chloride	**6.40**
Meat Peptone	**8.60**	Brilliant Green	**0.07**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 63 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Do not boil. Dispense into sterilized containers homogenizing the medium well enough to distribute the calcium carbonate. AVOID OVERHEATING. DO NOT AUTOCLAVE. The prepared medium should be stored at 2 - 8°C. The color is milky green with calcium carbonate precipitate.

The dehydrated medium should be homogeneous, free-flowing and cream with green tint in color. If there are any physical changes, discard the medium.

The growth of *Proteus* is inhibited by taking the pH to 6.5 or also by adding Novobiocin at 0.4%.

USES

BRILLIANT GREEN TETRATHIONATE BROTH is recommended by the European Pharmacopoeia in the Paragraph 2.6.13 Microbiological examination of non-Sterile products: test for specified microorganisms' as a selective enrichment broth for *Salmonella spp.*

Meat peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria. They also prevent the growth of the anaerobic lactose fermenters such as *Clostridium perfringens*, which could give false positive reactions at 44°C. The Calcium carbonate is a neutralizer that will absorb any toxic metabolites. Organisms that have the enzyme tetrathionate reductase will grow and multiply in this medium due to the presence of Potassium tetrathionate and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Once the sample is pre-enriched in Trypticasein Soy Broth (**Cat. 1224**), homogenized and incubated at 35 - 37°C for 18 - 24 hours, 1 ml of enriched culture is transferred to 10 ml of Brilliant Green Tetrathionate Bile Broth and incubated at 41 - 43°C C for 18 - 24 hours.

Sub-culture and incubate at 35 - 37°C for 18 - 72 hours to at least 2 of the following media for confirmation of *Salmonella spp.* Desoxycholate Citrate Agar (**Cat. 1067**); XLD Agar (**Cat. 1080**) or Brilliant Green Agar (**Cat. 1078**).

Results:

Desoxycholate Citrate Agar – well-developed, colorless colonies

XLD Agar – well-developed, red colonies, with or without black centers

Brilliant Green Agar – small, transparent, colorless, pink or opaque-white colonies, often surrounded by a pink or red zone.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 41 - 43°C during 18 - 24 hours.

Microorganisms	Growth		Concentration of the inoculum
	6 hours	24 hours	
Salmonella typhimurium ATCC 14028	>70%	>95%	approx. 1%
Escherichia coli ATCC 25928	<30%	<5%	approx. 99%

After 24 hours subculture to following plated media for confirmation.

	Brilliant Green Agar	Desoxycholate Citrate Agar	XLD Agar
Salmonella spp.	Small, transparent, colorless, pink or opaque white-colonies, often surrounded by a pink or red zone	Well developed, colorless colonies	Well developed, red colonies, with or without black centers

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

European Pharmacopoeia. 6th. Edition.

Microbiological examination of non-sterile products PS 137-140.

BRUCELLA AGAR

CAT. 1012

For the cultivation of *Brucella* in diverse clinical specimens, foods and othermaterials of sanitary interest

FORMULA IN g/l

Meat peptone	**10.00**	Yeast Extract	**2.00**
Casein Peptone	**10.00**	Dextrose	**1.00**
Sodium Chloride	**5.00**	Sodium Bisulfite	**0.10**
Bacteriological Agar	**15.00**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 43.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 5% sterile sheep defibrinated blood. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. Homogenize gently and dispense into Petri dishes. The prepared plates should be stored at 8 - 15ºC.

Brucella Agar can be made selective to yield higher numbers of positive isolations by aseptically adding two vials of the Brucella Supplement (**Cat. 6017**) previously reconstituted in 10 ml of 1:1 solution of methanol / sterile distilled water. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

Brucella Supplement (Cat. 6017)
(1 vial for 500 ml of the medium)

Nystatin.....50000 IU	Cycloheximide.....50 mg
Bacitracin.....12500 IU	Vancomycin.....10 mg
Polymyxin B.....2500 IU	Nalidixic Acid.....2.5 mg

For better growth, Polyenrichment Supplement (**Cat. 6011**) may be added if required.

USES

BRUCELLA AGAR, being rich in nutrients and growth factors, is very suitable to grow and isolate fastidious microorganisms.

It is used to successfully isolate *Brucella* from diverse specimens contaminated with microflora, both saprophytes and commensals, in clinical samples as well as in foods. This medium is also used to produce clostridial toxins. It can also be utilized in the isolation of many anaerobes both of human and animal origin. Food samples can be inoculated directly on the plates of Brucella Agar, while clinical specimens are more convenient as suspensions or macerations in sterile saline solutions.

The Meat peptone and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium bisulfite is the reducing agent. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose is the fermentable carbohydrate providing carbon and energy. The addition of blood provides extra growth factors for fastidious microorganisms. Bacteriological agar is the solidifying agent. The addition of the supplement enhances the medium's selectivity for the growth of *Brucella*.

Brucella species are level 3 pathogens and cause brucellosis, a zoonotic disease. It is usually transmitted through milk, dairy products, meat and direct contact with infected animals.

Inoculations and incubation at 35 ± 2°C should be made in duplicate - one plate under normal conditions and one plate under 5 - 10% CO_2. Observe after 24 - 72 hours.

Note: For an excellent medium for anaerobes, add 5 mg/ml of hemin and 10 mcg/ml of Vitamin K1 (phytomenadione) to the basal medium, culture and incubate under anaerobic conditions.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, adding the supplements Brucella Supplement (**Cat. 6017**), Polyenrichment Supplement (**Cat. 6011**), and 5% sterile sheep defibrinated blood after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere, and observed after 24 - 72 hours.

Microorganisms	Growth	Concentration of the inoculum
Brucella abortus ATCC 4315	Good	10^3-10^4
Brucella melitensis ATCC 4309	Good	10^3-10^4

Microorganisms	Growth	Concentration of the inoculum
Brucella suis ATCC 4314	Good	10^3-10^4

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Smith Louis Ds. The Pathogenic Anaerobic Bacteria. C. Thomas Pub. Springfield, Il, 1975.

Koneman, Allen, Dowell, and Sommers. Color Atlas and Textbook of Diagnostic Microbiology, J.B. Lippincott, Philadelphia, 1979.

BRUCELLA BROTH

CAT. 1223

For the cultivation of *Brucella* from diverse materials of medical and sanitary interest

FORMULA IN g/l

Meat peptone	**10.00**	Yeast Extract	**2.00**
Casein Peptone	**10.00**	Dextrose	**1.00**
Sodium Chloride	**5.00**	Sodium Bisulfite	**0.10**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 28.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BRUCELLA BROTH is a general purpose medium elaborated according to the APHA formula. Rich in nutrients and growth factors, it is very suitable to grow and isolate fastidious microorganisms, including *Campylobacter, Streptococcus* and *Neisseria*.

It is used extensively to isolate *Brucella* from diverse specimens contaminated with microflora, both saprophytes and commensals, in clinical samples as well as in foods. It can also be used in the development of many anaerobes, both of human and animal origin. It can also be used in blood culture bottle systems.

The Meat peptone and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium bisulfite is the reducing agent. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose is the fermentable carbohydrate providing carbon and energy.

Brucella species are level 3 pathogens and cause brucellosis, a zoonotic disease. It is usually transmitted through milk, dairy products, meat and direct contact with infected animals.

For cultivation of *Brucella*: inoculate and incubate at 35 ± 2°C in duplicate, one lot under normal conditions and one lot under 5 - 10% CO_2. Observe after 24 - 72 hours. Growth in tubes is indicated by turbidity compared with an uninoculated control.

For cultivation of other microorganisms incubate at the required temperature in a suitable atmosphere to encourage growth.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C, under 5 - 10% CO_2, and observed after 24 - 72 hours.

Microorganisms	Growth	Concentration of the inoculum
Brucella abortus ATCC 4315	Good	< 10^3
Brucella melitensis ATCC 4309	Good	< 10^3
Brucella suis ATCC 4314	Good	< 10^3

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Isenberg, H.D. (ed.) 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Hausler, W.J. (ed.). 1976. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.

BRUCELLA MEDIUM BASE

CAT. 1374

For the cultivation of *Brucella* from diverse materials of medical and sanitary interest

FORMULA IN g/l

Peptone	**10.00**	Sodium Chloride	**5.00**
Glucose	**10.00**	Bacteriological Agar	**15.00**
Beef Extract	**5.00**		
Final pH 7.5 ± 0.2 at 25°C			

PREPARATION

Suspend 45 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and add 5% horse serum, inactivated by heating at 56°C for 80 minutes. To obtain a selective medium add 2 vials of Brucella Selective Supplement Modified (**ffffffffffff**previously reconstituted in 10 ml of 1:1 w/w of ethanol and distilled water. Homogenize gently and pour into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and light in color. If there are any physical changes, discard the medium.

ffffffffffffffffffffffffffffffffffff Modiffffffffffffff
(1 vial for 500 ml of the medium)

Nystatin.....50000 IU	Natamycin:.....25.0 mg
Bacitracin.....12500 IU	Vancomycin:.....10.0 mg
Polymyxin B.....2500 IU	Nalidixic Acid:.....2.5 mg

USES

BRUCELLA BASE MEDIUM is prepared according to the formula described by Jones and Brinley Morgan for the cultivation and isolation of *Brucella*, including fastidious types. It is a medium rich in nutritive elements and growth factors that make it adequate for the growth and isolation of *Brucella spp.*

Beef extract and Peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. The addition of the supplement enhances the medium's selectivity for the growth of *Brucella*.

Brucella species are level 3 pathogens and cause brucellosis, a zoonotic disease. It is usually transmitted through milk, dairy products, meat and direct contact with infected animals.

It is widely used for the isolation of *Brucella* in highly contaminated materials, food materials and clinical samples.

Incubate at 35°C ± 2°C in an atmosphere of 5 - 10% CO_2 and observe alter 72 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, by adding the supplement, from type cultures after incubation at a temperature of 35°C ± 2°C, in an atmosphere of 5 - 10% CO_2, and observed after 72 hours.

Microorganisms	Growth
Brucella abortus ATCC 4315	Good
Brucella melitensis ATCC 4309	Good
Brucella suis ATCC 4314	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Kzudas and Mors, J.Bact. 66:502. 1953 Rennoux G. Ann. Inst. Pasteur, 87:325. 1954 Standard Methods for Examination of Diary Products. 10 th Ed. APHA, Inc. New York 1960 Smith Louis Ds. The pathogenic anaerobic Bacteria. C. Thomas Pub. Springfield, Il, 1975

BRYANT BURKEY BROTH BASE (MODIFIED WITH RESAZURIN)

CAT. 1247

For the detection of lactate fermenting Clostridial species in milk and dairy products

FORMULA IN g/l

Tryptone	**15.00**	Sodium Acetate	**5.00**
Beef Extract	**7.50**	L-Cysteine	**0.50**
Yeast Extract	**5.00**	Resazurin	**0.0025**
Final pH 5.9 ± 0.2 at 25°C			

PREPARATION

Suspend 33 grams of the medium in one liter of distilled water. Add 10 ml of 50% Sodium lactate. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes of 10 ml and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. If the medium presents a pink color in more than 1/3 under the surface when it is going to be used, regenerate the anaerobic conditions by heating at 100°C for 10 minutes. The color is yellow-pink.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

BRYANT-BURKEY BROTH BASE is used for the enumeration of spores of lactate fermenting Clostridia in milk and dairy products, particularly *Clostridium tyrobutyricum*. This bacterium is the one that causes the swelling of cheeses.

During milking process small numbers of butyric acid fermenting bacteria from silage are introduced into the raw milk. When the contaminated milk is used for producing cheese, the brines become contaminated with heat resistant Clostridia spores. During the ripening of salt brined, semi- and hard cheeses, (for example, Gouda, Edammer, Emmentaler, Gruyere, and Parmesan) late blowing gasogenic Clostridia ferment lactate into butyric acid, acetic acid and gas (CO_2 and H_2). The gas expands the cheese and causes a defect known as "late blowing" or butyric swelling.

The medium does not contain lactate so it must be added when the medium is prepared. Sodium lactate is fermented under anaerobic conditions by *C. tyrobutyricum* and other lactate-fermenting Clostridia and uses it as a source of carbon and energy, producing hydrogen and CO_2. Tryptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. The Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium acetate is the selective agent inhibiting Gram-negative bacteria and also promotes the growth of *C. tyrobutyricum*. L-Cysteine is the reducing agent and Resazurin is an oxidation indicator, turning from pink (aerobic) to colorless (under anaerobic conditions).

Before use, heat tubes and boil for 10 minutes to regenerate anaerobic conditions. Prepare decimal dilutions of the sample and inoculate into 10 ml of medium in tubes. Pour 2 ml of melted paraffin (60 - 65°C) into each tube, previously autoclaved at 121°C for 20 minutes.

Heat tubes at 75°C for 15 minutes to kill vegetative cells and active spores. Allow to cool to room temperature.

Read results after incubation at 37 ± 2°C for up to 7 days, considering the tubes with growth and gas production positive. To count the spores use the most probable number method (MPN).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37± 2°C and observed after for up to 7 days.

Microorganisms	Growth	Gas production
Clostridium tryobutyricum ATCC 132	Good	+
Clostridium perfringens ATCC 10543	Good	+
Staphylococcus aureus ATCC 25923	Moderate	-
Pseudomonas aeruginosa ATCC 27853	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

BRYANT M.P. and BURKEY L.A: 1956. The characteristics of lactate fermenting spore forming anaerobes from silage. J. Bact., 43 - 46

CERF. O. et BERGERE J.L. 1968. Numeration des spores de Clostridium et son application au lait et aux produits laiters. Numeration des différents groupes de Clostridium. Le lait, 48, 501-519.

BUFFERED PEPTONE WATER EUROPEAN PHARMACOPEIA

CAT. 1401

Recommended as a diluent for the homogenization of samples

FORMULA IN g/l

Disodium Hydrogen Phosphate Dihydrate	**7.20**	Potassium Dihydrogen Phophate	**3.60**
Sodium Chloride	**4.30**	Pancreatic Digest of Casein	**1.00**

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 16.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is transparent.

The dehydrated medium should be homogeneous, free-flowing and whitish in color. If there are any physical changes, discard the medium.

USES

BUFFERED PEPTONE WATER is recommended by the European Pharmacopoeia in the Paragraph 2.6.12 'Microbiological examination of non-sterile products: Microbial enumeration test', and in the Paragraph 2.6.13 'Microbiological examination of non-Sterile products: test for specified microorganisms' as a diluent for the homogenization of samples, effectiveness of culture media and validity of the counting method.

Pancreatic digest of casein provides nitrogen, vitamins, minerals and amino acids essential for growth. Potassium phosphates act as a buffer system and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

It is used in the preparation of the samples to dissolve or dilute water-soluble products (1:10 dilution) and non-fatty products insoluble in water (in general 1:10 dilution, but some may require larger volumes of Buffered Peptone Water)

Fatty products are homogenized with a suitable sterile surface-active agent such as Polysorbate or Tween 80 heated if necessary to no more than 40°C or, in exceptional cases, to no more than 45°C. Mix carefully and if necessary maintain the temperature in a water-bath. Add enough of the pre-warmed Buffered Peptone Water diluent to make a 1 in 10 dilution of the original product.

When testing, Buffered Peptone Water is used to prepare reference suspensions. Inoculate and incubate at 30 - 35°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 35°C and observed after 18 - 24 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 6538	Good
Escherichia coli ATCC 8739	Good
Bacillus subtilis ATCC 6633	Good
Candida albicans ATCC 10231	Good
Aspergillus brasiliensis ATCC 16404	Good
Salmonella typhimurium ATCC 14028	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

European Pharmacopoeia 7.0

BUFFERED PEPTONE WATER ISO 6579, ISO 22964

CAT. 1402

Recommended as a diluent for the homogenization of samples in the microbiological analysis of food

FORMULA IN g/l

Pancreatic Digest of Casein	**10.00**	Disodium Phosphate* Equivalent to 9.0 g of Disodium Hydrogen Phospate Dodeca-hydrate	**3.50**
Sodium Chloride	**5.00**	Monopotassium Phosphate	**1.50**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 20 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is very light amber.

The dehydrated medium should be homogeneous, free-flowing and white cream to slightly toasted in color. If there are any physical changes, discard the medium.

USES

BUFFERED PEPTONE WATER is a non-selective medium recommended as a preenrichment medium by the UNE-EN-ISO 6579 normative in food samples containing suspected contaminants such as *Salmonella* and in ISO 22964 in milk and milk products for *Enterobacter sakazakii*.

Salmonella can be present in small numbers and are usually found with considerably larger numbers of other Enterobacteriaceae or other families. Pre-enrichment is necessary to allow the detection of small numbers of *Salmonella* or injured *Salmonella*.

A feature common to all selective media is that sublethally injured organisms are not generally detected and therefore a recovery step must be included in examination procedures. This is of importance, particularly in the food industry as various processes such as heat, desiccation, preservation processes, pH changes, etc, cause sublethal injuries to *Salmonella*. The broth is rich in nutrients and produces high resuscitation rates for sublethally injured bacteria and intense growth.

Changes in pH may cause damages to bacteria growth. Buffered Peptone Water maintains a high pH over the enrichment period via the phosphate buffer system and allows repair of injured cells sensitive to low pH. Pancreatic digest of casein provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium pyruvate can be added as an additional source of energy, but may also have protective effects against hydrogen peroxide that aids in the resuscitation of stressed organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

The medium for *Salmonella* is designed for the selective enrichment with MKTTN Broth with Brilliant Green & Novobiocin (**Cat.1173**) and Rappaport Soy Broth (Vassiliadis) (ISO 6579) (**Cat.1174**). Inoculate and incubate at 37 ± 1°C for 18 hours.

The medium for *Enterobacter sakazakii* must be used as a preenrichment before inoculating Lauryl Sulfate Trypthose Broth Modified (m LST) (**Cat. 1445**). Inoculate the Buffered peptone water at 37 ± 1°C for 18 ± 2 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1°C and observed after 18 hours.

Microorganisms	Growth
Salmonella enteritidis ATCC 13076	Good
Salmonella typhi ATCC 19430	Good
Salmonella typhimurium ATCC 14028	Good
* *Enterobacter sakazakii* ATCC 29544	Good

* According to ISO 22964 Incubate at 37 ± 1°C during 18 ± 2 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

M.R. Pascual Anderson (1982) Techniques for Microbiological Analysis of Foods and Drinks, CeNAN.

Normative UNE-EN ISO 6579. Microbiology of food stuff for humans and animals. Horizontal method to detect Salmonella spp.

ISO/TS 22964. Milk and milk products — Detection of Enterobacter sakazakii

BUFFERED SALINE PEPTONE WATER

CAT. 1406

Recommended as a primary solvent for serial dilution

FORMULA IN g/l

Sodium Chloride	**8.50**	Disodium Phosphate	**0.044**
Casein Peptone	**1.00**	Sodium Phosphate	**0.023**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 9.57 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is colorless.

The dehydrated medium should be homogeneous, free-flowing and very light beige in color. If there are any physical changes, discard the medium.

USES

BUFFERED SALINE PEPTONE WATER is a non selective medium, therefore allowing an easy recovery of stressed microorganisms. Buffered Saline Peptone Water is rich in nutrients and produces high recovery of damaged cells and intensifies the growth of microorganisms. A feature common to all selective media is that sublethally injured organisms are not generally detected and therefore a recovery step must be included in examination procedures.

Changes in pH may damage bacteria growth. Buffered Saline Peptone Water maintains a high pH via the Phosphate buffer system and allows the repair of injured cells which are sensitive to low pH. Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Salmonella typhimurium ATCC 14028	Good
Staphylococcus aureus ATCC 25923	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Standard Methods for the Examination of Water and Wasterwater 15a edition, 1980

Juven, Cox Bailet, Rhomson, Charles and Schutze. 1984. J. Food Prot. 47:299

Andrews, Flowers, Silliker and Bailey. 2001. In Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington

BURKHOLDERIA CEPACIA AGAR BASE

CAT. 1347

For the selective isolation of *Burkholderia cepacia* from respiratory secretions of Cystic Fibrosis patients, and for the routine testing of non-sterile inorganic salts containing preservatives

FORMULA IN g/l

Sodium Pyruvate	**7.00**	Ammonium Sulfate	**1.00**
Peptone	**5.00**	Magnesium Sulfate	**0.20**
Potassium Dihydrogen Phosphate	**4.40**	Phenol red	**0.02**
Yeast Extract	**4.00**	Ferrous Ammonium Sulfate	**0.01**
Bile Salts	**1.50**	Crystal Violet	**0.001**
Disodium Phosphate	**1.40**	Bacteriological Agar	**12.00**

Final pH 6.2 ± 0.2 at 25°C

PREPARATION

Suspend 18.25 grams of the medium in 500ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Burkholderia Selective Supplement (**Cat. 6032**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is orange.

The dehydrated medium should be homogeneous, free-flowing and strawberry pink in color. If there are any physical changes, discard the medium.

Burkholderia Selective Supplement (Cat. 6032)
(Composition each vial for 500 ml)

Polymixin B.....75000 IU
Gentamicin.....2.5 mg
Ticarcillin.....50.0 mg

USES

BURKHOLDERIA CEPACIA AGAR BASE is a selective medium especially formulated for the isolation of *Burkholderia cepacia* (*Pseudomonas cepacia*), from clinical and non-clinical specimens. *Burkholderia cepacia* is a Gram-negative, oxidase positive, mobile and aerobic bacillus. It is normally found in water deposits and damp environments. This bacillus is an important opportunist pathogen and causes pulmonary infections in Cystic Fibrosis patients.

The organism may be present in small numbers in many non sterile products used in hospitals. It has been isolated from a number of water sources and can grow in distilled water with a nitrogen source because of its capacity to fix CO_2 from air. Suction catheters rinsed in a solution of acetic acid have reduced the transmission of *Burkholderia cepacia* and other *Pseudomonas*.

The medium contains Peptone which provides nitrogen, vitamins, minerals and amino acids essential for growth. Selective agents are added to improve *B. cepacia* recovery through the inihibition of common contaminants. Crystal violet inhibits Gram-positive cocci, especially enterococci and staphylococci. Bile salts inhibit most Gram-positive cocci except enterococci, and Ticarcillin and Polymyxin B inhibit Gram-negative bacilli. Phenol red facilitates detection of *B. cepacia*. Alkaline end products from the metabolism of pyruvate raise the pH of the medium, causing the color of the indicator to change from light orange to pink, or pink-red, in the growth area. In areas of heavy *B. cepacia* growth, the pink color intensifies. Magnesium sulphate, Ammonium sulphate and Ferrous sulfate provide sources of sulfates and metallic ions. Phosphate salts act as a buffer system. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 37°C for 48 to 72 hours. Colonies of *B. cepacia* are 1 - 2 mm in diameter and turn the medium to pink. Low numbers of colonies may not produce a color change of the medium. Occasional growth of some strains of *Candida species*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* and other *Pseudomonas species* may occur.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C and observed after 48 - 72 hours.

Microorganisms	Growth
Burkholderia cepacia ATCC 25608	Good
Pseudomonas aeruginosa ATCC 27853	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bahame, J. B. and Schroth, M. N. [1989): Spatial-temporal colonization pattems of a rhizobacterium on underground organs of potato Phytopatology. Vol.77:1 093-1100.

Barelmann, I.; Meyer, I.M.; Taraz, K. and Budzikiewicz, D. [1996): Cepaciachelin, a new catecholate siderophore from Burkholderia [Pseudomonas) cepacia. Z Natarfosch. Vol. 51: 627-630.

Bashan, Y. and Holguin, Gina. [1998): Proposal for the Division of plant growth–promoting rhizobacteria into two classifications: biocontrol-PGPB [plant growth–promoting bacteria) and PGPB. Soil Biol Biochem. Vol. 30[8-9): 1225-1228.

CALCIUM CASEINATE AGAR

CAT. 1069

Selective medium for the recovery of proteolytic microorganisms in foods

FORMULA IN g/l

Meat Peptone	**5.00**	Calcium Hydroxide	**0.15**
Sodium Chloride	**5.00**	Calcium Chloride	**0.05**
Beef Extract	**3.00**	Bacteriological Agar	**13.50**
Casein (Hammarsten)	**2.50**		

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 29.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Pour into Petri dishes swirling the medium to resuspend the precipitate. The prepared medium should be stored at 8 - 15°C. The color is whitish.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

CALCIUM CASEINATE AGAR is used as a selective medium for the recovery of proteolytic microorganisms in foods. The industrial food processes cause sublethal injuries to many microorganisms and, to aid in recovery, nutrient-rich media are used.

This medium contains casein, a raw milk source rich in amino acids and nitrogen which is degraded by the proteolytes to form clearer zones surrounding the colonies in an otherwise turbid medium. Meat peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Calcium hydroxide is the inhibitor. Calcium Chloride helps to maintain the pH of the medium. Bacteriological Agar is the solidifying agent.

Inoculation can be made by streaking the surface of the plate or by using the pour plate method. Incubate at 35 ± 2°C for 48 - 72 hours. Casein is degraded by proteolytic organisms and forms clear zones surrounding the colonies. The finished medium is turbid especially if 5 - 10 g/l of powdered milk is added.

Count the colonies with clearing zones only. Covering the surface of the plate with 5 - 10% Acetic acid can improve differentiation of colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 48 - 72 hours.

Microorganisms	Growth	Transparency halo (clearing)
Bacillus cereus ATCC 11778	Good	+
Pseudomonas aeruginosa ATCC 27853	Good	+
Proteus vulgaris ATCC 13315	Good	-
Escherichia coli ATCC 25922	Good	-
Enterobacter cloacae ATCC 13047	Good	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Frazier, W.C., a. RUPP, P: Studies on the proteolytic bacteria of milk. A. medium for the direct isolation of caseolytic milk bacteria. J. Bact. 16 57-63 (1928).

CAMPYLOBACTER AGAR BASE (PRESTON)

CAT. 1131

Selective medium for *Campylobacter jejuni* and *C. coli*

FORMULA IN g/l

Casein Peptone	**10.00**	Sodium Chloride	**5.00**
Beef Extract	**10.00**	Bacteriological Agar	**12.00**

Final pH 7.5 ± 0.2 at 25°C

PREPARATION

Suspend 18.5 grams of the medium in 475 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45°C and aseptically add 5-7% of lysed horse blood and one vial of Preston Campylobacter Supplement (**Cat. 6019**), previously reconstituted with 5 ml of 1:1 solution of acetone / sterile distilled water. Homogenize gently and dispense into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium, and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium is yellowish white.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Preston Campylobacter Supplement (Cat. 6019)
(One vial for 500 ml of medium)

Polymyxin B.....2500 IU
Cycloheximide.....50 mg
Rifampicin.....5 mg
Trimethoprim.....5 mg

CAUTION: Preston Campylobacter Supplement contains Cycloheximide and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

CAMPYLOBACTER AGAR BASE (Preston) is based on the formulation described by Bolton and Robertson, and designed for the isolation of *Campylobacter* species from human, animal, bird and environmental samples. The supplement is especially selective for *Campylobacter jejuni* and *C. coli*. The recovery of injured cells can be improved by pre-enrichment in broth medium, permitting sublethally injured organisms to repair lesions and to tolerate certain selective antibiotics.

Campylobacter spp. are of world wide significance in human and animal disease, especially *C. jejuni*, considered one of the main causes of acute bacterial diarrhea in man.

Casein peptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Polymyxin B inhibit Gram-negative microorganisms. Cycloheximide inhibits yeasts. Rifampicin inhibits Gram-positive organisms. Trimethoprim exerts its antibacterial action through the selective inhibition of bacterial dihydrofolate reductase (DHFR), which is an essential enzyme in all living cells. Bacteriological agar is the solidifying agent.

Inoculate the plates with the addition of Preston Campylobacter Supplement (**Cat. 6019**) and incubate at 42°C for 24 - 48 hours. Examine the plates and confirm the typical colonies as *Campylobacter jejuni* or *C. coli* by the standard method. The colonies first appear flat and grey with an irregular edge or raised and round.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with Preston Campylobacter Supplement (**Cat. 6019**) added, from type cultures after incubation at a temperature of 42°C and observed after 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Campylobacter jejuni ATCC 29428	Good
Campylobacter coli ATCC 33559	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bolton F.J. Hutchinson D.N. y Cioste D. (1984] clin. Microbiol. 19, 169-171

Bolton E.J. Robertson L. (1982] J. Clin Parth 35, 462-467

CAMPYLOBACTER AGAR BASE - BLOOD FREE (CCDA)

CAT. 1129

Selective medium for the isolation of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter laridis*

FORMULA IN g/l

Nutrient Broth N°2	**25.00**	Ferrous Sulfate	**0.25**
Casein Hydrolysate	**3.00**	Sodium Pyruvate	**0.25**
Bacteriological Charcoal	**4.00**	Bacteriological Agar	**12.00**
Sodium Desoxycholate	**1.00**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 22.75 grams of the medium in 500ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of C.C.D.A Supplement (Campylobacter Blood Free) (**Cat. 6053**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is black.

The dehydrated medium should be homogeneous, free-flowing and dark beige in color. If there are any physical changes, discard the medium.

C.C.D.A. Supplement (Cat. 6053)
(One vial for 500 ml of medium)

Cefoperazone.....16 mg
Amphotericin B.....5 mg

USES

CAMPYLOBACTER AGAR BASE (CCDA) BLOOD FREE is a modified formula described by Bolton *et* al., replacing blood with Charcoal, Sodium pyruvate and Ferrous sulfate. This medium supports the growth of most enteric Campylobacters, and recommended for the selective isolation of *Campylobacter jejuni*, *Campylobacter coli* and termophilic Campylobacteria, in foods and in clinical and non clinical specimens.

Campylobacter is considered the main cause of enteric illnesses. *Campylobacter spp.* can cause mild to severe diarrhea, with loose, watery stools frequently followed by bloody diarrhea. These pathogens are very infective and are transmitted by contaminated food or water.

The medium contains Ferrous sulfate, Sodium pyruvate and Charcoal to promote the growth of *Campylobacter species*, as they quench the toxic forms of oxygen (hydrogen peroxide) increasing the aerotolerance and enabling the oxygen sensitive strains to be readily isolated. Sodium desoxycholate partially or completely inhibits Gram-positive organisms, coliforms and *Proteus*. Nutrient broth N°2 and Casein hydrolysate provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent.

The addition CCDA Supplement: Cefoperazone increases selectivity and inhibits the growth of Gram-negative enteric bacilli and some Gram-positive species, whilst Amphotericin B suppresses yeasts and fungi that may grow at 37°C, a temperature shown to increase selectivity.

Inoculate and incubate at 42°C for 24 - 48 hours. *C. jejuni* produces gray, moist, flat, spreading colonies. *C. coli* colonies are creamy-gray, moist, slightly raised and tend to be discrete.

MICROBIOLOGICAL TEST

Inoculate and incubate at 42°C for 24 - 48 hours. *C. jejuni* produces gray, moist, flat, spreading colonies. *C. coli* colonies are creamy-gray, moist, slightly raised and tend to be discrete.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Campylobacter jejuni ATCC 29428	Good
Campylobacter coli ATCC 33559	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bolton F.J. Hutchinson D.N. y Cioste D. (1984) clin. Microbiol. 19,169-171 Bolton E.J., Roberstson L. (1982) J. Clin Parth 35, 462-67

CANDIDA CHROMOGENIC AGAR

CAT. 1382

Differential and selective chromogenic medium for the isolation and quick identification of *Candida spp.* of clinical importance

FORMULA IN g/l

Glucose	**20.00**	Chromogenic Mixture	**0.40**
Peptone	**10.00**	Bacteriological Agar	**15.00**
Chloramphenicol	**0.50**		
Final pH 6.1 ± 0.2 at 25°C			

PREPARATION

Suspend 45.9 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution AVOID OVERHEATING. DO NOT AUTOCLAVE. Dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is clear amber, slightly opalescent.
The dehydrated medium should be homogeneous, free-flowing and light beige in color If there are any physical changes, discard the medium.

USES

CANDIDA CHROMOGENIC AGAR is an alternative chromogenic formulation to the traditional media for the detection and isolation of *Candida spp.*

In this chromogenic medium, the three different species of *Candida albicans*, *Candida tropicalis* and *Candida krusei* can be differentiated due to the chromogenic substrates present within the medium. Candida Chromogenic Agar allows the easy and rapid identification and differentiation of all 3 species by producing easy-to-read results in one plate, since they present different colored colonies.

Colonies of *Candida albicans* are green, those of *Candida krusei* are purple-pink and those of *Candida tropicalis* are blue.

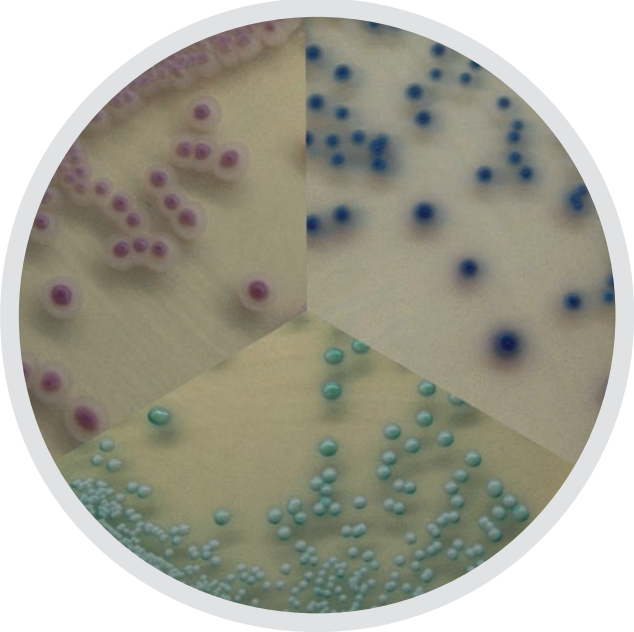
In the medium Glucose is the fermentable carbohydrate providing carbon and energy. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum. The chromogenic

mixture allows the identification and differentiation of all 3 species by producing easy-to-read results in one plate, since they present different colored colonies, Bacteriological agar is the solidifying agent.

The different species of *Candida* produce different kinds of infections. Candidiasis, the most common opportunistic fungal infection is frequently caused by *Candida albicans*. *Candida tropicalis* and *Candida glabrata* infections occur less often. *Candida spp.* are present in clinical specimens due to environmental contamination, colonization, or a disease process. *Candida albicans* is the most common and is usually susceptible to the antigfungal agents' azole group. However, *Candida glabrata*, *Candida tropicalis* and *Candida krusei* are azole tolerant, thus the rapid identification of the different species of is essential for its correct diagnosis and treatment.

Candida krusei
ATCC 34135

Candida tropicalis
ATCC 1369



Candida albicans
ATCC 10231

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 37°C and observed after 24, 48 and 72 hours.

Microorganisms	Growth	Colony Color
Candida tropicalis ATCC 1369	Good	Blue
Candida albicans ATCC 10231	Good	Green
Candida krusei ATCC 34135	Good	Purple-Pink
Candida parasilosis ATCC 22019	Good	Light White – Purple
Candida glabrata ATCC 2001	Good	Light White - Purple

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Sheehan, D.J. et. al.(1999) Current and Emerging Azole Antifungal Agents Clinical Microbiology Reviews, 12 (1): 40-79

Odds, F.C. (1988) Candida and candidiasis, 2nd ed, Baillière Tindall, London, England.

Ibrahim E.H. et al. (2001) The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest, 118 (1): 146-55

CARY-BLAIR MEDIUM

CAT. 1529

Transport medium recommended for the collection and transport of clinical specimens

FORMULA IN g/l

Sodium Chloride	**5.00**	Disodium Phosphate	**1.10**
Sodium Thioglycollate	**1.50**	Calcium Chloride	**0.09**
Agar N° 2	**5.50**		
Final pH 8.4 ± 0.2 at 25°C			

PREPARATION

Suspend 13.2 grams of the medium in one liter of distilled water. Mix well. Heat with frequent agitation and boil for one minute until completely dissolved. Dispense into screw-capped test tubes and place in flowing steam for 15 minutes. Allow to cool at room temperature and tighten the caps to avoid water loss. The prepared medium should be stored at 2 - 8°C. The color is white opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

CARY-BLAIR MEDIUM is recommended for the collection and transport of fecal and rectal samples, maintaining viability of *Salmonella* and *Shigella* in fecal samples. This medium has a low oxidation/reduction potential, which assures bacterial survival for long periods of time.

Cary-Blair Medium has a low nutrient content and a phosphate buffer, together with the Sodium thioglycollate, that inhibit the massive growth of strains such as *Escherichia coli* and *Klebsiella aerogenes*. Agar N°2 is the solidifying agent.

Due to its high pH, Cary-Blair Medium has been described as especially good for epidemiological studies of *Vibrio parahemolyticus*, allowing long-term survival (up to 35 days at temperatures from 22 - 31°C) of rectal swabs. Long recovery times have been reported for *Pasteurella pestis* (75 days) as well as for Salmonellae and Shigellae (49 days). Cotton swabs placed at the bottom of the transport medium tube are used for the collection of the samples.

Inoculate sterile swabs with suspensions of test organisms containing 1000 - 10000 CFU / 0.1ml. Place in the medium and incubate at room temperature for up to 72 hours. Remove swabs and streak on prepared Trypticasein Soy Agar (**Cat. 1068**) with defibrinated blood.

The survival of bacteria in a transport medium depends on various factors such as bacteria type and concentration in the specimen, transport medium formulation, and transport temperature and duration. Optimal growth and typical morphology can only be expected if direct inoculation and appropriate cultivation are followed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at different temperatures (4°C and room temperature) up to 72 hours.

Microorganisms	Recovery at 4°C	Recovery at 25°C
Neisseria meningitidis ATCC 13090	≥ 50	≥ 50
Neisseria gonorrhoeae ATCC 19424	≥ 50	≥ 50
Streptococcus pneumoniae ATCC 6301	≥ 50	≥ 50
Shigella flexneri ATCC 12022	≥ 50	≥ 50
Bordetella pertussis ATCC 9340	≥ 50	≥ 50
Haemophilus influenzae ATCC 19418	≥ 50	≥ 50

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Cary, S.G. and E.B. Blair 1964. New transport medium for shipment of clinical specimens. J. Bacteriol.

Cary, S.G., M.S. Mathew, M.H. Fusillo, and C. Hasking 1965 Survival of Shigella and Salmonella in a new transport medium. Am. J. Clin. Path.

CETRIMIDE AGAR BASE EUROPEAN PHARMACOPOEIA

CAT. 1102

For the selective isolation and identification of *Pseudomonas aeruginosa*

FORMULA IN g/l

Pancreatic Digest of Gelatin	**20.00**	Cetrimide	**0.30**
Dipotassium Sulfate	**10.00**	Bacteriological Agar	**13.60**
Magnesium Chloride	**1.40**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 45.3 grams of the medium in one liter of distilled water. Add 10 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is white-opaque.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

CETRIMIDE AGAR BASE is recommended by the European Pharmacopoeia for the selective isolation and identification of *Pseudomonas aeruginosa*. This medium promotes the production of fluorescein (pyoverdin), a green-yellow fluorescent pigment that oxidizes to yellow. It is water-soluble and, unlike pyocyanin (blue-green pigment), it is not soluble in chloroform. The pigment diffuses throughout the medium and the fluorescent yellow-green color is observed.

Strains of *Pseudomonas aeruginosa* are identified from specimens because, in addition to their colonial morphology and the characteristic grape-like odor of aminoacetophenone, they produce pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment. *P. aeruginosa* is the only specie of *Pseudomonas* or Gram-negative rod known to excrete pyocyanin.

Gelatin pancreatic digest provides nitrogen, vitamins, minerals and amino acids essential for growth. Glycerol is the carbon source. Magnesium chloride and Dipotassium Sulfate enhance the production of pyocyanin, pyoverdin and fluorescein.

Cetrimide is the selective agent as it inhibits the growth of the accompanying microbial flora.

The European Pharmacopoeia method recommends to inoculate the plates at 30 - 35°C for 18 - 72 hours and to incubate *E. coli* as a negative control at 30 - 35°C.

The identification of *P. aeruginosa* is completed by performing the oxidase test. Add a few drops of a freshly prepared , N-dimethyl-p phenylenediamine monohydrochloride solution to the growth on the nutrient agar slant. Oxidase positive cultures develop a pink color which successively becomes maroon, dark red, and black in 10 to 30 minutes.

Pseudomonas aeruginosa
ATCC 27853

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with 10 ml of glycerol, from type cultures after incubation at a temperature of 30 - 35°C and observed after 18 - 72 hours.

Microorganisms	Growth	Colony Color	Inoculum (cfu/ml)	Recovery Rate (%)
Escherichia coli ATCC 25922	Inhibited	-	$>10^5$	≤ 0.01
Escherichia coli ATCC 8739	Inhibited	-	$>10^5$	≤ 0.01
Pseudomonas aeruginosa ATCC 27853	Good	Yellow-green	10^3-10^5	≥ 30
Pseudomonas aeruginosa ATCC 9027	Good	Yellow-green	10^3-10^5	≥ 30
Staphylococcus aureus ATCC 25923	Inhibited	-	$>10^5$	≤ 0.01
Staphylococcus aureus ATCC 6538	Inhibited	-	$>10^5$	≤ 0.01

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD EP

King, Ward and Raney. J. Lab. and Clin. Med. 44:301. 1954. Brown and Lowbury. J. Clin. Path. 18:752. 1965.

Lowbury. J. Clin. Path. 4:66. 1951. Lowbury and Collins. J. Clin. Path. 8:47. 1955.

European Pharmacopoeia 7.0

CHAPMAN STONE AGAR

CAT. 1017

Selective and differential medium for the isolation of staphylococci in foods

FORMULA IN g/l

Ammonium Sulfate	**75.00**	D-Mannitol	**10.00**
Sodium Chloride	**55.00**	Dipotassium Phosphate	**5.00**
Gelatin	**30.00**	Yeast Extract	**2.50**
Casein Peptone	**10.00**	Bacteriological Agar	**15.00**

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 202.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 10 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

CHAPMAN STONE AGAR is used for the isolation of pathogenic staphylococci in foods. It is similar to Staphylococcus N° 110 Agar (**Cat. 1032**), but contains Ammonium sulfate to detect the gelatinase activity (Stone's reaction).

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. D-Mannitol is the fermentable carbohydrate providing carbon and energy. Sodium chloride, in high concentrations, inhibits most bacteria except staphylococci. Gelatin is a protein derived by the hydrolysis of collagen, found abundantly in bones, skin, tendons, cartilage and animal tissue. It is used in culture media to determine gelatinolysis by bacteria. The gelatinoses produced by the microorganisms hydrolyze the gelatin liquefying a solid medium or preventing the gelation of a medium containing gelatin. Bacteriological agar is the solidifying agent.

The samples suspected of containing pathogenic staphylococci are inoculated heavily and incubated for 30 ± 2°C for 18 - 48 hours. Any pigmented colony (yellow or soft orange) that is surrounded by a clear zone is probably a pathogenic *Staphylococcus*.

The staphylococcal colonies are yellow, yellow-gold or orange, ferment mannitol, coagulase-positive, produce beta-hemolysis in media such as Blood Agar and are gelatinase-positive (positive Stone´s reaction).

Pale colonies, practically lacking in color or not producing pigment, should not be considered as positives, even if they are surrounded by a clear zone (halo).

It is recommended to pick the colony and emulsify it in 0.1 - 0.2 ml Brain Heart Infusion Broth (**Cat. 1400**) and perform the coagulase test.

At the same time it is convenient to add a drop of Bromocresol purple to the colony site in order to determine mannitol fermentation: a yellow color formation is a positive reaction. The zones or clear halos around the colonies indicate degradation by the enzyme gelatinase (gelatin hydrolysis).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Mannitol Fermentation	Halo
Escherichia coli ATCC 25922	Inhibited	-	-
Staphylococcus epidermidis ATCC 12228	Good	-	+
Staphylococcus aureus ATCC 25923	Good	+	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Chapman J. Bact. 1945. 50: 201 Recommended Methods for the Microbiological Examination of Foods APHA. Inc. New York 1958. Standards Methods for Examination of Dairy Products, 1st Ed. APHA. Inc. New York, 1960.

CHLORAMPHENICOL AGAR (YGC AGAR) ISO 7954

CAT. 1301

Selective medium for the isolation and enumeration of molds in milk and dairy products

FORMULA IN g/l

Dextrose	**20.00**	Chloramphenicol	**0.10**
Yeast Extract	**5.00**	Bacteriological Agar	**12.00**
Final pH 6.6 ± 0.2 at 25°C			

PREPARATION

Suspend 37.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.
The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

CHLORAMPENICOL AGAR (Yeast Glucose Chloramphenicol Agar) is recommended by the International Dairy Federation (FIL-IDF), International Organization for Standardization (ISO), and Deutsche Institute für Normung (DIN) for the selective isolation and enumeration of yeasts and molds in milk and dairy products.

The antibiotic method for enumerating yeasts and molds in dairy products is the preferred method of choice as it results in a better recovery of injured fungal cells.

Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Dextrose is the fermentable carbohydrate providing carbon and energy and Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum. Bacteriological agar is the solidifying agent.

Inoculate sample dilutions (0.01, 0.001) and incubate at 25°C ± 1°C during 3, 4 and 5 days, and count colonies, differentiating yeast from molds by colony morphology.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25°C ± 1°C after 3, 4 and 5 days.

Microorganisms	Growth	Inoculum (cfu/ml)	Recovery Rate (%)
Candida albicans ATCC 10231	Good	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9763	Good	10^3-10^5	≥ 70
Escherichia coli ATCC 25922	Inhibited	$>10^5$	≤ 0.01
Staphylococcus aureus ATCC 25923	Inhibited	$>10^5$	≤ 0.01

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

FIL-IDF(1991) Standard 94B. Enumeration of yeast and moulds. Colony Count Technique at 25°C.

ISO (1981) ISO/DIS 6611: Milk and Milk products: Enumeration of yeast and molds colony counts technique at 25°C.

ISO 7954- Microbiology – General Guidance for enumeration of yeasts and molds. Colony count technique at 25°C

DIN Standard 10186. Mikrobiologische Milch Untersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen.

CLED AGAR (CYSTINE LACTOSE ELECTROLYTE DEFICIENT)

CAT. 1016

For the inhibition of *Proteus* swarming in the cultivation of Gram-positive and Gram-negative urinary tract bacteria

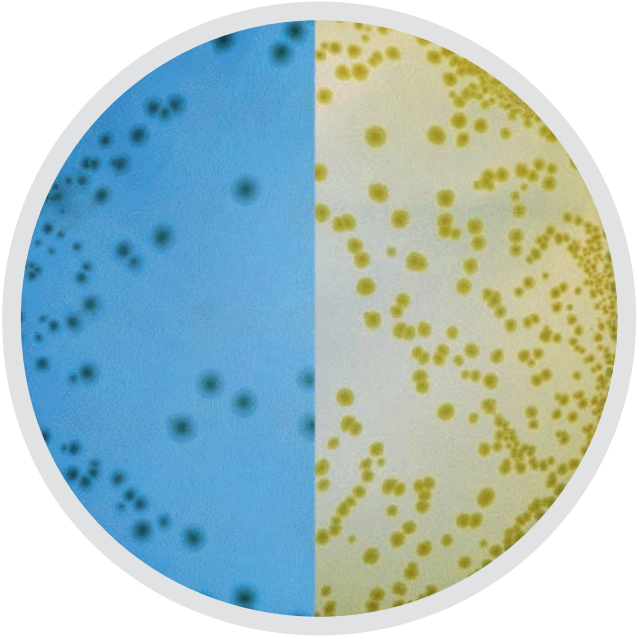
FORMULA IN g/l

Lactose	**10.00**	L-Cystine	**0.128**
Casein Peptone	**4.00**	Bromothymol Blue	**0.02**
Gelatin Peptone	**4.00**	Bacteriological Agar	**15.00**
Beef Extract	**3.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 36 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. When the medium is solidified, invert the plates to avoid excess moisture. The prepared medium should be stored at 8 - 15°C. The color is green.

The dehydrated medium should be homogeneous, free-flowing and greenish beige in color. If there are any physical changes, discard the medium.



Proteus vulgaris ATCC 29905 | *Escherichia coli* ATCC 25922

USES

CLED AGAR is a non-selective differential plating medium for the growth and enumeration of urinary tract microorganisms. Omitting sodium chloride inhibits the *Proteus* swarming and supports the growth of the vast majority of bacteria causing urinary tract infections, and is used to differentiate and identify them. The presence of bacterial contaminants like Diphtheroids, lactobacilli and other microbes indicate the degree of care taken with the handling of the urine specimen.

Beef Extract and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. L-Cystine is added as a growth supplement for cystine dependent coliforms. Differentiation of lactose fermenters and lactose non fermenters is achieved using Bromothymol blue as a pH indicator. Organisms that ferment lactose will lower the pH and change the color of the medium from green to yellow. Bacteriological agar is the solidifying agent.

The microorganisms which cause infection in the urinary tract are generally abundant and of only one species. *E. coli* is the organism most frequently isolated. The seeding of the sample can be done by the dilution method or by streaking on the surface

of agar with a calibrated loop. Count the colonies after 24 - 48 hours of incubation at a temperature of 35 ± 2°C. Report the number of colonies per ml of urine. A count of 100.000 (10⁵)/ml or more is an indication of a significant clinical urinary tract infection.

CHARACTERISTICS OF THE COLONIES

Staphylococcus aureus:	*Proteus:*	*Escherichia coli:*
Deep yellow colonies of 0.75 mm diameter.	Translucent blue. Smaller than *E. coli.*	Large, elevated, yellow and opaque. Center more intense yellow. Yellow agar. (non-lactose fermenting strains: blue colonies).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Medium color
Enterobacter aerogenes ATCC 13048	Good	Light yellow-blue
Escherichia coli ATCC 25922	Good	Yellow
Proteus vulgaris ATCC 29905	Good (swarming inhibited)	Blue-blue green
Staphylococcus aureus ATCC 25923	Good	Light yellow - •
Enterococcus faecalis ATCC 19433	Good	Light yellow - •

• = without changes

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Bebis, T. D. J. Med. Lab. Technol, 26-38-41. 1968. Mackey, J. R. and Sandys, G.H. 1965.

B.M.H. 1 1173. Mackey, J.R. and Sandys, G.H. 1966. B.M.H. 1 1173. Guttman, D. and Nailer G.R.E., 1967 B.M.J. 2 343-345.

CLED AGAR WITH ANDRADE'S INDICATOR

CAT. 1303

Modification of CLED Agar with Andrade's indicator for the cultivation of pathogens from urine specimens

FORMULA IN g/l

Lactose	**10.00**	L-Cystine	**0.128**
Casein Peptone	**4.00**	Andrade's Indicator	**0.10**
Gelatin Peptone	**4.00**	Bromothymol Blue	**0.02**
Beef Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 7.5 ± 0.2 at 25°C			

PREPARATION

Suspend 36.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is dark blue.

The dehydrated medium should be homogeneous, free-flowing and green-beige in color. If there are any physical changes, discard the medium.

USES

CLED AGAR WITH ANDRADE'S INDICATOR is a non-selective solid medium for the cultivation of pathogens from urine specimens. It has a composition similar to CLED Agar, but with Andrade's indicator added. It is based on the principle of lactose fermentation, lowering the pH of the medium with acid production. It improves colony detection and microorganism identification by the addition of the acid fuchsin, which differentiates between lactose fermenting and non-lactose fermenting bacteria.

Beef extract, Casein peptone and Gelatin peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. L-Cystine is added as a growth supplement for cystine-dependent coliforms. Differentiation of lactose-fermenters and lactose non-fermenters is achieved using bromothymol blue and Andrade's indicator as pH indicators. Bacteriological Agar is the solidifying agent.

Inoculate immediately after urine collection and incubate at 35 ± 2°C for no longer than 24 hours, if lactose-fermenters predominate, the whole medium may turn pink, masking the presence of non-lactose fermenters.

CHARACTERISTICS OF THE COLONIES

Escherichia coli:	*Proteus mirabilis:*	*Staphylococcus aureus:*
Bright pink, semi-translucent colonies with surrounding pink halo.	Blue-green, translucent colonies.	*Smooth, entire, opaque bright golden yellow colonies. Lactose fermenting.*

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed for no longer than 24 hours.

Microorganisms	Growth	Colony color
Proteus mirabilis ATCC 10975	Good	Blue-green, translucent
Escherichia coli ATCC 25922	Good	Bright pink, semi-translucent
Staphylococcus aureus ATCC 25923	Good (swarming inhibited)	Golden Yellow

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bevis T.D. (1968) J. Med. Lab. Technol.25.38-41. Furniss A.L., Lee J.V. and Donovan T.J. (1978) P.H.L.S. Monograph series, London, H.M.S.O.,11.

CLOSTRIDIUM DIFFICILE AGAR BASE

CAT. 1447

For the isolation of *Clostridium difficile* when used with supplements

FORMULA IN g/l

Peptone Proteose	40.00	Magnesium Sulfate	0.10
Fructose	6.00	Sodium Chloride	2.00
Disodium Hydrogen Phosphate	5.00	Bacteriological Agar	15.00
Potassium Dihydrogen Phosphate	1.00		

Final pH 7.4± 0.2 at 25°C

PREPARATION

Suspend 34.5 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Clostridium Difficile Supplement (ﬀﬀﬃﬃﬃﬃ),ﬃﬃiously reconstituted in 5 ml of sterile distilled water and 7% sterile defibrinated horse blood. Homogenize gently and dispense into Petri dishes. Be careful to avoid bubble formation when adding the blood and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium with the blood added is red. Sheep blood may be used instead of horse blood, but some strains of the organisms will show a slightly reduced growth recovery.

The dehydrated medium should be homogeneous, free flowing and beige in color. If there are any physical changes, discard the medium.

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(Composition: one vial for 500 ml)

D-Cycloserine............ 125.0 mg
Cefoxitin...........................4.0 mg

USES

CLOSTRIDIUM DIFFICILE AGAR BASE when used with supplement is a selective medium for the isolation of *Clostridium difficile* from fecal specimens.

Clostridium difficile was first isolated from meconium and infant feces in 1935 by Hall and O'Toole, who proposed the name "difficile" because it was very difficult to isolate. Keighley associated *Clostridium difficile* with colitis and diarrhea without pseudomembranous changes after antibiotic therapy following gastrointestinal operations.

Peptone Proteose provides nitrogen, vitamins, minerals and amino acids essential for growth. Fructose is the fermentable carbohydrate used to enhance recovery and growth of *C. difficile.* Potassium dihydrogen phosphate and Disodium hydrogen phosphate act as a buffer system. Magnesium Sulfate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Horse blood provides essential growth factors in Clostridium Difficile Agar. Bacteriologycal Agar is the solidifying agent.

The combination of Clostridium Difficile Agar with the supplement is based on the formulation proposed by George W. L, Sutter V.L., Goldstein E.C.J., Ludwig S.L. and Finegold S.M. The selective agents D-Cycloserine, and Cefoxitin inhibit the growth of most Enterobacteriaceae, as well as *Enterococcus faecalis*, staphylococci, gram-negative, non-sporing anaerobic bacilli, and Clostridia species (except *Clostridium difficile*), which may be found in large quantities in fecal samples.

Incubate at 35 - 37°C and observe after 18 - 48 hours in aerobic conditions. After 48 hours *Clostridium difficile* colonies grow circular, raised, opaque grey, sometimes with irregular borders, and 4 - 6 mm in diameter.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with Clostridium Difficile Supplement (**Cat. 6061**) and 7% defibrinated Horse Blood added, after incubation at a temperature of 35 - 37°C and observed after 18 - 48 hours.

Microorganisms	Growth
Clostridium difficile NCTC 11204	Good
Staphylococcus aureus ATCC 25923	Good
Escherichia coli ATCC 25922	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

George W. L., Sutter V.L., Goldstein E.C.J., Ludwig S.L and Finegold S.M (1978) Lancet. i. 802-803

Hall I. and O'Toole E. (1935) am. J. Dis. Child. 49. 390.

Keighley M.R.B, Burdon D.W., Alexander Williams J. et al (1978) Lancet ii. 1165-1167.

CLOSTRIDIUM PERFRINGENS AGAR BASE (m-CP)

CAT. 1132

For the enumeration and isolation of *Clostridium perfringens* in water samples

FORMULA IN g/l

Tryptose	**30.00**	Magnesium Sulfate $MgSO_4$ $7H_2O$	**0.10**
Yeast Extract	**20.00**	Bromocresol Purple	**0.04**
Sucrose	**5.00**	Bacteriological Agar	**15.00**
L-Cysteine Hydrochloride	**1.00**		
Final pH 7.6 ± 0.2 at 25°C			

PREPARATION

Suspend 71.14 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add the agents described below. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Clostridium Perfringens Supplement (Cat. 6020)
(One vial for 500 ml of the medium)
D-Cycloserine .....200 mg

Other reagents

Polymyxin sulfate .....25 mg

Indoxyl ß - D-glucoside..... 60 mg (dissolved in 8 ml of distilled water).

Phenolphthalein diphosphate ..... 20 ml (0.5% sterile solution).

$FeCl_3$ $6H_20$..... 2 ml (4.5% sterile solution).

Note: * The reconstituted Clostridium Perfringens Supplement (**Cat. 6020**) contains 200 mg of D-Cycloserine

USES

CLOSTRIDIUM PERFRINGENS AGAR BASE (m-CP) is a medium used for the rapid isolation and presumptive identification of *Clostridium perfringens* from water samples. m-CP Agar was first described by Bisson and Cabelli for the rapid quantisation of *Clostridium perfringens* from various water samples (seawater, drinking water and sewage) for human consumption and from environmental water samples. It is recommended in European Council Directive 98/83/EC for testing the quality of water intended for human consumption by the membrane filtration technique.

The medium was shown to give better recovery of *Clostridium perfringens* from water and sewage samples. It can be used for monitoring all types of waters. *C. perfringens* is present in large numbers, in water and sewage and its spores are resistant to wastewater treatment practices, extremes in temperature, and environmental stress. The medium has been recommended for the examination of chlorinated waters and untreated water containing industrial wastes lethal to non-spore forming bacteria, sewage sludge, and situations in which the detection of remote as well as recent pollution is desirable.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sucrose is a complex carbohydrate energy source. L-Cysteine hydrochloride is the reducing agent and Bromocresol purple is the pH indicator. Bacteriological Agar is the solidifying agent.

Filter the water sample using a 0.45 µm membrane, and inoculate and incubate at 44 ± 1°C for 21 ± 3 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with the supplements added, from type cultures after incubation at a temperature of 44 ± 1°C and observed after 21 ± 3 hours.

Microorganisms	Growth	Colony Color
Clostridium perfringens ATCC 13124	Good	Opaque yellow or a color change to pink or red after 20 - 30 seconds exposure to ammonium hydroxide vapors

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Armon, R., and Payment, P., 1988, A modified m-CP medium for enumerating Clostridium perfringens from water samples

BOE Nº 45 DE 21 DE FEBRERO DE 2003

Bisson, J.W., and Cabelli, V.J., 1979, Membrane filter enumeration method for Clostridium perfringens: Applied and Environmental Microbiology, v. 37. no.1. p. 55-66

E.U. (1998) 98/83/EC of Council of 3rd of November 1998 on the quality of water intended for human consumption. Off. J. Eur. Commun., L330, 32-54

COLUMBIA AGAR BASE EUROPEAN PHARMACOPOEIA

CAT. 1104

For the isolation and cultivation of fastidious microorganisms and the determination of hemolytic reactions

FORMULA IN g/l

Pancreatic Digest of Casein	**10.00**	Heart Pancreatic Digest	**3.00**
Yeast Extract	**5.00**	Maize Starch	**1.00**
Meat Peptic Digest	**5.00**	Bacteriological Agar	**13.50**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 42.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The medium is generally enriched with sterile defibrinated blood (sheep, rabbit or horse), serum or some other material. Cool to 45 - 50°C and aseptically add 5 - 10% sterile defibrinated blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium without blood is clear amber, slightly opalescent. The color of the prepared medium with blood is cherry red opaque.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

To make the medium more selective, the following supplements may be added if desired:

CNA Staph/Streph Supplement (**Cat. 6016**) for staphylococci, streptococci and pneumococci isolation

Brucella Supplement (**Cat. 6017**) for *Brucella* isolation

USES

COLUMBIA AGAR BASE is a highly nutritive general purpose medium for the cultivation of fastidious organisms, especially when used as a base for Blood Chocolate Agar. It can also be used as a selective isolation medium by adding antimicrobial agents. Columbia Agar Base is used extensively as a medium base for a variety of culture formulations in medical bacteriology. The hemolytic reactions in blood agar are genuinely defined. The majority of the common pathogenic bacteria, however, grow well without the addition of blood.

Pancreatic digest of casein, Meat peptic digest and Heart pancreatic digest provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract and Maize starch are source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. The blood is another source providing growth factors for the microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary according to the type of blood or base medium used. For example, defibrinated sheep blood gives best results for Group A streptococci.

The European Pharmacopoeia recommends in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" that the Reinforced Clostridial Medium (**Cat. 1007**) should be incubated under anaerobic conditions at 30 - 35°C for 48 hours. After incubation, make subcultures from each tube on Columbia Agar and incubate under anaerobic conditions at 30 - 35°C for 48 hours. The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This should be confirmed by identifications tests.

If no anaerobic growth of microorganisms is detected on Columbia agar or the catalase test is positive, the product complies with the test.

With the addition of 5 - 10% sterile defibrinated blood and, especially with Polyenrichment Supplement (**Cat. 6011**) and VCN Supplement (**Cat. 6013**), whilst the patient is receiving antibiotic treatment, Columbia Agar Base becomes an excellent chocolate agar that can be used to isolate pathogenic *Neisseria*, gonococci and meningococci, as good as or better than Thayer-Martin Medium.

Depending on the selection of inhibitors, alternative supplements to VCN may be VCNT (**Cat. 6026**) or VCAT (**Cat. 6014**).

Inoculate and incubate at 30 - 35, under 5 - 10% CO_2 during 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with 5% sterile defibrinated sheep blood added, from type cultures after incubation at a temperature of 30 - 35°C, under 5 - 10% CO_2, and observed after 48 hours.

Microorganisms	Growth	Hemolysis
Neisseria meningitidis ATCC 13090	Good	———
Staphylococcus aureus ATCC 25923	Good	Beta/Gamma
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta
**Clostridium sporogenes* ATCC 11437	Good	———

*According European Pharmacopoeia incubate at 30 - 35 °C under 5 - 10% CO_2 for 48 - 72 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Ellner, Stossel, Drakeford and Vasi. AM J. Clin. Path. 45:502-504. 1966.

European Pharmacopoeia. 7.0

COLUMBIA BROTH

CAT. 1229

For the cultivation of a wide variety of fastidious microorganisms or as a general use broth

FORMULA IN g/l

Peptone Mixture	**20.00**	TRIS Aminomethane	**0.83**
Sodium Chloride	**5.00**	Sodium Carbonate	**0.60**
Tryptic Digest of Beef Heart	**3.00**	L-Cysteine HCl Hydrochloride	**0.10**
TRIS Aminomethane HCl	**2.86**	Magnesium Sulfate (Anhydrous)	**0.10**
Dextrose	**2.50**	Ferrous Sulfate	**0.02**
Final pH 7.5 ± 0.2 at 25°C			

PREPARATION

Suspend 35 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

COLUMBIA BROTH, being highly nutritive, is used for the cultivation of microorganisms in general, as even the most fastidious grow in it. This medium is prepared according to the formulation described by Morello and Ellner in their study of Columbia Broth.

A medium developed for blood cultures, it is superior to commonly used general purpose broths for faster growth of *Staphylococcus aureus*, *E. coli* and streptococci (*Viridans* and *Enterococcus*). Columbia Broth, in the presence of CO_2 and supplemented with the anticoagulant SPS (Sodium polyanetholsulfonate), is an excellent blood culture medium.

Peptone mixture and Tryptic Digest of Beef Heart provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. L-Cysteine HCl is the reducing agent. The medium is buffered with TRIS Aminomethane and TRIS Aminomethane HCl. Ferrous sulfate is added to facilitate organism growth. Magnesium sulfate is a magnesium ion required in a large variation of enzymatic reactions. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium carbonate is a neutralizer that will absorb any toxic metabolites.

Inoculate and incubate at 35°C ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good
Staphylococcus aureus ATCC 25923	Good
Streptococcus pneumoniae ATCC 6303	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

J.A. Morello & P.D. Ellner (1969). New medium for blood cultures. (Appl. Microb. 17: 68-70) H.D. Isenberg (ed) (1992). Clinical microbiology procedures handbook, vol. 1 (American Soc. for Microbiol., Washington, D.C.) P.R. Murray, E.J. Baron, M.A. Pfaller, et al. Manual of clinical microbiology, 6th ed. (American Soc. for Microbiol., Washington, D.C.)

COLUMBIA CNA AGAR BASE

CAT. 1152

Recommended for the isolation of Gram-positive cocci of clinical samples and other materials when used with blood

FORMULA IN g/l

Peptone Mixture	**20.00**	Nalidixic Acid	**0.015**
Sodium Chloride	**5.00**	Colistin Sulfate	**0.01**
Beef Extract	**3.00**	Bacteriological Agar	**15.00**
Corn Starch	**1.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 44 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. AVOID OVERHEATING. Cool to 45 - 50°C and aseptically add 5 - 10% sterile defibrinated sheep blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood. The prepared medium should be stored at 8 - 15ºC. The color is clear amber, slightly opalescent. The color with blood is red

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

COLUMBIA CNA AGAR BASE is a modification of the Columbia Agar Base with the selective antimicrobial agents Colistin Sulfate and Nalidixic Acid added (CNA). These agents inhibit the growth of Enterobacteriaceae and *Pseudomonas* while they allow the growth of yeast, staphylococci, streptococci and pneumococci.

Colistin breaks the cell membrane of Gram-negative microorganisms, especially *Pseudomonas species*. The Nalidixic acid blocks the DNA replication of susceptible bacteria and acts against many Gram-negative bacteria. The growth of most anaerobic bacteria is promoted by growth nutrients and stimulants such as nitrogen, vitamins, minerals and amino acids contained in the Peptone mixture and Beef extract. Corn Starch increases growth of *Neisseria spp.*, and enhances the hemolytic reactions of some streptococci. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. Blood is an additional source providing the microorganisms with growth factors and is the basis for determining haemolytic reactions. Hemolytic patterns may vary according to the blood or base medium types used. For example, defibrinated sheep blood allows the recovery of *Thermophilus* species and gives best results for Group A streptococci.

Some Gram-negative microorganisms, like *Gardnerella vaginalis*, and some species of bacteria can grow in Columbia CNA Agar with added blood.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed after 18 - 24 hours. Prepared plates with and without 5% sheep blood.

Microorganisms	Growth	Growth with 5% of sheep blood	Hemolysis
Staphylococcus aureus ATCC 25923	Good	Good	Beta
Proteus mirabilis ATCC 12453	Inhibited	Inhibited	
Streptococcus pneumoniae ATCC 6305	Good	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Good	Beta

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ellener, P.C., C.J. Stoessel, E. Drakeford, and F. Vassi 1966. A new culture medium for medical bacteriology. Am J. Clin Pathol. 45:502-504.

Ruoff, k., l. 1995 Streptococcus

CORN MEAL AGAR

CAT. 1164

For chlamydospore production by *Candida albicans* and for the culture of phytopatological fungi

FORMULA IN g/l

Corn Meal Infusion	**2.00**	Bacteriological Agar	**15.00**
Final pH 6.0 ± 0.2 at 25°C			

PREPARATION

Suspend 17 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is opaque and white.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

CORN MEAL AGAR is a general-purpose medium used for the cultivation of fungi.

Candida albicans is the etiological agent in Candidiasis, which ranges from a mild to severe skin, nail, and mucous membrane infections. One of the most important differentiating characteristics of *C. albicans* is its capacity to form chlamydospores on some media. Chlamydospore production is an important characteristic for diagnosis used in the identification of *C. albicans*.

Corn Meal infusion provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent.

Corn Meal is valuable for the morphologic differentiation of many yeast-like organisms. It suppresses the vegetative growth of many fungi and at the same time stimulates the sporulation. Corn Meal Agar allows *Candida albicans* to produce chlamydospores, which is one of the best criterions for identification. Walker and Huppert reported that the addition of 1% Tween 80 enhanced chlamydospore formation.

MICROBIOLOGICAL TEST

The following results were obtained of the medium from type cultures after incubation at a temperature of 25 ± 2°C and observed after 48 - 60 hours.

Microorganisms	Growth	Chlamydospores
Candida albicans ATCC 10231	Good	+
Aspergillus brasiliensis ATCC 16404	Good	-
Saccharomyces cerevisiae ATCC 9763	Good	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

McGinnis. 1980. Laboratory handbook of medical mycology. Academic Press, New York, N.Y.

Walker and Huppert. 1960. Tech. Bull. Reg. Med. Technol. 30:10.

Haley and Callaway. 1978. Laboratory methods in medical mycology. HEW Publication No. (CDC) 78-8361. Center for Disease Control, Atlanta, Ga.

Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Campbell and Stewart. 1980. The medical mycology handbook. John Wiley & Sons, New York,N.Y.

CTA MEDIUM

CAT. 1502

For the maintenance of strains and in motility and carbohydrate fermentation studies

FORMULA IN g/l

Casein Peptone	**20.00**	Yeast Extract	**0.20**
Sodium Chloride	**5.00**	Phenol red	**0.017**
L-Cystine	**0.50**	Bacteriological Agar	**2.50**
Sodium Sulfite	**0.50**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 28.5 grams of the medium in one liter of distilled water. If desired, add 0.5 to 1.0% carbohydrate for a specific fermentation test. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118°C for 15 minutes. Cool to 50°C, mix well and dispense into tubes. The prepared medium should be stored at 2 - 8°C. The color is red to pink.

The dehydrated medium should be homogeneous, free-flowing and beige pink in color. If there are any physical changes, discard the medium.

USES

CTA MEDIUM (Cystine Tryptic Agar) is a nutrient base, semisolid medium which contains peptones rich in tryptophane and vitamins. It is used for the determination of motility of fastidious microorganisms, for fermentation tests with the addition of carbohydrates and for the classification of yeasts, being able to determine fermentation reactions of fastidious microorganisms, e.g. pathogenic *Neisseria*.

Uninoculated Tube | *Escherichia coli* ATCC 25923 | *Staphylococcus aureus* ATCC 25923

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. L-Cystine and Sodium sulfite are the reducing agents. Phenol red is the pH indicator. Bacteriological agar is the solidifying agent.

The semisolid consistency of the medium is suitable for detecting the motility of some microbes. With the addition of a 1% concentration of a specific carbohydrate, it is recommended for the differentiation of fastidious microorganisms by means of fermentation reactions. Without the addition of carbohydrates, it is recommended as a holding medium for fastidious microorganisms at 25°C.

The fastidious organisms such as *Neisseria, Pasteurella,* pneumococci, streptococci, *Brucella, Corynebacteria*, and *Vibrio* grow well in CTA Medium without adding carbon dioxide, serum, or any other enrichment substances.

The stabbed cultures of motile organisms grow out from the line of inoculation. The non-motile microorganisms remain only within the inoculated area, while the surrounding agar remains clear.

For fermentation tests with members of *Neisseria*, inoculate the surface of the tubes only. *Neisseria species* usually produce acid in the area of stabs (upper third) only. If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present. If in doubt about a tube containing a *Neisseria* species, a Gram stain and oxidase test should be performed on the growth.

The facultative microorganisms such as streptococci and strictly anaerobic microorganisms can be inoculated by stabbing at half the depth of the tube.

The acid reactions can be easily observed as the produced acid does not spread immediately throughout the entire tube. The majority of cultures display an alkaline reaction when there is no fermentable carbohydrate present.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Motility
Escherichia coli ATCC 25922	Good	+
Staphylococcus aureus ATCC 25923	Good	-
Neisseria gonorrhoeae ATCC 19424	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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CZAPEK-DOX MODIFIED AGAR

CAT. 1015

For the cultivation of fungi and bacteria using sodium nitrate as a sole source of nitrogen

FORMULA IN g/l

Sucrose	**30.00**	Potassium Sulfate	**0.35**
Sodium Nitrate	**2.00**	Ferrous Sulfate	**0.01**
Magnesium Glycero-phosphate	**0.50**	Bacteriological Agar	**12.00**
Potassium Chloride	**0.50**		
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 45.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent with uniform flocculent precipitate.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

CZAPEK-DOX MODIFIED AGAR is commonly used for the cultivation of fungi and chlamydospore formation by *C. albicans*. For the cultivation of acidophilic organisms, such as yeasts, the acidity of the medium may be increased. It is also used for taxonomic studies of *Aspergillus, Penicillium* and *Actinomycetes*.

Czapek-Dox Modified Agar is a semi-synthetic medium, which contains sodium nitrate as a sole source of nitrogen. It has the advantage of a chemically defined formulation, which has been modified in its original formula by substituting magnesium sulfate and potassium phosphate with the magnesium glycerophosphate in this formula to prevent the precipitation of magnesium phosphate. The medium is prepared with inorganic nitrogen sources and chemically defined carbon sources only.

Sucrose is the sole fermentable carbohydrate providing carbon and energy. Sodium Nitrate is the sole nitrogen source. Potassium salts act as a buffer system. Potassium chloride contains essential ions. Magnesium glycerophosphate and Ferrous sulfate are sources of cations. Bacteriological agar is the solidifying agent.

In general, the medium should be cooled to 45 - 50°C before pouring in order to avoid excess water moisture on the plates. Dispense approximately 12 ml in a 90 mm diameter Petri dish. Store the plates in an inverted position. Inoculate with a straight needle, taking the precaution to invert the plates in order to protect the medium surface from airborne spores.

Time and temperature of incubation vary considerably according to the fungi. As a general rule, incubate for 1-2 weeks at room temperature (approximately 25°C). Most *Penicillium* grow best between 20 - 25°C; *Aspergillus* species grow well at around 30°C, but *Aspergillus fumigatus* grows well at 50°C and *C. albicans* at 25°C during 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30°C and observed after 1 - 5 days.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Saccharomyces cerevisiae ATCC 9763	Good
Bacillus subtilis ATCC 6633	Moderate
Candida albicans ATCC 10231	Good
Staphylococcus aureus ATCC 25923	Moderate

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Thom and Raper. Manual of Aspergilli. Williams and Wilkins Co., Baltimore, MD 1945.

Smith G. An Introduction to Industrial Mycology 5th Ed. Arnold LR London, 1960.

CZAPEK-DOX MODIFIED BROTH

CAT. 1250

For the cultivation of fungi and bacteria using sodium nitrate as a sole source of nitrogen

FORMULA IN g/l

Sucrose	**30.00**	Potassium Chloride	**0.50**
Sodium Nitrate	**3.00**	Magnesium Glycero-phosphate	**0.50**
Dipotassium Sulfate	**1.00**	Ferrous Sulfate	**0.01**
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 35 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is colorless, and may present a slight precipitate.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

CZAPEK-DOX MODIFIED BROTH is commonly used for the cultivation of fungi and *Candida albicans*.

It is similar to Czapek-Dox Modified Agar (**Cat. 1015**), without the agar, and is used to grow bacteria and fungi, which are capable of using Sodium nitrate as a sole source of nitrogen.

Czapek-Dox Modified Broth is a semi-synthetic medium containing Sodium nitrate as a sole source of nitrogen. It has the advantage of a chemically defined formulation, which has been modified in its original formula by substituting Magnesium sulfate and Potassium phosphate with the Magnesium glycerophosphate to prevent the precipitation of Magnesium phosphate. The medium is elaborated with inorganic sources of nitrogen and chemically defined sources of carbon only. It is useful in a variety of microbiological procedures, including soil microbiology, and fungi and mold resistance tests. This medium will yield moderately good growth of most saprophytic Aspergilli.

Sucrose is the sole fermentable carbohydrate providing carbon and energy. Sodium nitrate is the sole nitrogen source. Potassium salts act as a buffer system. Potassium chloride contains essential

ions. Magnesium glycerophosphate and Ferrous sulfate are sources of cations.

Times and temperatures of incubation vary considerably according to the fungi. As a general rule, incubate from 1 - 2 weeks at room temperature (approximately 25°C). Most *Penicillium* grow best between 20 - 25°C; *Aspergillus* species grow well at around 30°C, but *Aspergillus fumigatus* grows well at 50°C, and *C. albicans* at 25°C during 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 2°C and observed after 1 to 5 days.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Saccharomyces cerevisiae ATCC 9763	Good
Bacillus subtilis ATCC 6633	Moderate
Candida albicans ATCC 10231	Good
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Thom y Raper. Manual of Aspergilli. Williams and Wilkins Co. Baltimore Md. 1945.

Smith G. An Introduction to Industrial Mycology 5th Ed Arnold LR London 1960.

DCLS AGAR (DESOXYCHOLATE, CITRATE, LACTOSE, SUCROSE)

CAT. 1045

Moderately selective medium for the isolation of *Salmonella* and *Shigella* from fecal specimens and urine

FORMULA IN g/l

Sodium Citrate	**10.50**	Beef Extract	**3.00**
Proteose Peptone	**7.00**	Sodium Desoxycholate	**2.50**
Sodium Thiosulfate	**5.00**	Neutral Red	**0.03**
Lactose	**5.00**	Bacteriological Agar	**12.00**
Sucrose	**5.00**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is orange-red.

The dehydrated medium should be homogeneous, free-flowing and pink-beige in color. If there are any physical changes, discard the medium.

USES

DCLS AGAR is a selective medium for the primary isolation of *Salmonella* and *Shigella* from fecal specimens and urine.

The Gram-positive organisms, coliforms and *Proteus* are completely or partially inhibited by Sodium citrate, Sodium thiosulfate and Sodium desoxycholate. Proteose Peptone and Beef extract provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose and Sucrose are the fermentable carbohydrates, providing carbon and energy. Neutral red is the pH indicator. Bacteriological agar is the solidifying agent.

It can be used with direct streaking or with an enrichment for *Salmonella* in Sodium Selenite Broth (**Cat. 1222**) or Selenite Cystine Broth (**Cat. 1220**). It is preferable to inoculate in duplicate: one heavily and the other diluted. Incubation is for 24 hours at 35 ± 2°C. If negative, reincubate for another 24 hours.

The presence of two carbohydrates in the formulation assures the formation of red colonies of those organisms, which ferment one or both of the carbohydrates.

Red colonies: Coliforms

Transparent colonies, colorless to slightly pink: *Salmonella, Shigella*

The majority of *Shigella* organisms yield colorless colonies, but some strains of *S. flexneri*, as well as other species of *Shigella*, grow rapidly giving colonies that are a weak pink but are distinguished easily from *Proteus* or the coliforms. If *Salmonella* or *Shigella* are suspected, the colonies should be subcultured on other media for identification, such as Kligler Iron Agar (**Cat. 1042**), Nitrate Motility Medium Base (**Cat. 1565**) or Triple Sugar Iron Agar (**Cat. 1046**)

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color	Precipitate
Escherichia coli ATCC 25922	Inhibited	Pink-red	-
Salmonella typhimurium ATCC 14028	Good	Colorless/ pale pink	-

Microorganisms	Growth	Colony Color	Precipitate
Salmonella choleraesuis ATCC 13312	Good	Colorless/ pale pink	-
Salmonella enteritidis ATCC 13076	Good	Colorless/ pale pink	-
Proteus vulgaris ATCC 13315	Moderate	Colorless/ pink	±

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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DESOXYCHOLATE AGAR

CAT. 1020

For the isolation and differentiation of Gram-negative enteric bacilli

FORMULA IN g/l

Peptone Mixture	**10.00**	Sodium Desoxycholate	**1.00**
Lactose	**10.00**	Ferric Ammonium Citrate	**1.00**
Sodium Chloride	**5.00**	Neutral Red	**0.033**
Dipotassium Phosphate	**2.00**	Bacteriological Agar	**16.00**
Sodium Citrate	**1.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 46 grams of the medium in one liter of distilled water. Soak for 10 - 15 minutes. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and pinkish beige in color. If there are any physical changes, discard the medium.

NOTE: Overheating may increase the degree of inhibition.

USES

DESOXYCHOLATE AGAR is a selective and differential medium for the isolation and differentiation of Gram- negative enteric bacilli. Leifson demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora.

The Desoxycholate and Citrate salts inhibit the development of the Gram-positive organisms. The Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Dipotassium phosphate acts as a buffer system. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Neutral red is a pH indicator. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours. The recovery of organisms is sometimes facilitated by adding a thin layer over the inoculated and solidified agar. Differentiation of enteric bacilli is based on the fermentation of lactose. Lactose fermenters acidify the medium and, under Neutral red, form red or pink colonies. The colonies of the microorganisms which do not ferment lactose such as *Salmonella, Shigella* and *Proteus* are colorless.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18- 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Pink with bile precipitate
Salmonella typhimurium ATCC 14028	Good	Colorless
Staphylococcus aureus ATCC 25923	Inhibited	—

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Standard Methods for the Examination of Dairy Products. 1 ed. APHA, Inc. New York, 1960. Standard Methods for the Examination of Water and Wastewater, APHA, Inc. New York, 1 960.

DESOXYCHOLATE CITRATE AGAR EUROPEAN PHARMACOPOEIA

CAT. 1067

Moderately selective and differential medium for the isolation of enteric pathogens, especially *Salmonella* and many *Shigella* species

FORMULA IN g/l

Sodium Citrate	**20.00**	Sodium Desoxycholate	**5.00**
Lactose Monohydrate	**10.00**	Ferric Citrate	**1.00**
Meat Peptone	**10.00**	Neutral Red	**0.02**
Beef Extract	**10.00**	Bacteriological Agar	**13.50**
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 69.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and pinkish-beige in color. If there are any physical changes, discard the medium.

Salmonella typhimurium
ATCC 14028

USES

DESOXYCHOLATE CITRATE AGAR is a modification of Desoxycholate Agar Leifson's formula and is ideal for the investigation of pathogenic *Enterobacteria* in highly contaminated foods. It is particularly useful in isolating *Salmonella* and many *Shigella spp.*

The Gram-positive organisms, coliforms and many *Proteus spp.* are highly inhibited by the increased concentration of Sodium citrate and Sodium desoxycholate. Ferric citrate aids in the detection of H_2S production. Meat peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate. Neutral red is a pH indicator. Bacteriological agar is the solidifying agent.

It is used in conjunction with Brilliant Green Tetrathionate Bile Broth (**Cat. 1253**) as a confirmation of *Salmonella spp.* Inoculate and incubate at 35 ± 2 °C for 18 - 24 hours. It is recommended to seed the sample heavily on the plate. Lactose-fermenting bacteria form red colonies in the presence of Neutral red. Lactose-fermenting colonies may have a desoxycholate precipitation zone around them. Lactose non-fermenters will appear as colorless colonies. H_2S producers will have black centers. *Salmonella typhi*, *S. paratyphi* and *Shigella* types yield well-developed colorless colonies while lactose-positive organisms like *Escherichia coli* are pink to red. A previous enrichment in Selenite Cystine Broth (**Cat. 1220**) or Sodium Selenite Broth (**Cat. 1222**) can also be used.

The European Pharmacopoeia recommends in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" to subculture in this medium after incubation in Brilliant Green Tetrathionate Bile Broth (**Cat. 1253**), at 41 - 43°C for 18 - 24 hours and incubate at 35 - 37 °C for 18 - 72 hours. The probable presence of salmonellae is indicated by the growth of cultures having the following appearance in this medium: well-developed and colourless colonies. Precise confirmation may be carried out by appropriate biochemical and serological test. The product passes the test if colonies of the type described do not appear or if the confirmatory biochemical and serological test are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color	H_2S
Escherichia coli ATCC 25922	Partially Inhibited	Pink with bile precipitate	-
Salmonella enteritidis ATCC 13076	Good	Colorless	+
**Salmonella typhimurium* ATCC 14028	Good	Colorless	+
Shigella flexneri ATCC 12022	Moderate	Colorless	-
Enterococcus faecalis ATCC 19433	Inhibited	-	-

*According to European Pharmacopeis incubate at 35 - 37°C for 18 - 72 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

European Pharmacopoeia, 6th Ed. 2007

Leifson E. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. 40: 581-599.

Farmer III, J.J. and MT. Kelly. 1991 Enterobacteriaceae. P. 360-383. In A. Balows, W. J. Hausler, Jr., K.L. Hermann, H.D. Isenberg and H.J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology.

DESOXYCHOLATE LACTOSE AGAR

CAT. 1025

Differential and slightly selective medium for the isolation of Gram-negative enteric bacilli

FORMULA IN g/l

Bacteriological Peptone	**10.00**	Sodium Desoxycholate	**0.50**
Lactose	**10.00**	Neutral Red	**0.03**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Sodium Citrate	**2.00**		
Final pH 7.1 ± 0.2 at 25°C			

PREPARATION

Suspend 42.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is red-orange. Prepared plates may present a slight precipitate.

The dehydrated medium should be homogeneous, free-flowing and pink-beige in color. If there are any physical changes, discard the medium.

NOTE: Overheating may increase the degree of inhibition.

USES

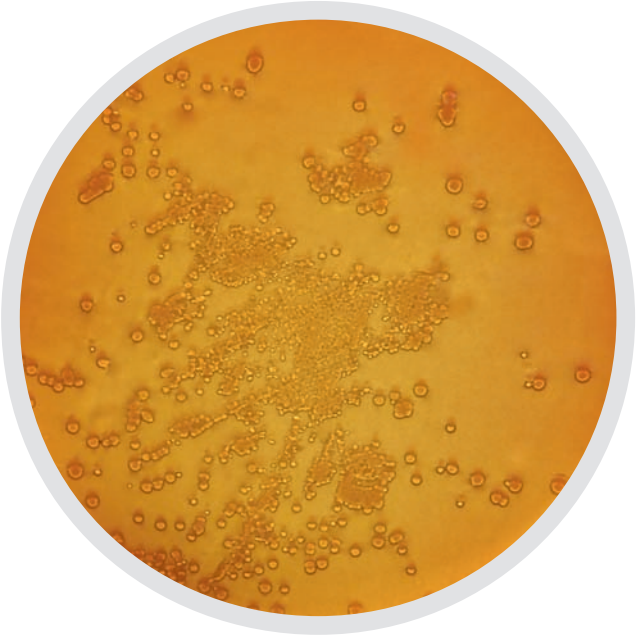
DESOXYCHOLATE LACTOSE AGAR is a differential and slightly selective medium for the isolation of Gram-negative enteric bacilli. It is used to isolate and enumerate coliforms from water, wastewater, milk and dairy products.

The Desoxycholate and Citrate salts inhibit the development of Gram-positive organisms. The Bacteriological peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Neutral red is a pH indicator. Bacteriological agar is the solidifying agent.

In general, it is used for the enumeration of coliforms by the dilution method. This is accomplished by adding 1 ml of the desired dilution to an empty Petri dish and pouring it on the cooled (45 - 50°C) medium. If the product to be tested has not been diluted (e.g. pasteurized milk), it can be added directly to the melted medium and poured plates.

It is convenient to put a second layer of medium on the plate after initial solidification. Incubate at 35 ± 2°C for 18 - 24 hours.

Coliform colonies are lenticular, pink or bright red. Differentiation is made on the basis of the lactose fermentation: lactose fermenters in the presence of neutral red give red colonies while non-fermenters give colorless colonies (*Salmonella* and *Shigella*). If no second layer is applied, the colonies of *Escherichia coli* which develop on the surface of the plate are large and pink while Enterobacter aerogenes are pale with a pink center.



Escherichia coli
ATCC 25922

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color	Precipitate
Escherichia coli ATCC 25922	Good	Red	+
Klebsiella pneumoniae ATCC 13883	Good	Red	+
Enterobacter cloacae ATCC 13047	Good	Pink	±
Salmonella typhimurium ATCC 14028	Good	Colorless	-

Microorganisms	Growth	Colony Color	Precipitate
Shigella flexneri ATCC 12022	Good	Colorless	-
Enterococcus faecalis ATCC 11700	Inhibited	—	-
Staphylococcus aureus ATCC 12923	Null		-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Standard Methods for the Examination of Dairy Products. Eleventh Edition APHA Inc. New York 1960.

Recommended Methods for the Microbiological Examination of Foods APHA Inc. New York 1960.

American Public Health Association. 1960. Standard methods for the examination of water and wastewater, 1 1th ed. American Public Health Association, Washington, D.C.

DEXTROSE AGAR

CAT. 1021

For the cultivation of a wide variety of microorganisms with or without blood, and for the general laboratory purposes

FORMULA IN g/l

Peptone Mixture	**10.00**	Beef Extract	**3.00**
Dextrose	**10.00**	Bacteriological Agar	**15.00**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 43 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

NOTE: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45 - 50°C.

USES

DEXTROSE AGAR is a medium suitable to cultivate a wide variety of microorganisms with or without added blood. The high dextrose concentration yields abundant growth in a shorter period of time than other media as well as lessening the less time than other media and shortens lag period of older cultures. Although is a medium for general use, it is not appropriate for haemolyses' studies due to the high content of dextrose.

The Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth.

Dextrose is the fermentable carbohydrate, providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 48 hours.

The addition of 5% defibrinated blood allows the isolation of many fastidious bacteria, including *Haemophilus* and *Neisseria*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good
Staphylococcus aureus ATCC 25923	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Recommended Methods for the Microbiological Examination of Foods APHA Inc., New York.

Compendium of Methods for the Microbiological Examinations of Food. 3rd edition APHA 1992.

DEXTROSE BROTH (GLUCOSE BROTH)

CAT. 1203

For the cultivation of fastidious microorganisms and for the study of glucose fermentation

FORMULA IN g/l

Peptone	**10.00**	Sodium Chloride	**5.00**
Dextrose	**10.00**	Beef Extract	**3.00**
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 28 grams of the medium in one liter of distilled water. If desired, add 0.1 - 0.2 % of bacteriological agar. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

DEXTROSE BROTH (GLUCOSE BROTH), being highly nutritious, is used to cultivate fastidious microorganisms and specimens containing a low inoculum, as well as to detect gas formation from enteric bacilli through dextrose fermentation.

Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate, providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Adding 0.1 - 0.2% agar to Dextrose Broth helps anaerobic growth, and the dispersion of reducing substances and CO_2 formed in the environment. The low agar concentration is suitable for aerobic growth, in the clear upper zone, and for microaerophilic and anaerobic growth in the lower, flocculent agar zones.

Inoculate medium, with or without the agar added, and incubate at 35 ± 2°C. Read growth and gas production is at 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Gas Production
Shigella flexneri ATCC 12022	Good	-
Escherichia coli ATCC 25922	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Norton, 1932. Bacteriology of pus. J. Lab. Clin. Med.

MacFaddin J.D. 1985 Media for isolation cultivation identification maintenance of medical bacteria. Williams & Wilking, Baltimore. MD.

DICHLORAN GLYCEROL AGAR (DG 18) ISO 21527-2

CAT. 1161

For the enumeration and isolation of xerophilic fungi in dry and semi-dry foods

FORMULA IN g/l

Dextrose	**10.00**	Chloramphenicol	**0.10**
Peptone	**5.00**	Dichloran	**0.002**
Monopotassium Phosphate	**1.00**	Bacteriological Agar	**15.00**
Magnesium Sulfate	**0.50**		
Final pH 5.6 ± 0.2 at 25°C			

PREPARATION

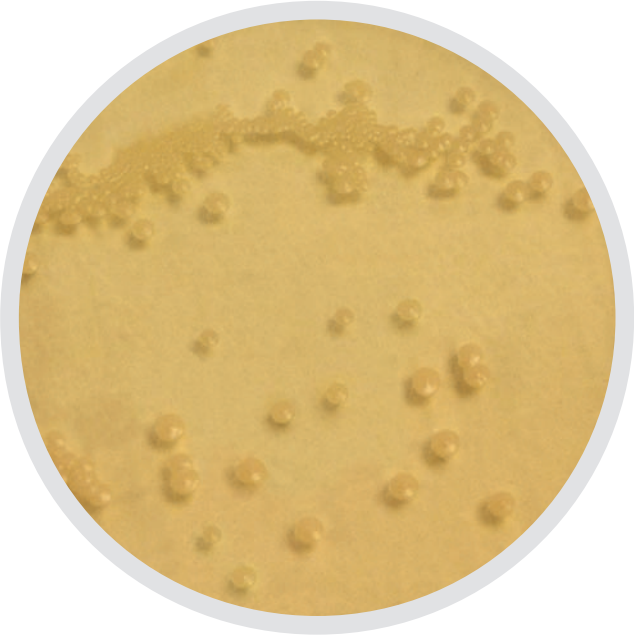
Suspend 31.6 grams of the medium in one liter of distilled water. Add 175 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and pour into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

DICHLORAN GLYCEROL AGAR BASE (DG 18) is a selective medium based on the formulation of Hocking and Pitt. It is recommended for the enumeration and isolation of xerophilic molds from dried and semi-dried foods, such as fruits, spices, cereals, nuts, meat and fish products

Glycerol reduces the water activity from 0.999 to 0.95, thereby reducing bacterial growth, and is also the carbon source. Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide



Saccharomyces cerevisiae
ATCC 9763

bacterial spectrum. Bacteria growth inhibition and spreading of more-rapidly growing molds restriction aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. Dichloran prevents the fast spreading of mucoraceous fungi, improving the colony count. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphate acts as a buffer system. Magnesium sulfate provides sulfur and other trace elements. Bacteriological agar is the solidifying agent.

ISO 21527 recommends the use of two different plates. The first should be spread by transferring 0.1 ml of the test sample, if the sample is liquid, or 0.1 ml of the initial suspension, if the sample is not liquid, to the plate. The second plate must be spread by transferring to it 0.1 ml of the first decimal dilution (10^{-1}), if liquid, or 0.1 ml of the 10^{2} dilution, if not. Inoculate and incubate at 25 ± 1°C and examine for growth after 5 - 7 days. If the presence of *Xeromyces bisporus* is suspected, incubate the plates for 10 days. After incubation select the dishes containing < 150 colonies and count them. If fast growing molds are a problem, count colonies after 2 days and again after 5 - 7 days of incubation. This number can be reported as number of xerophilic colonies per gram of food.

Other studies have shown this medium to be a good general purpose one, giving good results for yeasts and molds isolated from foods.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature 25 ± 1°C and observed after 5 - 7 days.

Microorganisms	Growth
Saccharomyces cerevisiae ATCC 9763	Good
Rhodotorula mucilaginosa DMS 70403	Good (orange)
Mucor racemosus ATCC 42647	Moderate
Bacillus subtilis ATCC 6633	Inhibited
Escherichia coli ATCC 25922	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 21527-2: Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 2: Colony count technique in products with water activity less than or equal to 0.95

Hocking, A.D.,and Pitt,J.L. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Enviornm. Microbiol 39, 488-492.

DIFFERENTIAL REINFORCED CLOSTRIDIAL BROTH (DRCM)

CAT. 1416

For the enumeration of all clostridia by the MPN method in food, water and other material

FORMULA IN g/l

Beef Extract	**8.00**	Yeast Extract	**1.00**
Meat Peptone	**5.00**	L-Cysteine Hydrochloride	**0.50**
Casein Peptone	**5.00**	Sodium Disulfite	**0.50**
Sodium Acetate	**5.00**	Ferric Ammonium Citrate	**0.50**
Starch	**1.00**	Resazurin Sodium Salt	**0.002**
Glucose	**1.00**		

Final pH 7.1 ± 0.2 at 25°C

PREPARATION

Suspend 27.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is reddish-brown.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

DIFFERENTIAL REINFORCED CLOSTRIDIAL BROTH (DRCM) is used to determine the count of sulfite-reducing bacteria by the MPN technique.

Beef extract, Meat peptone and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Glucose is the fermentable carbohydrate providing carbon and energy. L-Cysteine hydrochloride is the reducing agent. Starch absorbs any toxic metabolites produced. Resazurin is an oxidation indicator, turning from pink (aerobic) to colorless (anaerobic conditions), used as an indicator to monitor anaerobiosis. Ferric ammonium citrate and Sodium disulfite are H_2S indicators.

Clostridia reduce sulfite to sulfide, the iron sulfide produced causes the culture medium to turn black. As other bacteria can also produce sulfide, vegetative forms must first be removed from the culture by a relevant treatment (e.g. pasteurization), and the anaerobic spore-forming microorganisms must then be identified. To inhibit the growth of most non-spore-forming microorganisms add 70 IU/ ml polymyxin to the broth.

Incubate at 30°C and observe after 4 - 7 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 4 - 7 days.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	-
Bacillus cereus ATCC 11778	Moderate	-
Clostridium perfringens ATCC 10543	Good	+
Clostridium perfringens ATCC 13124	Good	+
Clostridium sporogenes ATCC 19404	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

GIBBS, B.M.: The detection of Clostridium welchii in the Differential Clostridial Medium technique. - J. Appl. Bact., 36; 23-33 (1973).

HIRSCH, A., a. GRINSTED, E.: Methods for the growth and enumeration of anaerobic spore-formers from cheese, with observations on the effect on nisin. - J. Dairy Res., 21; 101-110 (1954).

DNase TEST AGAR (DEOXYRIBONUCLEASE ACTIVITY)

CAT. 1028

For the detection of deoxyribonuclease activity to aid in the identification of bacteria isolated from clinical specimens

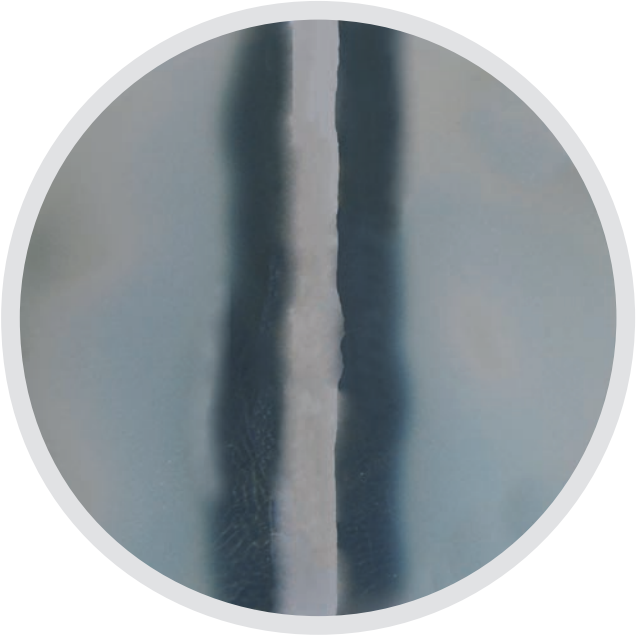
FORMULA IN g/l

Casein Peptone	**15.00**	Deoxyribonucleic Acid	**2.00**
Soy Peptone	**5.00**	Bacteriological Agar	**15.00**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 42 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.



Staphylococcus aureus
ATCC 25923

USES

DNase TEST AGAR is used to differentiate microorganisms using correlation between coagulase positive and DNase activity. This differential medium is especially recommended for the identification of pathogenic staphylococci.

Casein peptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Deoxyribonucleic Acid enables the detection of DNase that depolymerize DNA. Bacteriological agar is the solidifying agent.

Inoculate making a heavy band streak (2 cm in length) of the test organism on the surface of the plate. You can simultaneously place 4 to 5 different samples on the same plate. Incubate for 18 - 24 hours at 35 ± 2°C. After satisfactory growth, add a drop of 1N hydrochloric acid or a few drops of 0.1% toluidine blue solution. With some strains it is necessary to increase the concentration of HCl to 2N to obtain a good positive reaction. In the presence of diluted hydrochloric acid, the reaction with DNA in the culture medium forms a hazy precipitate. Growth with deoxyribonuclease production appear surrounded by a zone or a clear halo containing fractions of soluble nucleotides from the degradation of DNA, which are not precipitated by the hydrochloric acid. Toluidine Blue form colored complexes with polymerised DNA.

Results:

In the presence of hydrochloric acid:

DNase-positive: when there is clear zone surrounding the inoculum, streak with the rest of the plate remaining opaque. The positive reaction takes approximately 5 minutes to form.

DNase-negative: Absence of a clear halo around the inoculum streak.

In the presence of toluidine blue:

DNase-positive: Appearance of a pink halo surrounding the inoculum streak. The rest of the plate remains blue.

DNase-negative: Absence of the pink halo surrounding the inoculum streak.

Nevertheless, for some fastidious organisms it may be necessary to add blood. The addition of diluted hydrochloric acid forms a well defined but opaque halo with DNase-positive organisms. The DNase medium with blood should not be used in the study of hemolytic reactions and should only be added if absolutely necessary.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	DNase Test Transparency
Staphylococcus epidermidis ATCC 12228	Good	-
Staphylococcus aureus ATCC 6538	Good	+
Staphylococcus aureus ATCC 25923	Good	+
Serratia marcescens ATCC 8100	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Blair E.B. Emerson, J.S. and Tull, S.C. Am. J.Clin.Poth, 47:30-39, 1957. Disalvo Med. Tech. Bull. 9:191. 1958.

Weckman and Catting J. Bact. 73: 747. 1957.

E. COLI COLIFORMS CHROMOGENIC MEDIUM

CAT. 1340

Selective medium for the simultaneous detection of *E. coli* and other coliforms in water and food samples

FORMULA IN g/l

Sodium Chloride	**5.00**	Sorbitol	**1.00**
Phosphate Buffer	**4.90**	Chromogenic Mixture	**0.36**
Bacteriological Peptone	**3.00**	Tergitol-7	**0.10**
Sodium Pyruvate	**1.00**	Bacteriological Agar	**10.00**
Tryptophan	**1.00**		
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 26.4 grams of the medium in one liter ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Allow to cool at 45 - 50°C and dispense in Petri dishes. The prepared medium should be stored at 8 - 15ºC, protected from light. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

E. COLI-COLIFORMS CHROMOGENIC MEDIUM is a selective medium for the detection of *E. coli* and other coliforms in waters and foods.

The interaction of ingredients in the medium, such as peptone, sorbitol, etc, grants a quick colony growth, including infectious ctoliforms. Tergitol-7 inhibits Gram-positive bacteria. Sodium chloride maintains the osmotic balance and the Phosphate

is the buffer. Bacteriological agar is the solidifying agent. The chromogenic mixture contains chromogenic substrates as Salmon-GAL and X-glucuronide. Coliform enzymes produced, such as galactosidase and glucuronidase, cleave these substrates, resulting in the different coloration of certain bacteria colonies.

The ß-D-galactosidase cleaves Salmon-GAL substrate, and gives a salmon to red color to the coliform colonies.

The cleaves both substrates Salmon-Gal and X-glucuronide, giving a dark blue to violet color to the colonies, easily distinguishable from other coliform colonies that have a salmon to red color.

The addition of tryptophan to the medium allows the performance of the Indole test for further *E. coli* confirmation.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

NOTE: Some *Shigella* strains contains the enzyme ß-D-glucuronidase and can grow as light blue colonies. The negative *E. coli* b-ß-glucuronidase colonies are salmon-pink, e.g. *E. coli* O157:H7.

Escherichia coli ATCC 25922 *Citrobacter freundii* ATCC 8090

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Blue-dark violet
Escherichia coli ATCC 11775	Good	Blue-dark violet
Citrobacter freundii ATCC 8090	Good	Salmon
Salmonella enteritidis ATCC 13076	Good	Colorless
Enterococcus faecalis ATCC 19433	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Alonso, J.L. Soriano, K., Amoros I., Ferrus, M.A. 1998 Cevartitatine determination of E. coli and fecal coliforms in water using a chromogenic medium.

J. Environ. Sci Health 33.

EC MEDIUM ISO 7251

CAT. 1522

For the detection and enumeration of coliform organisms in water

FORMULA IN g/l

Tryptose	**20.00**	Dipotassium Phosphate	**4.00**
Lactose	**5.00**	Bile Salts n° 3	**1.90**
Sodium Chloride	**5.00**	Monopotassium Phosphate	**1.50**
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 37.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in tubes with Durham gas collecting vials for gas detection and boil for 5 minutes. Sterilize in autoclave at 121°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8 °C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

EC MEDIUM was developed by Hajna and Perry, for the selective identification of coliform bacteria and *Escherichia coli* in water, foodstuffs and other materials. It is recommended by ISO 7251, for the enumeration of *E. coli* with MPN tecnique.

This medium improves the detection methods of the coliform group, in particular of *E. coli*, and is used to investigate drinking water, wastewater treatment systems and generally for water-quality monitoring, as well as shellfish and other foods. It is used in many Standard Methods for Food and Water testing.

The medium can be used at 35 ± 2°C for detection of coliform organisms or at 44.5°C for isolation of *E. coli*.

The Bile salts act as selective agent inhibiting Gram-positive bacteria, bacilli and enterococci but allowing *E. coli* to develop. The Potassium salts have a high buffering capacity. Tryptose provides the nutrients for growth and Lactose is the fermentable carbohydrate as carbon and energy source. Sodium chloride maintains the osmotic balance.

Inoculate sample into tubes and incubate at 37°C for 24 hours. Lactose fermentation with gas production is evidence of the presence of coliforms. If growth from positive tubes is re-inoculated and re-incubated at 44.5°C for 24 hours, and yields positive growth, confirmation of *E. coli* can then be made by using the appropriate biochemical tests (indole, citrate, etc.).

Formation of gas at 37°C …… coliforms.

Formation of gas at 37°C & 44.5°C …….. *E. coli*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 44.5 ± 2°C and observed after 24 ± 2 hours.

E.coli ATCC 25922

Enterococcus faecalis ATCC 19433

Microorganisms	Growth	Gas production
Bacillus subtilis ATCC 6633	Inhibited	-
Enterobacter aerogenes ATCC 13048	Inhibited	-
Escherichia coli ATCC 25922	Good	+
Enterococcus faecalis ATCC 19433	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Hajna and Perry 1944 A.P.H.A.

Ray B. 1986 Impact of bacterial injury and repair in food microbiology. Its past, present and future J. Food Prot.

ISO 7251 Microbiology -- General guidance for enumeration of presumptive Escherichia coli -- Most probable number technique

EE BROTH ISO 21528-1

CAT. 1362

For the detection and enumeration of Enterobacteriaceae by the MPN method

FORMULA IN g/l

Dehydrated Ox Bile	**20.00**	Glucose Monohydrate	**5.00**
Pancreatic Digest of Gelatin	**10.00**	Potassium Dihydrogen Phosphate	**2.00**
Disodium Hydrogen Phosphate	**6.45**	Brilliant Green	**0.0135**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 43.5 grams of the medium in one liter of distilled water. Dispense into appropriate containers and sterilize as soon as possible, either by using vapor flow during 10 minutes, or in autoclave at 121°C for 5 minutes. Cool immediately under tap water without contaminating the medium. The prepared medium should be stored at 2 - 8°C. The color is green.

The dehydrated medium should be homogeneous, free-flowing and light green in color. If there are any physical changes, discard the medium.

USES

EE BROTH is a medium recommended by the ISO normative 21528 for the detection and enumeration of Enterobacteriaceae by the MPN method (Most probable number method).

Pancreatic digest of gelatin and Glucose are the nitrogen and energy sources. Dehydrated ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria. Sodium phosphate and potassium phosphate act as a buffer system.

ISO 21528-1:2004 outlines a method, including preenrichment, for the detection of Enterobacteriaceae. It can be applied to products for human consumption and the feeding of animals, as well as environmental samples in the area of food production and food handling. This method is used when the microorganisms sought are expected to need resuscitation before enrichment, and when the number sought is expected to be in the range 1 to 100 per milliliter or per gram of test sample.

For the enumeration of Enterobacteriaceae by the MPN method: inoculate 3 tubes of the sample in Buffered Peptone Water (**Cat.1402**), using the adequate dilutions to obtain the detection of the parameters required for the product for each dilution of the sample. Sub-cultivate in EE broth with Durham gas collecting tubes and incubate at 30 ± 1°C (milk and lactic products), or 37 ± 1°C (other food samples) for 24 hours. Sub-cultivate each tube in Violet Red Bile Agar w/Glucose (VRBG) (**Cat.1092**) and incubate at 30 ± 1°C /milk and lactic products, or 37 ± 1°C (other food samples) for 24 hours. Detection of Enterobacteriaceae will be confirmed with the presence of gases in EE Broth and red-purple colonies in VRBG Agar.

Determination must be carried out from the number of positive tubes of selected dilutions using an MPN table and calculation of the Enterobacteriaceae count per gram or milliliter of sample.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 1°C or 37 ± 1°C and observed after 24 hours.

Microorganisms	Growth	Yellow Acid production
Enterobacter aerogenes ATCC 13048	Good	+
Escherichia coli ATCC 25922	Good	+
Salmonella enteritidis ATCC 13076	Good	± (could be delayed)
Staphylococcus aureus ATCC 25923	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 21528-1. Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of Enterobacteriaceae -- Part 1: Detection and enumeration by MPN technique with pre-enrichment

Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient identifiable information. London. December 1997.

The European Parliament and the Council of the European Union. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union. L226.

ELLIKER MEDIUM

CAT. 1539

For the cultivation of streptococci and lactobacilli in dairy products

FORMULA IN g/l

Tryptone	**20.00**	Sodium Chloride	**4.00**
Yeast Extract	**5.00**	Gelatin	**2.50**
Dextrose	**5.00**	Sodium Acetate	**1.50**
Lactose	**5.00**	Ascorbic Acid	**0.50**
Sucrose	**5.00**		

Final pH 6.8 ± 0.2 at 25°C

PREPARATION

Suspend 48.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and pale amber in color. If there are any physical changes, discard the medium.

USES

ELLIKER MEDIUM, also known as Lactobacilli Broth, is a medium recommended for the general cultivation of streptococci and lactobacilli.

Testing dairy products for lactic acid bacteria facilitates the determination of acid levels, evaluation of lactic starter cultures and helps to control the quality of cured cheese, cultured milks and uncultured products. The medium is prepared according to the formula of Elliker, which has a slightly acidic pH and contains sufficient nutrients to support the growth of these Gram-positive microorganisms.

Gelatin, Tryptone and Yeast extract provide the essential nutrients for growth. Lactose, Sucrose and Dextrose are the fermentable carbohydrates providing carbon and energy. Ascorbic acid provides adequate acid conditions. Sodium chloride supplies essential electrolytes for transport and osmotic balance and Sodium acetate is the selective agent inhibiting Gram-negative bacteria and also acts as a buffer system.

Inoculate and incubate and 35 ± 2°C for 18 - 48 hours, except for *Streptococcus cremoris* which is incubated at 30 ± 2°C.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Lactobacillus casei ATCC 7469	Good
Lactobacillus lactis ATCC 8000	Good
**Streptococcus cremoris* ATCC 9596	Good

* *Streptococcus cremoris* is incubated at a temperature of 30 ± 2°C.

STORAGE

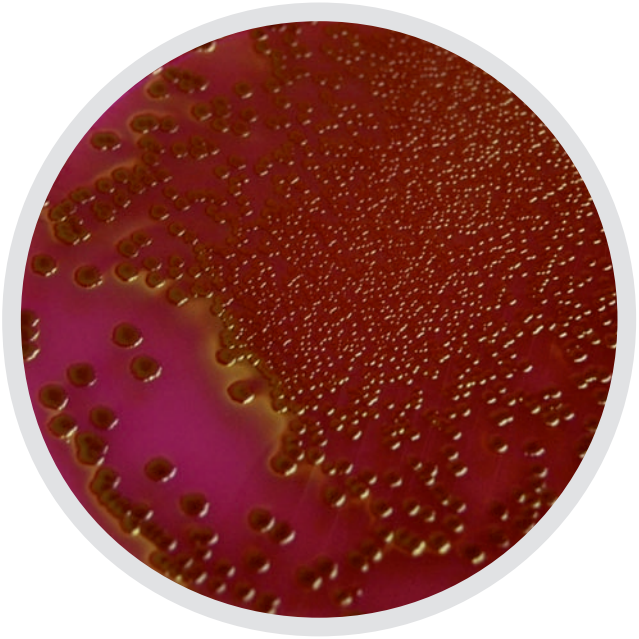
Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Elliker, P.R.A. W. Anderson and G. Hannesson 1956. An agar culture medium for lactic acid streptococci and lactobacilli. J. DairySci. 39:1611 Splittstoesg.

Vanderzant C. and D.F. Splittstoes 1992. Compendium of methods for the microbiological association of good, APHA 3rd edition.

CAUTION: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, the same applies if breathing becomes difficult or if swallowed.



Escherichia coli
ATCC 25922

ENDO AGAR BASE

CAT. 1118

For the detection of coliforms and other enteric microorganisms in water, dairy products and food in general

FORMULA IN g/l

Bacteriological Peptone	**10.00**	Sodium Sulfite	**2.50**
Lactose	**10.00**	Bacteriological Agar	**10.00**
Dipotassium Phosphate	**3.50**		
Final pH 7.5 ± 0.2 at 25°C			

PREPARATION

Suspend 36 grams of the medium in one liter of distilled water. Add 5 ml of an alcoholic solution at 10% (w/v) of basic fuchsin in 95% ethyl alcohol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC protected from light. The color of the prepared medium is amber with a pink tint.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

NOTE: Basic fuchsin is a potential carcinogen and pretvs should be taken to avoid inhalation of the dye powder as well as contact with skin.

USES

ENDO AGAR BASE is a differential and moderately selective culture medium for the detection and confirmation of coliforms and other enteric microorganisms in waters, milk, dairy and other food products.

It uses fuchsin to differentiate between positive lactose-fermenting and lactose non-fermenting bacteria. Acetaldehyde production by lactose-fermenting organisms such as *E. coli* produce characteristic red colonies and a red surrounding area, marked by its reaction with Sodium sulphite in the presence of fuchsin. Lactose non-fermenters form colorless, transparent colonies.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Dipotassium phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

Incubate plates, protected from light, at 35 ± 2°C for 18 - 24 hours. If negative after 24 hours, reincubate an additional 24 hours. To confirm presumptive positive coliforms, tubes of Endo Agar Base can be inoculated, incubated at 35 ± 2°C for 18 - 24 hours and examined for acid and gas production.

Rapid lactose fermenters produce red colonies with a metallic sheen. Slow lactose fermenters produce red colonies. Lactose non fermenters produce colorless colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with basic fuchsin added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Good	Red
Salmonella typhi ATCC 6539	Good	Colorless
Shigella sonnei ATCC 25931	Good	Colorless
Escherichia coli ATCC 25922	Good	Red with metallic sheen

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Endo S. 1904 uber ein verfahren Zum Nachweiss der Typhusbacillen

A.P.H.A. 1975 Standard methods for the examination of water and wastewater. 14th edition.

Standard Methods for the Examination of Water and Wastewater" (1992).

ENDO LES AGAR BASE

CAT. 1137

For the detection and enumeration of coliforms in water using the membrane filter technique

FORMULA IN g/l

Lactose	**9.40**	Sodium Sulfite	**1.60**
Tryptose	**7.50**	Yeast Extract	**1.20**
Casein Peptone	**3.70**	Monopotassium Phosphate	**1.00**
Meat Peptone	**3.70**	Sodium Desoxycholate	**0.10**
Sodium Chloride	**3.70**	Sodium Lauryl Sulfate	**0.05**
Dipotassium Phosphate	**3.30**	Bacteriological Agar	**15.00**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 50.25 grams of the medium in one liter of distilled water. Add 8 ml of an alcoholic solution at 10% (w/v) of basic fuchsin in 95% ethanol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is pinkish once the fuchsin is added.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

NOTE: Basic fuchsin is a potential carcinogen and precautions should be taken to avoid inhalation of the dye powder as well as contact with skin.

CAUTION: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, also if breathing becomes difficult or if swallowed.

USES

ENDO LES AGAR BASE is a modification of ENDO AGAR BASE (**Cat. 1118**), for testing water by the membrane filter technique. It uses Lauryl Sulfate Broth (**Cat. 1310**) as previous enrichment, obtaining greater growth. LES stands for Lawrence Experimental Station. It is a standard formula for testing waters and is also specified in the coliforms fermentation technique.

Like Endo Agar, it uses fuchsin to differentiate between positive lactose-fermenting and lactose non fermenting bacteria. Acetaldehyde production by lactose fermenting organisms such as *Escherichia coli* produce characteristic red colonies and a red surrounding area, marked by its reaction with Sodium sulfite in the presence of fuchsin. Lactose non-fermenters form colorless, transparent colonies.

Casein and Meat peptones, and Tryptose provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Lactose is the fermentable carbohydrate providing carbon and energy. Potassium phosphates act as a buffer system. Sodium desoxycholate inhibit growth of Gram-positive bacteria. Sodium lauryl sulphate partially inhibits organisms other than coliforms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Use the membrane filter technique to inoculate filters and pre-incubate on pads saturated with Lauryl Sulfate Broth (**Cat. 1310**) at 35 ± 2°C for 1.5 - 2.5 hours. Transfer filters to plates of Endo Les Agar Base and incubate at 35 ± 2°C for 18 - 24 hours.

Rapid lactose fermenters produce red colonies with a metallic sheen. Slow lactose fermenters produce red colonies. Lactose non fermenters produce colorless colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with alcoholic solution of basic fuchsin added, from type cultures after incubation at a temperature of 35°C ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Red with metallic sheen

Microorganisms	Growth	Colony Color
Salmonella typhimurium ATCC 14028	Good	Pink
Staphylococcus aureus ATCC 25923	Marked to Complete Inhibition	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

APHA (1980) Standard Methods for the Examination of Water and Wastewater.15th. Ed. Washington, D.C.

ENTEROBACTER SAKAZAKII ISOLATION CHROMOGENIC AGAR (ESIA) ISO 22964

CAT. 1446

For the isolation of presumptive *Enterobacter sakazakii* in infant milk products

FORMULA IN g/l

Pancreatic Digest of Casein	**7.00**	α-D-glucopyranoside	**0.15**
Sodium Chloride	**5.00**	Crystal Violet	**0.002**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Sodium Desoxycholate	**0.60**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 30.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45 - 50°C, homogenize gently and dispense into Petri dishes in amounts of 15 ml. The prepared medium should be stored at 8 - 15ºC, protected from light for up to 14 days. The color is purplish blue.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

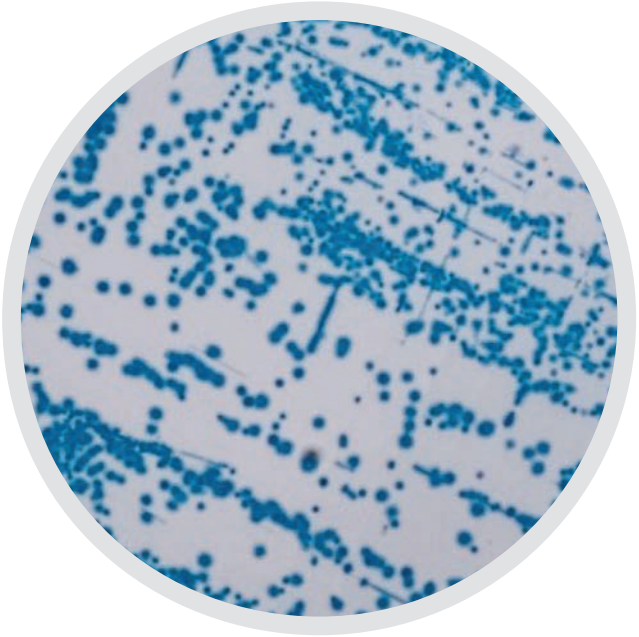
ENTEROBACTER SAKAZAKII ISOLATION CHROMOGENIC AGAR (ESIA) is a selective medium for the detection of *Enterobacter sakazakii* in milk powder and powered infant formula. The ISO normative 22964 recommends this medium for the isolation of *Enterobacter sakazakii*.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium desoxycholate and Crystal violet inhibit gram-positive bacteria. 5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside is the chromogenic substrate.

Enterobacter sakazakii is currently considered and emerging pathogen responsible for, un-weaned babies, risking severe meningitis and necrotic enterocolitis that can be the cause of mortality rate between 40 - 80%.

The pathogenicity of *Enterobacter sakazakii* for un-weaned babies' makes it necessary to review the manufacturing process of the milk-based products specialized for babies, guaranteeing the absence of the bacteria in the final product.

Additional prevention measures at a hospital include the sanitary hygiene of the prepared food; reducing the time between the preparation and its administration, to impede the multiplication of microorganisms.



Enterobacter sakazakii
ATCC 29544

After incubation of the inoculated LAURYL SULPHATE TRYPTOSE BROTH MODIFIED (mLST) (**Cat. 1445**), streak a loopful (ca. 10 µl) onto the surface of the *Enterobacter sakazakii* isolation chromogenic agar plate. Incubate the plate at 44 °C ± 1 °C for 24 hr ± 2 hr. After incubation, examine the chromogenic plate for the presence of typical colonies of presumptive *Enterobacter sakazakii*. Typical colonies are small to medium sized green to blue-green colonies. Non-typical colonies are often slightly

transparent and violet coloured. The typical colony colors of green - blue greenish should be confirmed in TSA Agar (**Cat. 1068**) in which they present yellow color. A biochemical confirmation is needed from the yellow pigmented colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 44°C ± 1°C and observed after 24 ± 2 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Transparent - red/violet
Escherichia coli ATCC 8739	Good	Transparent - red/violet
Enterobacter sakazakii ATCC 29544	Good	Green-blue - greenish
Staphylococcus aureus ATCC 25923	Inhibited	
Enterococcus faecalis ATCC 29212	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO normative 22964:2006 Milk and milk products detection of Enterobacter sakazakii

GUILLAUME-Gentil, O., Sonnard, V. Kandahai, M.C., Mauragg, J.D. and Jootsen, H. A simple and Rapad Cultural Method for Detection of Enterobacter Sakazakii in environmental samples. Journal of Food. Protection, 68 (1), 2005, pp. 64-69.

ENTEROCOCCUS CONFIRMATORY AGAR

CAT. 1018

For the confirmation of enterococci presence in water and other sources of sanitary interest

FORMULA IN g/l

Dextrose	5.00	Sodium Azide	0.40
Yeast Extract	5.00	Methylene Blue	0.01
Casein Peptone	5.00	Bacteriological Agar	15.00
Final pH 8.0 ± 0.2 at 25°C			

PREPARATION

Suspend 30.4 grams of the medium in one liter of distilled water. Soak for 10 - 15 minutes. Dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position in order to obtain 1.5 - 2.0 cm depth butts. The prepared medium should be stored at 8 - 15ºC. The color is blue-greenish.

The dehydrated medium should be homogeneous, free-flowing and beige with a blue tint in color. If there are any physical changes, discard the medium.

CAUTION: this medium contains Sodium azide, is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

ENTEROCOCCUS CONFIRMATORY AGAR is used to confirm the presence of enterococci in water and other sources of sanitary interest.

The presence of intestinal enterococci, also known as fecal streptococci, is an indicator for fecal contamination, especially when the contamination occurred a long time ago and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead at the time of analysis.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium azide and Methylene blue are inhibitors of Gram-positive bacteria, and Methylene blue is also the pH indicator. Bacteriological agar is the solidifying agent.

To the prepared test tubes, in order to cover half of the slanted surface, aseptically add a volume of either Enterococcus Selective Broth (**Cat.1204**) or Enterococcus Confirmatory Broth (Same formulation as this medium but without the agar). Using growth from KAA Presumptive Broth (**Cat. 1209**), inoculate both the surface and the broth in the ConfirmatoryAgar/Broth mixture tube.

The tubes are incubated at 35 ± 2°C for 18 hours and are examined to detect the presence of small pinpoint colonies. Perform a Gram stain and observe under a microscope looking for large chains of ovoid cells. Immediately perform a catalase test by adding 5 ml of H_2O_2 to the tube in study. If there is no generation of gases (negative test), this constitutes the confirmation of enterococci in the sample.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited

Microorganisms	Growth
Enterococcus faecalis ATCC 19433	Good
Enterococcus faecium ATCC 8043	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Winter and Sandholzer U.S Det. Interioir Fishery, Leaflet 201 Part II, Nov. 1946

Ewing W.H. 1986. Edwards and Ewing's identification of Enterobacteriaceae 4th Edition.

ENTEROCOCCUS SELECTIVE AGAR (ENTEROCOCCOSEL AGAR)

CAT. 1070

Selective medium for the enrichment and isolation of enterococci from diverse clinical materials and of highly contaminated products of sanitary importance.

FORMULA IN g/l

Casein Peptone	**15.00**	L-Cystine	**0.20**
Soy Peptone	**5.00**	Sodium Azide	**0.20**
Dextrose	**5.00**	Sodium Sulfite	**0.20**
Sodium Chloride	**4.00**	Crystal Violet	**0.0002**
Sodium Citrate	**1.00**	Bacteriological Agar	**12.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 42.6 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. Cool to 45 - 50°C, mix well and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium is clear amber slightly opalescent, with a violet hue.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.

USES

ENTEROCOCCUS SELECTIVE AGAR (ENTEROCOCCOSEL AGAR) is basically the same as Enterococcosel Broth (**Cat. 1204**) to which has been added 1.2% agar.

It is a sensitive enrichment medium for the isolation of streptococci from specimens containing numerous other flora. Many organisms such as saprophytic *Neisseria, Staphylococcus, Haemophilus*, non-hemolytic streptococci, and a certain number of Enterobacteriaceae are inhibited wholly or partially, permitting satisfactory fluorescence studies of Group A streptococci in 18 - 24 hours.

Casein and Soy peptones provide essential nutrients for growth. Dextrose is the fermentable carbohydrate energy source. Sodium chloride maintains the osmotic balance. Sodium citrate provides additional carbon. Sodium azide is an inhibitor. Sodium sulfite when reduced produces H_2S. L-Cystine lowers the oxidation-reduction potential by removing oxygen to maintain a low Eh. Crystal violet is a pH indicator. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

Adding 0.5% of sterile defibrinated sheep or rabbit blood notably increases its nutritional power and hemolytic studies can be conducted. These conditions yield good results in the isolation and identification of different groups of *Streptococcus* such as the alpha and beta-hemolytic, and the non-hemolytic.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Enterococcus faecalis ATCC 19433	Good
Enterococcus faecium ATCC 27270	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Washington, D.C. 2nd Ed., 1974.

Facklam and Carly, 1985, Mannual of Clinical Miocrobiology, Lennette and others (Eds). 4th Ed. ASM, Washington DC.

ENTEROCOCCUS SELECTIVE BROTH (ENTEROCOCCOSEL BROTH)

CAT. 1204

For the selective growth from clinical samples

FORMULA IN g/l

Casein Peptone	**15.00**	Sodium Sulfite	**0.20**
Soy Peptone	**5.00**	L-Cystine	**0.20**
Dextrose	**5.00**	Sodium Azide	**0.20**
Sodium Chloride	**4.00**	Crystal Violet	**0.0002**
Sodium Citrate	**1.00**		

Final pH 7.4 ± 0.2 at 25°C

PREPARATION

Suspend 30.6 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in 10 ml screw-capped tubes and sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT as the medium will become too inhibitory. The prepared medium should be stored at 2 - 8°C. The color is clear amber with violet tint.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with the skin. Wear gloves and eye/face protection.

USES

ENTEROCOCCUS SELECTIVE BROTH (ENTEROCOCCOSEL BROTH) is a sensitive enrichment medium for the isolation of enterococci from specimens containing numerous other flora. Many organisms such as saprophytic *Neisseria, Staphylococcus, Haemophilus*, non-hemolytic streptococci, and a certain number of Enterobacteriaceae are inhibited wholly or partially.

Casein and Soy peptones provide essential nutrients for growth. Dextrose is the fermentable carbohydrate energy source. Sodium chloride maintains the osmotic balance. Sodium citrate provides additional carbon. Sodium azide is an inhibitor. Sodium sulfite when reduced produces H_2S. L-Cystine lowers the oxidation-reduction potential by removing oxygen to maintain a low Eh. Crystal violet is a pH indicator.

Clinical material is inoculated into this selective medium and tubes are incubated at 35°C for 18 - 24 hours in a normal atmosphere. The growth of streptococci can be determined by the formation of a granular precipitate at the bottom of the tube, with the liquid above being clean or slightly turbid. At this point, perform a Gram stain and restreak on Trypticasein Soy Agar (**Cat. 1068**) blood plates or Blood Agar Base (**Cat. 1108**) to determine the type of hemolysis and to purify the culture.

To differentiate streptococci and pneumococci place bacitracin and optochin discs in the area of the inoculum on the Blood Agar plates and incubate for 18 - 24 hours at 35 ± 2°C under the recommended conditions.

Perform a Gram stain, catalase and bile solubility tests on characteristic colonies taken from the Blood Agar plate or from the growth obtained from the broth.

The presence of variable length chains of Gram-positive cocci inhibited by bacitracin in a low concentration, catalase negative and insoluble in bile or bile salts, constitute a valid presumptive identification of Group A beta-hemolytic streptococci. The definitive identification of the streptococci groups can be made by performing other biochemical tests such as esculin hydrolysis, pyruvate hydrolysis, etc. Also, serological typing, using Lancefield antisera methods, or more conveniently, the techniques of co-agglutination of Edwards and Larson can be performed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Enterococcus faecalis ATCC 19433	Good
Enterococcus faecium ATCC 27270	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Washington, D.C. 2nd Ed., 1974

Facklam and Carly, 1985, Mannual of Clinical Miocrobiology, Lennette and others (Eds). 4th Ed. ASM, Washington DC.

EOSIN METHYLENE BLUE AGAR (EMB)

CAT. 1039

For the isolation, cultivation and differentiation of Gram-negative enteric bacilli from clinical and other specimens

FORMULA IN g/l

Bacteriological Peptone	**10.00**	Eosin Y	**0.4**
Lactose	**5.00**	Methylene Blue	**0.065**
Sucrose	**5.00**	Bacteriological Agar	**13.50**
Dipotassium Phosphate	**2.00**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 36 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well, avoiding the formation of bubbles and dispense carefully into Petri Dishes. DO NOT OVERHEAT. The prepared medium should be stored at 8 - 15°C. The color is tournasol blue. Sterilization reduces the methylene blue, leaving the medium orange in color. The normal purple may be restored by gently mixing. The reduced medium should be shaken to oxidize the methylene blue; otherwise a dark zone from the top extending downwards will gradually appear.

The dehydrated medium should be homogeneous, free flowing and purple-rose flocculent precipitate in color. If there are any physical changes, discard the medium.

USES

EOSIN METHYLENE BLUE AGAR is a differential medium similar to Levine EMB Agar (**Cat. 1050**) and is used for the isolation of Enterobacteria. The use of Eosin Y and Methylene Blue enable differentiation between lactose-fermenting and non-fermenting organisms. It is widely used in medical bacteriology, in techniques recommended by APHA and for the detection and enumeration of coliforms, contaminants of foods and drinking water. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sucrose is added to Lactose as a fermentable carbohydrate to detect coliforms that ferment sucrose more readily than lactose. Eosin Y and Methylene blue dyes are both partial inhibitors of Gram-positive bacteria and pH indicators. Due to the lactose and sucrose, the medium can be differential in primary culture: Salmonellae and Shigellae which are lactose-negative can be differentiated from other lactose-negative and sucrose-positive organisms such as *Proteus vulgaris, Citrobacter* and *Aeromonas*. Dipotassium phosphate acts as a buffer system and Bacteriological agar is the solidifying agent.

For the isolation of enteric pathogens from clinical samples, inoculate onto a small area of one quadrant of EMB Agar and streak for isolation, allowing discrete colonies to develop. Incubate at 35 ± 2°C and observe at 24 hours and again at 48 hours. *Salmonella* and *Shigella* colonies are translucent and amber colored or colorless. Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and a greenish metallic sheen. Other coliforms such as Enterobacter form mucoid, pink colonies. Strains of *Enterococcus faecalis* are partially inhibited on this medium and appear as colorless colonies. As the medium is moderately inhibitory some staphylococci, streptococci and yeast may grow. Also some Gram-negative non-fermenting bacilli may appear as non-lactose fermenters. Further Biochemical tests are necessary for genus identification.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Good	Pink
Escherichia coli ATCC 25922	Good	Green with metallic shine
Salmonella typhimurium ATCC 14028	Good	Colorless
Pseudomonas aeruginosa ATCC 10145	Good	Colorless
Staphylococcus aureus ATCC 25923	Inhibited	Colorless

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

American Public Heath Association. Diagnostic Procedures and Reagents. 2nd Ed. APHA, Inc. New York, 1950

A.P.H.A Examination of dairy products. 10th Ed. APHA, Inc. New York, 1953. Society of American Bacteriologists. Manual of Microbiological Methods MacGraw-Hill New York, 1957..

ESTY BROTH

CAT. 1254

For the cultivation of *Streptococcus thermophilus* in yogurt

FORMULA IN g/l

Disodium Glycerophosphate	**19.00**	Yeast Extract	**2.50**
Soy Peptone	**5.00**	Ascorbic Acid	**0.50**
Tryptone	**5.00**	Magnesium Sulfate	**0.25**
Beef Extract	**5.00**		
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 37.25 grams of the medium in 950 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 5 grams of lactose (**Cat. 1905**), previously reconstituted in 50 ml of distilled water. Sterilize lactose solution by membrane filtration. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ESTY BROTH is a medium recommended for the growth of lactic streptococci and their bacteriophages from yogurt and other dairy products.

This medium is a recommended for *Streptococcus thermophilus* isolation and enumeration in yogurt. Lactic streptococci produce acid and are difficult to grow. They are nutritionally fastidious and demand complex culture media for optimum growth. The Glycerophosphate present in high concentrations acts as a pH regulator and inhibits *Lactobacillus bulgaricus* development while the Ascorbic Acid promotes the growth of lactic streptococci. It is recommended for the maintenance of the initial cultures that produce acids in their metabolism.

Beef extract, Tryptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Disodium glycerophosphate is a buffering agent. Ascorbic acid promotes the growth of Lactic streptococci. Magnesium sulphate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication, and also acts as a buffer. Bacteriological Agar is the solidifying agent.

Inoculate with 1 ml of inoculum and incubate at 30 ± 2°C during 72 hours for mesophilic streptococci and for *Streptococcus thermophilus* at 35 ± 2°C during 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Lactobacillus bulgaricus ATCC 11842	Inhibited
Streptococcus thermophilus ATCC 14486	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Reiter B., and J.D. Oram. 1962. Nutritional studies on cheese starters. I. Vitamin and aminoacid requirements of single strain starters. J. Dairy Res.

International Dairy Federation 1981 Identification and enumeration of microorganisms in fermented milks.

ESTY MEDIUM

CAT. 1555

Selective medium for the enumeration of *Streptococcus thermophilus* in yogurt

FORMULA IN g/l

Disodium Glycerophosphate	**19.00**	Yeast Extract	**2.50**
Soy Peptone	**5.00**	Ascorbic Acid	**0.50**
Tryptone	**5.00**	Magnesium Sulfate	**0.25**
Beef Extract	**5.00**	Bacteriological Agar	**11.00**
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 48.30 grams of the medium in 950 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 5 grams of lactose (**Cat. 1905**), previously reconstituted in 50 ml of distilled water. Sterilize lactose solution by membrane filtration. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ESTY MEDIUM is a recommended medium for the growth of lactic streptococci and their bacteriophages from yogurt and other dairy products. This medium has been recommended by the International Dairy Federation for this use. This medium is recommended for *Streptococcus thermophilus* isolation and enumeration in yogurt. Lactic streptococci produce acid and are difficult to grow. They are nutritionally fastidious and demand complex culture media for optimum growth. The Glycerophosphate present in high concentrations acts as a pH regulator and inhibits *Lactobacillus bulgaricus* development while the Ascorbic Acid promotes the growth of lactic streptococci.

Beef extract, Tryptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Disodium glycerophosphate is a buffering agent. Ascorbic acid promotes the growth of Lactic streptococci. Magnesium sulphate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication, and also acts as a buffer. Bacteriological Agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Lactobacillus bulgaricus ATCC 11842	Inhibited
Streptococcus thermophilus ATCC 14486	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Terzaghi, B.E. and W. E. Sandine. 1975 Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol 29:807-813.

International Dairy Federation 1981. Identification and enumeration of micro-organisms in fermented milks. Joint IDF/ISO/AOAC.

EUGON AGAR

CAT. 1036

For the eugonic growth of most microorganisms

FORMULA IN g/l

Casein Peptone	**15.00**	L-Cystine	**0.70**
Dextrose	**5.50**	Sodium Sulfite	**0.20**
Soy Peptone	**5.00**	Bacteriological Agar	**15.00**
Sodium Chloride	**4.00**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 45.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118°C for 15 minutes. Cool to 45 - 50°C and, if desired, aseptically add 5-10 % sterile defibrinated sheep blood and two vials of Polyenrichment Supplement (**Cat. 6011**), previously reconstituted with 1 vial of Polyenrichment Restoring Solution (vial B). Homogenize gently and dispense into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

EUGON AGAR is a medium recommended for the growth of a wide variety of microorganisms. It yields a high level of growth of microorganisms (eugonic growth) even with bacteria, which are more difficult to cultivate, such as *Haemophilus, Neisseria, Pasteurella, Brucella, Lactobacillus*, etc. It is very useful in medical bacteriology and food testing, such as cured meat, dairy products and other foods. Likewise, this medium is ideal for cultivating delicate pathogenic microorganisms and for obtaining high counts of bacterial cultures in the preparation of antigens and vaccines.

L-Cystine and Sodium sulfite are added to stimulate growth. Casein and Soy peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. The addition of blood provides extra growth factors for fastidious microorganisms and the Polyenrichment supplement (**Cat. 6011**) is a supplement specifically formulated for *Neisseria, Francisella and Brucella*. .

The non-enriched medium is recommended for the rapid growth of lactobacilli linked to cured meat products, dairy products and other foods.

The addition of defibrinated blood, chocolated or not, permits the development of *Histoplasma capsulatum* and *Nocardia*. The medium also is used for the analysis of clinical materials such as blood and cerebrospinal or pleural fluids that generally contain pure cultures.

Inoculate sample and incubate for at 35 ± 2°C for 18 - 48 hours. *Candida albicans* and *Aspergillus brasiliensis* should be incubated at 30 ± 2°C.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 40 - 48 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good
Streptococcus pneumoniae ATCC 6303	Good
Streptococcus pyogenes ATCC 19615	Good
Brucella abortus ATCC 4315	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Vera H.J. Bact. 54:14. 1947. Pelczar and Vera Milk Plant Monthly, 38-30. 1949.

Frank J. Bact. 70:269, 1955. Ramos C., Mario "Manual of Milk and Lactides". Edition of Author, Berna 12:201. Mexico 6. D.F., 1976.

EVA BROTH (ETHYL VIOLET AZIDE, LITSKY)

CAT. 1230

Selective medium for the confirmation and detection of enterococci and as a detector of fecal contamination in water

FORMULA IN g/l

Peptone Mixture	**20.00**	Dipotassium Phosphate	**2.70**
Dextrose	**5.00**	Sodium Azide	**0.40**
Sodium Chloride	**5.00**	Ethyl Violet	**0.0008**
Monopotassium Phosphate	**2.70**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 35.8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense 10 ml amounts into test tubes and sterilize in autoclave at 121°C for 15 minutes. It is recommended to use a large inoculum as the medium is very selective and is used in the second phase of confirmation. The prepared medium should be stored at 2 - 8°C. The color is greyish.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

EVA BROTH is a selective medium specific for the detection and confirmation of enterococci in water, other specimens and foods. It is an indication of fecal contamination. EVA Broth, in conjunction with Rothe Broth (Glucose Broth with Azide - **Cat. 1238**) as the presumptive medium, is used to enumerate fecal enterococci in water, soil and food products by the MPN technique.

The presence of intestinal enterococci is an indicator for faecal contamination, especially when the contamination occurred long ago and the less resistant coliform bacteria, including E*scherichia coli*, are already dead when the analysis is carried out.

Sodium azide and Ethyl violet inhibit all Gram-positive bacilli and Gram-positive cocci except enterococci. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphates act as a buffer system, and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

The tubes are inoculated with the appropriate dilutions in series of 3 tubes for each dilution and incubated at 35 ± 2°C for 24 - 48 hours. The appearance of turbidity and, eventually, the formation of a violet (purple) button of growth at the bottom of the tube are characteristics of enterococci growth.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aureus ATCC 25923	Inhibited
Streptococcus pyogenes ATCC 19615	Inhibited
Enterococcus faecalis ATCC 29212	Good
Enterococcus faecalis ATCC 19433	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Litsky W. Mallmann W.L Fifield C.W. A.J.P.H 1953. 43. 873-879

Mallman and Seligman. 195 A.J.P.H 40:286.

EWING MALONATE BROTH MODIFIED

CAT. 1212

For the differentiation of coliforms and other enteric organisms

FORMULA IN g/l

Sodium Malonate	**3.00**	Dipotassium Phosphate	**0.60**
Ammonium Sulfate	**2.00**	Monopotassium Phosphate	**0.40**
Sodium Chloride	**2.00**	Dextrose	**0.25**
Yeast Extract	**1.00**	Bromothymol Blue	**0.025**
Final pH 6.7 ± 0.2 at 25°C			

PREPARATION

Suspend 9.3 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is green.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

EWING MALONATE BROTH MODIFIED is prepared following Leifson's formula and modified with the addition of Yeast extract and Dextrose, for the differentiation of coliforms and other enteric organisms. It is widely used for the differentiation of *Enterobacter* and *Escherichia coli* based on the use of malonate.

Examples of microorganisms with positive malonate activity are *Enterobacter, Klebsiella* and strains of *Arizona*. Some examples of those not able to use malonate are *Escherichia, Salmonella* and *Serratia*, amongst others.

Malonate utilization as a carbon source, in conjunction with Ammonium sulphate as a nitrogen source during growth, produces Sodium hydroxide and thereby increased alkalinity, which changes the color of the medium from green to blue due to the pH indicator Bromothymol blue. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphates act as a buffer system. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Inoculate the broth with the suspect culture and incubate at 35 ± 2°C for 18 - 48 hours in an aerobic atmosphere.

The organisms that do not utilize malonate do not produce a color change and the medium remains the original green color. Some malonate-negative strains produce a yellow color due to the fermentation of Dextrose, which increases acidity, and the medium turns yellow at a pH of 6.0.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Medium Color
Enterobacter aerogenes ATCC 13048	Good	Blue
Escherichia coli ATCC 25922	Good	Green
Klebsiella pneumoniae ATCC 13833	Good	Blue
Salmonella typhimurium ATCC 14028	Good	Green
Salmonella arizonae ATCC 13314	Good	Blue

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Leifson, E. J. 26:329, 1993 Ewing. W. H. Identification of Enterobacteriaceae, Burgess Publishing Co., Minneapolis, Minn., 1972.

FECAL COLIFORMS AGAR BASE (m-FC)

CAT. 1127

For the cultivation and enumeration of fecal coliforms in water by the membrane-filtration technique at a high temperature

FORMULA IN g/l

Lactose	**12.50**	Yeast Extract	**3.00**
Tryptose	**10.00**	Bile Salts N° 3	**1.50**
Proteose Peptone N°3	**5.00**	Aniline Blue	**0.10**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 52.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Cool to 45 - 50°C and aseptically add 2 vials, each vial for 500 ml of the medium, of Fecal Coliforms Supplement (**Cat. 6023**), previously reconstituted in 5 ml of 1% 0.2 N NaOH solution. Boil for one minute until complete dissolution. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium without supplement is gray-blue. The color of the prepared medium with supplement is cranberry red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Fecal Coliforms Supplement (Cat. 6023)
(Composition: each vial for 500 ml)
Rosolic Acid .....50 mg.

USES

FECAL COLIFORMS AGAR BASE (m-FC) is prepared according to the formula proposed by Geldreich, Clark and Bert. It is used for the cultivation and enumeration of fecal coliforms microorganisms. This medium is suitable for the membrane filter technique at a high temperature.

Many standard procedures specify the use of Fecal coliforms Media for testing water and foods. Fecal coliforms are differentiated from other coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.

Proteose and Tryptose provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Lactose is the fermentable carbohydrate as a carbon and energy source. Bile salts inhibit growth of Gram-positive bacteria. Sodium chloride maintains the osmotic balance. Aniline Blue and Rosolic Acid are the differential indicators and suppress the growth of Gram-positive bacteria. Bacteriological agar is the solidifying agent.

Place the membrane filter, which the sample has been filtered through, on the medium following the membrane-filter technique. Incubate for 24 ± 2 hours, one lot as a control at 35 ± 2°C, the rest at 44.5 ± 0.5°C. Observe coliforms and count the colonies. The differential indicator system (aniline blue and rosolic acid) gives the colonies of fecal coliforms a blue color, while the rest of the microorganisms will become gray to cream-colored.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with Fecal Coliforms Supplement (**Cat. 6023**) added, from type cultures after incubation at a temperature of 35 ± 2°C and 44.5 ± 0.5 °C, and observed after 24 ± 2 hours. Following the membrane-filtration technique.

Microorganisms	Growth		Colony Color
	44.5°C	35°C	
Escherichia coli ATCC 25922	Good	Good	Blue
Salmonella typhimurium ATCC 14028	Inhibited	Good	Gray
Shigella flexneri ATCC 12022	Inhibited	Good	Gray
Enterococcus faecalis ATCC 19433	Inhibited	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Geldreich, Clark and Kabler, 1963. USPHS, HEW. Personal Communication.

Geldreich, Clark, Huff and Bert, 1965. Journal of American Water Works Association, 57:208..

FECAL COLIFORMS BROTH BASE (m-FC)

CAT. 1121

For the cultivation and enumeration of fecal coliforms in water by the membrane-filtration technique at a high temperature

FORMULA IN g/l

Lactose	12.50	Yeast Extract	3.00
Tryptose	10.00	Bile Salts N° 3	1.50
Proteose Peptone N°3	5.00	Aniline Blue	0.10
Sodium Chloride	5.00		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 37.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Cool to 45 - 50°C and aseptically add 2 vials, each vial for 500 ml of the medium, of Fecal Coliforms Supplement (**Cat. 6023**), previously reconstituted in 5 ml of 1% 0.2 N NaOH solution. Boil for one minute until complete dissolution. Cool to 45 - 50°C and pour 2 ml of the broth medium onto each sterile absorbent pad placed on Petri dishes. DO NOT AUTOCLAVE. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium without supplement is gray-blue. The color of the prepared medium with supplement is red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Fecal Coliforms Supplement (Cat. 6023)
(Composition each vial for 500 ml)
Rosolic Acid ..... 50 mg

USES

FECAL COLIFORMS BROTH BASE (m-FC) is prepared according to the formula proposed by Geldreich, Clark and Bert, and is used for the cultivation and enumeration of fecal coliform microorganisms. This medium is suitable for the membrane filtration technique at a high temperature. Many standard procedures specify the use of Fecal Coliforms Media for testing water and foods.

Fecal coliforms are differentiated from other coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.

Proteose and Tryptose provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group essential for bacterial growth. Lactose is the fermentable carbohydrate as a carbon and energy source. Bile salts inhibit growth of Gram-positive bacteria. Sodium chloride maintains the osmotic balance. Aniline Blue and Rosolic Acid are the differential indicators and suppress the growth of Gram-positive bacteria.

Place the membrane filter, which the sample has been filtered through, on the upper part of the saturated pad with the medium in the Petri dish (55 mm diameter). Close the dish. Incubate for 24 ± 2 hours, one lot as a control at 35 ± 2°C, the rest at 44.5 ± 0.5°C. Observe coliforms and count the colonies.

The differential indicator system (aniline blue and rosolic acid) gives the colonies of fecal coliforms a blue color, while the rest of microorganisms will become gray to cream-colored.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with Fecal Coliforms Supplement (**Cat. 6023**) added, from type cultures after incubation at both temperatures of 35± 2°C and 44.5 ± 0,5 °C, and observed after 24 ± 2 hours. Following the membrane filtration technique.

Microorganisms	Growth		Colony Color
	44.5°C	35°C	
Escherichia coli ATCC 25922	Good	Good	Blue
Salmonella typhimurium ATCC 14028	Inhibited	Good	Gray
Shigella flexneri ATCC 12022	Inhibited	Good	Gray
Enterococcus faecalis ATCC 19433	Inhibited	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Geldreich, Clark and Kabber, 1963. USPHS, HEN. Personal Communication.

Geldreich, Clark, Huff and Bert, 1965. Journal of American water works Association, 57:208..

FMM BROTH

CAT. 1264

For the cultivation of *Flexibacter maritimus*

FORMULA IN g/l

Sodium Chloride	**19.40**	Potassium Bromide	**0.08**
Magnesium Chloride	**5.90**	Strontium Chloride	**0.034**
Casein Peptone	**5.00**	Boric Acid	**0.022**
Sodium Sulfate	**3.24**	Sodium Acetate	**0.01**
Calcium Chloride	**1.80**	Sodium Fluoride	**0.0024**
Potassium Chloride	**0.55**	Ammonium Nitrate	**0.0016**
Yeast Extract	**0.50**	Disodium Phosphate	**0.008**
Sodium Bicarbonate	**0.16**	Sodium Silicate	**0.004**

Final pH 7.4± 0.2 at 25°C

PREPARATION

Suspend 36.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is light amber.

The dehydrated medium should be homogeneous, free-flowing and whitish in color. If there are any physical changes, discard the medium.

USES

FMM Broth is a medium used for the cultivation of *Tenacibaculum maritimum* (formerly *Flexibacter maritimus*) which is responsible for Flexibacteriosis marina, one of the most important bacterial diseases affecting marine lives around the world. This has an important economic impact to aquaculture producers. The infection has a variety of clinical manifestations depending on the species and age of fish, the most significant symptom being the presence of gross lesions on the body surface. It is, currently, the main pathological problem in turbot and sole cultivation affecting all ages of fish.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. The rest of the components provide the salinity requirements that make the medium similar to seawater.

The isolated bacteria are biochemically characterized by conventional methods in plates and tubes. They are Gram-negative filamentous bacillus, oxidize and catalyse positive, nitrate reductors and incapable of producing sulphydric acid. They are negative for the test of indole and methyl red. To confirm the taxonomic position perform molecular and seriological tests (PCR).

Incubate at 25°C during 72 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25°C and observed after 72 hours.

Microorganisms	Growth
Flexibacter maritimus sp	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Dinis, M.T., Ribeiro, L., Soares, F. & Sarasquete, C. (1999) A review on the cultivation potential of Solea senegalensis in Spain and in Portugal. Aquaculture 176, 27-38.

Dopazo, C.P. & Barja, J.L.. (2002) Diagnosis and identification of IPNV in salmonids by molecular methods. En: Molecular Diagnosis of Salmonid Diseases. pp. 23-48. C. Cunningham (ed). Kluwer Academic Publishers. Dordrecht, Holanda.

Osorio, C. and Toranzo, A.E. 2002. DNA-based diagnostics in Sea Framing. In: R. Nagabhushanam and M. Fingerman (Editors), Recent Advances in Marine Biotechnology Series Vol.7: Seafood safety anf Human Health. Science Publishers, Inc. Plymouth, UK. pp. 253-310.

GC AGAR BASE

CAT. 1106

For the selective isolation and cultivation of gonococci and *Haemophilus* species when used with hemoglobin and supplements

FORMULA IN g/l

Peptone Mixture	**15.00**	Corn Starch	**1.00**
Sodium Chloride	**5.00**	Monopotassium Phosphate	**1.00**
Dipotassium Phosphate	**4.00**	Bacteriological Agar	**10.00**

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 7.2 grams of the medium in 100 ml of distilled water to make a double-strength base. Mix well and allow to stand for 5 minutes. Heat with frequent agitation and boil for one minute. Sterilize in autoclave at 121°C for 15 minutes. Also, autoclave 100 ml of a 2% haemoglobin solution elaborated by gradually adding water to 2 grams of dry haemoglobin to obtain a uniform suspension, before exposing it to the autoclave's heat. Cool both flasks to 50°C and aseptically add the haemoglobin solution to GC Agar Base and mix gently. Aseptically add Polyenrichment Supplement (**Cat. 6011**), previously reconstituted with 1 vial of Polyenrichment Restoring Solution (vial B). Mix carefully to

avoid bubble formation. This completed medium is the general purpose Chocolate Agar. Pour into plates or tubes with screw caps. Allow tubes to solidify with a long slant. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

The medium can be also prepared adding the following supplements:

1. VCN Supplement (**Cat. 6013**). This supplement turns the medium into Thayer Martin Medium.
2. VCAT Supplement (**Cat. 6014**). This supplement is used for the isolation of *Neisseria*.
3. VCNT Supplement (**Cat. 6026**). This supplement is also used for the isolation of *Neisseria*.
4. LCAT Supplement (**Cat. 6012**). This supplement is used for the isolation of pathogen *Neisseria*. It must be added after adding lysed horse blood instead of haemoglobin and Polyenrichment Supplement.

NOTE: VCAT Supplement and VCNT Supplement contains different antibiotics (one each) and different concentrations of the same antibiotics (three) within both. The election must be made according to the selectivity required.

When VCN Supplement, VCAT Supplement and VCNT Supplement are added, Polyenrichment Supplement and 2% haemoglobin solution must be used.

USES

GC AGAR BASE is used with various additives for the isolation and cultivation of pathogenic microorganisms such us *Neisseria gonorrhoeae, Haemophilus influenzae* and *N. meningitidis*.

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Corn Starch absorbs any toxic metabolites produced. Dipotassium and Monopotossaium Phosphates act as buffer systems. Sodium Chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological Agar is the solidifying agent.

GC Agar Base is employed with the addition of haemoglobin and supplements for the preparation of Chocolate Agar and Thayer-Martin Medium.

Chocolate Agar is prepared with the addition of 2% haemoglobin. The addition of Haemoglobin provides hemin (X factor), required by *Haemophilus* species and promotes the growth of *Neisseria* species.

A chemical enrichment composed of cofactors, vitamins and nicotinamide adenine dinucleotide (NAD) is also required for the growth of *Haemophilus* and *Neisseria spp.* If required, antimicrobial supplements are added as inhibitors for an improved selectivity of the medium.

Thayer-Martin Medium is recommended for the primary isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens with mixed flora taken from throat, vagina, rectum and urethra samples. It is designed to reduce the overgrowth of gonococci and meningococci by contaminants, to suppress saprophytic *Neisseria* species growth and to encourage pathogenic *Neisseria* growth. The typical colonies of *N. gonorrhoeae* on Thayer-Martin Medium are white-gray, opaque, sometimes shiny, finely granular in appearance, variable in size (1 - 2 mm), round with entire or lobate edges and mucoid after 48 hours of incubation.

The specimen should be placed on the surface of the plate making sure that a heavy inoculum is contained in a relatively small area. Streaking out from this area will produce well-isolated colonies. Incubate in a humid atmosphere of 5 - 10% CO_2 and at 35°C for 40 - 48 hours. For suspect isolated colonies, perform a Gram stain and oxidase test.

In carbohydrate studies using the CTA Medium (**Cat. 1502**) with selected 1% sugars, *N. gonorrhoeae* ferments only glucose with acid production but no gas production. *N. meningitidis* ferments both glucose and maltose with acid but no gas production. The carbohydrate tests are carried out incubating the medium for 1 - 4 days at 35°C, aerobically and without CO_2.

The antimicrobial agents in selective formulas such as Thayer-Martin Medium inhibit some strains of *N. gonorrhoeae*, therefore it is wise to streak non-selective Chocolate Agar plates to culture these organisms.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with Polyenrichment Supplement (**Cat. 6011**) and haemoglobin added, after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere and observed after 40 - 48 hours.

Microorganisms	Growth
Haemophilus influenzae ATTC 19418	Good
Neisseria meningitidis ATCC 13090	Good
Neisseria gonorrhoeae ATCC 19424	Good
Streptococcus pneumoniae ATCC 6303	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bailey and Scott. Diagnostic Microbiology. Fifth Edition, 1978. The C.V. Mosby Company. St. Louis, USA. Preparation of Transgrow.

Sept. 15. 1971. Venereal Disease Research Lab., C.D.C. Atlanta, Ga., USA.

Thayer, J. D. Martin J. E., 1966. Improved medium selective for the cultivation of N. gonorrhoeae and N. meningtidis. Public Health Rep. 81. 559-562..

GELATIN LACTOSE MEDIUM

CAT. 1526

For the confirmation of *Clostridium perfringens*

FORMULA IN g/l

Gelatin	**120.00**	Yeast Extract	**10.00**
Tryptose	**15.00**	Phenol red	**0.05**
Lactose	**10.00**		

Final pH 7.5 ± 0.2 at 25°C

PREPARATION

Suspend 155 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is dark red.

The dehydrated medium should be homogeneous, free-flowing and red in color. If there are any physical changes, discard the medium.

USES

GELATIN LACTOSE MEDIUM is used to determine the identity of presumptive *Clostridium perfringens*. It contains lactose to test lactose fermentation and gelatin to test liquefaction.

The lactose fermentation is indicated by the presence of gas bubbles as well as a color change of the medium from red to yellow. The Gelatin is a protein derived by the hydrolysis of collagen, and is found abundantly in bones, skin, tendons, cartilage and animal tissue. It is used in culture media to determine gelatinolysis by bacteria, the gelatinoses produced by the microorganisms hydrolyze the gelatin liquefying a solid medium. Tryptose provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Lactose is the fermentable carbohydrate, producing acid indicated by the Phenol red indicator. Phenol red changes to yellow when acid is produced and to red when an alkalinisation of the medium is produced. Cracks or bubbles in the medium indicate gas production.

Inoculate and incubate at 35 ± 2°C for 24 – 48 hours. To read gelatinase, refrigerate until well chilled and compare to non-inoculated tubes. Tubes positive for gelatinase will remain liquid. *C. perfringens* usually liquefies the gelatin after 24 - 44 hours.

The isolated colonies which are not motile, reduce nitrate, ferment lactose and produce liquefaction of gelatine in 48 hours are presumptively identified as *Clostridium perfringens*.

NOTE: Not all *Clostridium perfringens* strains reduce nitrate to nitrite.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Color change to yellow (Gas Production)	Gelatinase
Clostridium perfringens ATCC 13124	+	+
Clostridium sporogenes ATCC 25781	-	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

APHA. 3rd Edition Compendium of methods for the microbiological examination of foods.

Métodos Analíticos del Laboratorio del Instituto Nacional del Consumo (CICC). Alimento I Ministerio de Sanidad y Consumo 1.999.

Department of Health NHS Executive: The Caldicott Committee. Report on the review of patientidentifiable information. London. December 1997.

GIOLITTI-CANTONI BROTH

CAT. 1232

For the selective enrichment of *Staphylococcus aureus* in food samples

FORMULA IN g/l

D-Mannitol	**20.00**	Lithium Chloride	**5.00**
Tryptone	**10.00**	Sodium Chloride	**5.00**
Beef Extract	**5.00**	Sodium Pyruvate	**3.00**
Yeast Extract	**5.00**	Glycine	**1.20**

Final pH 6.9 ± 0.2 at 25°C

PREPARATION

Suspend 54.2 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense 19 ml amounts into test tubes and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 0.3 ml of 3.5% Potassium Tellurite (**Cat. 5208**) or 1 ml of 1% Potassium Tellurite to each tube. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

GIOLITTI-CANTONI BROTH with Potassium tellurite added is prepared according to the formula of Giolitti and Cantoni, for the enumeration, using the MPN Method, and for the selective enrichment of coagulase positive staphylococci in foodstuffs and dried baby milk. It was designed by Giolitti and Cantoni to facilitate the growth of *Staphylococcus aureus* by incorporating Sodium pyruvate in the formula, even when present in low numbers in food samples.

The International Dairy Federation recommends this medium in a procedure for detecting *S. aureus* in dairy products, using it as an enrichment medium.

The Tryptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Mannitol is the fermentable carbohydrate providing carbon and energy. The growth of Gram-negative bacteria is inhibited by Lithium chloride, whilst the Gram-positive bacilli are inhibited by Glycine and the Potassium tellurite. The high level of Potassium tellurite is necessary to suppress the high numbers of contaminating organisms that could be expected.

Duplicate tubes should be inoculated with 1 ml of each serial dilution and the caps tightened. Incubate at 35 ± 2°C for 40 - 48 hours, examining the tubes daily.

The test is considered negative for *S. aureus* if no blackening of the medium is observed. If blackening is present throughout or at the bottom of the tube, subculture to an isolation medium, such as Baird Parker Agar Base (**Cat. 1100, 1319**), and observe for positive growth of black colonies surrounded by a clearing zone.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with 3.5% Potassium Tellurite or 1% Potassium Tellurite added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 40 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Micrococcus luteus ATCC 10240	Inhibited
Staphylococcus aureus ATCC 6538	Good (black)
Staphylococcus aureus ATCC 25923	Good (black)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Giolitti, C. and Cantoni, C. (1966) "A Medium for the Isolation of Staphylococci from Foodstuffs", J. Appl. Bact. 29, 395.

International Dairy Federation. 1978 IDF Standard GOA: 1978.

GIOLITTI-CANTONI BROTH ISO 6888-3

CAT. 1287

Liquid medium for the enumeration in accordance to the MPN method and selective enrichment of *Staphylococcus aureus* according to ISO 6888-3

FORMULA IN g/l

D-Mannitol	**20.00**	Sodium Chloride	**5.00**
Casein Peptone	**10.00**	Sodium Pyruvate	**3.00**
Beef Extract	**5.00**	Glycine	**1.20**
Lithium Chloride	**5.00**	Polysorbate 80	**1.00**
Yeast Extract	**5.00**		

Final pH 6.9 ± 0.2 at 25°C

PREPARATION

Suspend 55.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute in 19 ml amounts into and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 0.1 ml of 1% Potassium Tellurite solution per tube. The medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

GIOLITTI CANTONI BROTH (ISO 6888-3) is a modified formula of a medium formulated by Giolitti and Canton in 1996. It is recommended by ISO 6888-3 for the enumeration and detection of coagulase-positive staphylococci from food and animal feeding stuff, using the MPN Method.

Casein peptone and Beef Extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Mannitol is the fermentable carbohydrate providing carbon and energy. Lithium chloride inhibits the growth of Gram-negative bacteria. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene and formalin. The growth of staphylococci is encouraged by Sodium pyruvate and Glycine. Gram-negative contaminants are inhibited by Potassium tellurite.

Incubate the initial suspension for 24 ± 2 hr at 37°C. If no blackening develops, incubate for a further 24 ± 2 hr. Growth of staphylococci can be recognized by a black coloration of the

culture medium due to the reduction of tellurite to tellurium. Subcultivate tubes that present blackening in plates of Baird Parker Agar (**Cat. 1319**).

Incubate at 37 ± 2°C and observe after 24 - 48 hours.

This method is recommended for products where staphylococci are expected to be stressed and in low numbers such as dried products. Coagulase-positive staphylococci will mostly be *Staphylococcus aureus*, but *Staphylococcus intermedius* and some strains of *Staphylococcus hyicus* are also coagulase-positive.

The confirmation of staphylococci which produce coagulase is based on a strong positive coagulase reaction, but it is also known that some strains of coagulase-positive staphylococci give weak positive coagulase reactions. These latter strains can be confused with other bacteria but can be differentiated by the use of additional tests such as one for the production of thermonuclease.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with tellurite added, after incubation at a temperature of 37 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Micrococcus luteus ATCC 10240	Inhibited
Staphylococcus aureus ATCC 6538	Good (black)
Staphylococcus aureus ATCC 25923	Good (black)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

International Standard ISO 6888-3 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase – positive staphylococci (Staphylococcus aureus and other species) Part3: Detection and MPN technique for low numbers.

GLUCOSE CHLORAMPHENICOL AGAR

CAT. 1094

Selective medium for the isolation and enumeration of yeasts and molds in milk and dairy products

FORMULA IN g/l

Glucose	**20.00**	Chloramphenicol	**0.20**
Yeast Extract	**5.00**	Bacteriological Agar	**15.00**

Final pH 6.6 ± 0.2 at 25°C

PREPARATION

Suspend 40.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is light amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

GLUCOSE CHLORAMPHENICOL AGAR is recommended by the International Dairy Federation (FIL-IDF) for the isolation and enumeration of yeasts and molds in milk and dairy products. This medium has been adopted by the DIN and ISO standards.

Yeast extract is the water-soluble portion of hydrolyzed yeast and is a source of vitamins, particularly of the B-group, and other growth nutrients that stimulate yeast and mold development. Glucose is the fermentable carbohydrate as a carbon and energy source. Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum. Bacteriological agar is the solidifying agent.

Inoculate 0.1 ml of sample on medium surface. Incubate at 25 - 30°C and examine after 3 - 7 days. Report as number of colonies per gram of food.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30°C and observed after 3 - 7 days.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Candida albicans ATCC 2091	Good
Staphylococcus aureus ATCC 25923	Inhibited
Aspergillus spp.	Good
Lactobacillus casei ATCC 9595	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

FIL-IDF(1991) Standard 94B. Enumeration of yeast and moulds. Colony Count Technique at 25°C.

ISO (1981) ISO/DIS 6611: Milk and Milk products: Enumeration of yeast and moulds colony count technique at 25°C.

DIN Standard 10186. Mikrobiologische Milchuntersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen.

GLUCOSE CHLORAMPHENICOL BROTH

CAT. 1258

Selective medium for the isolation and enumeration of yeasts and molds in milk and dairy products using the MPN technique

FORMULA IN g/l

Glucose	**20.00**	Chloramphenicol	**0.20**
Yeast Extract	**5.00**		
Final pH 6.6 ± 0.2 at 25°C			

PREPARATION

Suspend 25.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium stored at 2 - 8°C. The color is light amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

GLUCOSE CHLORAMPHENICOL BROTH is recommended by the International Dairy Federation (FIL-IDF) for the isolation and enumeration of yeast and molds in milk and dairy products, using the most probable number (MPN) method.

Yeast extract is the water-soluble portion of hydrolyzed yeast and is a source of vitamins, particularly of the B-group, and other growth nutrients that stimulate yeast and mold development. Glucose is the fermentable carbohydrate as a carbon and energy source. Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum.

Inoculate with a series of dilutions as per indicated in the MPN technique. Incubate at 25 - 30°C and examine after 3 - 7 days. Report as number of colonies per gram of food.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30°C and observed after 3 - 7 days.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Candida albicans ATCC 2091	Good
Staphylococcus aureus ATCC 25923	Inhibited
Aspergillus spp.	Good
Lactobacillus casei ATCC 9595	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

FIL-IDF(1991) Standard 94B. Enumeration of yeast and moulds. Colony Count Technique at 25°C.

ISO (1981) ISO/DIS 6611: Milk and Milk products: Enumeration of yeast and moulds colony count technique at 25°C.

DIN Standard 10186. Mikrobiologische Milchuntersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen.

GN ENRICHMENT BROTH (HAJNA)

CAT. 1248

For the selective enrichment of Gram-negative microorganisms, especially *Shigella spp.* and *Salmonella spp.* from all types of research materials

FORMULA IN g/l

Tryptose	**20.00**	D-Mannitol	**2.00**
Sodium Citrate	**5.00**	Potassium Dihydrogen Phosphate	**1.50**
Sodium Chloride	**5.00**	Dextrose	**1.00**
Dipotassium Hydrogen Phosphate	**4.00**	Sodium Desoxycholate	**0.50**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 39 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8ºC. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

GN ENRICHMENT BROTH (HAJNA) was developed by Hajna for the selective enrichment of enteric Gram-negative microorganisms. GN stands for Gram-negative. It is intended for use in the detection of *Salmonella spp.* and *Shigella spp.* from clinical and non-clinical specimens.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Mannitol and Dextrose are the fermentable carbohydrates providing carbon and energy. Mannitol is provided in a higher concentration than dextrose to enhance the growth of mannitol-fermenting species, such as *Salmonella* and *Shigella*, and limits the growth of *Proteus* and other dextrose-fermenting bacteria. Sodium desoxycholate and Sodium citrate inhibit the growth of Gram-positive organisms. Potassium phosphate is a reagent with a very high buffering capacity. Most Potassium phosphate buffer solutions consist of mixtures of the monobasic and dibasic forms of Potassium phosphate to varying degrees, depending on the desired pH.

Inoculate sample and incubate at 35 ± 2°C and observe after 6 and 24 hours.

If *Proteus* and *Pseudomonas aeruginosa* are present, the growth of these in the first hours of incubation is very scarce. The growth of *Salmonella* and *Shigella* is good. Due to this, the medium must be observed after 6 hours.

Growth in broth media is indicated by turbidity as opposed to an uninoculated control. Subculture onto appropriate media in order to isolate pathogens for identification.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 6 and 24 hours.

Microorganisms	Growth
Shigella flexneri ATCC 12022	Good
Salmonella typhimurium ATCC 14028	Good
Escherichia coli ATCC 25922	Good
Enterococcus faecalis ATCC 11700	Partially Inhibited
Bacillus cereus ATCC 11778	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Hajna, A.A. 1955. A new enrichment broth medium for Gram-negative organisms of the intestinal group. Public Health Lab. 13:83-89.

MacFaddin, J.F. 1985 Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. p. 357-359. Williams & Wilkins, Baltimore, MD.

HEART INFUSION BROTH

CAT. 1323

General purpose medium used for the growth of fastidious microorganisms

FORMULA IN g/l

Beef Heart Infusion	**10.00**	Sodium Chloride	**5 .00**
Tryptose	**10.00**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

HEART INFUSION BROTH (HIB) is a non-selective, general purpose medium used for the cultivation of many pathogenic and fastidious microorganisms. Highly pathogenic organisms, such as meningococci and pneumococci, can be grown on an infusion medium without enrichments.

The nutritionally rich base of Beef heart infusion and Tryptose provide nitrogen, vitamins, minerals and amino acids essential for the growth of a variety of microorganisms, and supply nutritional requirements for the growth of fastidious microorganisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Incubate at 35 ± 2°C and observe after 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Streptococcus pneumoniae ATCC 6305	Good
Streptococcus pyogenes ATCC 19615	Good
Staphylococcus aureus ATCC 25923	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Elliott, Kaysner, Jackson and Tamplin. 1995. In FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.

Vanderzant and Splittstoesser (ed.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

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HEKTOEN ENTERIC AGAR

CAT. 1030

For the isolation and differentiation of Gram-negative enteric bacteria

FORMULA IN g/l

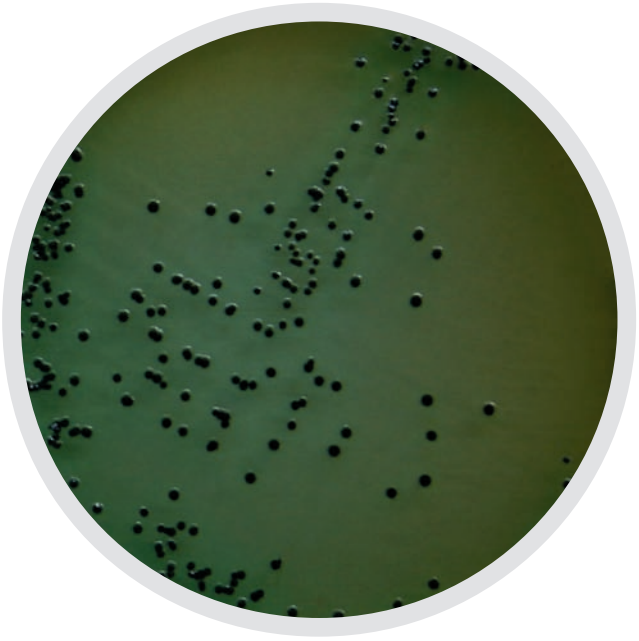
Meat Peptone	**12.00**	Yeast Extract	**3.00**
Sucrose	**12.00**	Salicin	**2.00**
Lactose	**12.00**	Ferric Ammonium Citrate	**1.50**
Bile Salts N°3	**9.00**	Acid Fuchsin	**0.10**
Sodium Chloride	**5.00**	Bromothymol Blue	**0.064**
Sodium Thiosulfate	**5.00**	Bacteriological Agar	**14.00**

Final pH 7.5 ± 0.2 at 25°C

PREPARATION

Suspend 76 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 55 - 60°C and pour into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is dusky green.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.



Salmonella typhimurium
ATCC 14028

USES

HEKTOEN ENTERIC AGAR is a differential and selective medium used for isolating and differentiating enteric pathogens such as *Salmonella* and *Shigella*, both of which cause a variety of serious human gastrointestinal diseases; and other Gram-negative Enterobacteriaceae.

It is used particularly in foods where multi-steps are followed to isolate the pathogens of gastroenteritis. The nutrients for growth are provided by the Meat peptone and Yeast extract. The increased content of the Peptone and the three fermentable carbohydrates (Lactose, Sucrose, Salicin) as sources of carbon and energy reduce the inhibitory action of the Bile salts on *Salmonella* and *Shigella spp.* The lactose concentration in this medium is higher than in many other media used for enterics since this helps the visualization of enteric pathogens and minimizes the problem of delayed lactose fermentation. Bromothymol blue and Acid fuchsin are pH indicators. Sodium thiosulfate provides Sulphur, and Ferric ammonium citrate is the indicator for H_2S production. H_2S positive colonies are black-centered. Sodium chloride maintains the osmotic balance.

The specimen is seeded by streaking directly on the surface of the medium, or by is first being enriched in Tetrathionate Broth (**Cat. 1114**), Selenite Cystine Broth (**Cat. 1220**) or GN Broth (**Cat. 1248**) and incubated at 35 ± 2°C for 18 - 24 hours. It is recommended to seed the sample on other selective media at the same time for Enterobacteriaceae because a larger number of positive cultures will be obtained. These media can be, for example, Eosin Methylene Blue Agar (**Cat. 1039**), MacConkey Agar (**Cat. 1052**), SS Agar (**Cat. 1064**), Brilliant Green Agar (**Cat. 1010**), Desoxycholate Lactose Agar (**Cat. 1025**), or XLD Agar (**Cat. 1080**).

Although suppressed, partially inhibited *E. coli* and other organisms which use lactose, sucrose, and/or salicin with the production of acid, give colonies whose tones vary from yellow

to orange to salmon. The *Salmonella* and *Shigella* are green or green-blue. *Proteus* is not inhibited but produces a green-yellow colony when it grows. The colonies of *Proteus* and *Salmonella* may present a black center and clear edges if they form iron sulfide as a result of H_2S production.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color	Inoculum (cfu/ml)	Recovery (cfu/ml)
Enterobacter aerogenes ATCC 13048	Acceptable	Orange	10^3-10^5	≥ 30
Escherichia coli ATCC 25922	Acceptable	Orange	<10^5	Not limited
Salmonella enteritidis ATCC 13076	Good	Blue-green	10^3-10^5	≥ 20
Salmonella typhimurium ATCC 14028	Good	Blue-green with black center	10^3-10^5	≥ 20
Shigella flexneri ATCC 12022	Good	Blue-green	10^3-10^5	≥ 5
Enterococcus faecalis ATCC 11700	Inhibited	-	>10^5	≤ 0.01

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

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Isenberg, Kominos & Siegel. Appl. Microbiol. 18:656. 1969. Hoben, Aston & Peterson Appl. Microbiol. 26:126. 1973.

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Goo et al Appl. Microbiol. 26:288, 1973.

INDOLE NITRATE MEDIUM (TRYPTICASEIN NITRATE MEDIUM)

CAT. 1504

For the differentiation of microorganism on the basis of indole production and the reduction of nitrate to nitrite

FORMULA IN g/l

Casein Peptone	**20.00**	Potassium Nitrate	**1.00**
Disodium Phosphate	**2.00**	Bacteriological Agar	**1.00**
Dextrose	**1.00**		

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. To perform motility and gas detection tests add 2 grams of agar. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into regular test tubes, half-fill them and sterilize in autoclave at 121°C for 15 minutes. If the prepared medium is semisolid allow solidification in tubes in a vertical position. Use the medium during the first 2 days after preparation. If kept longer, heat until boiling in a water bath to regenerate the medium. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

INDOLE NITRATE MEDIUM (Trypticasein Nitrate Medium) is a semisolid medium used to determine nitrate reduction and indole production by a wide variety of organisms. The reduction of nitrate is an important biochemical tool in the identification of many microorganisms. Nitrate reduction is an important characteristic of most members of the Enterobacteriaceae family.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Potassium nitrate acts as the substrate for determining nitrate reduction. Dextrose is the fermentable carbohydrate providing carbon and energy.

This medium is used to identify Gram-negative bacilli using 2 tests: one for Indole production and another for Nitrate reduction.

The test for indole should be conducted after 24 - 48 hours incubation (or after good bacterial growth) at a temperature of 35 ± 2°C and by adding a few drops of Kovacs Reagent (**Cat. 5205**). A positive test is indicated by the formation of a pink-to-red color in the reagent layer after a few minutes.

Indole Nitrite Medium can be used for nitrite tests with members of the Enterobacteriaceae family but is not recommended for the indole test with these organisms since they reduce nitrate to nitrite, thus preventing indole detection.

Nitrate reduction tests are conducted using Griess Reagent consisting of 2 solutions:

Solution A	Solution B
Sulfanilic acid .....8 g	Dimetil-α-naphtylamine.....5 g
Acetic acid 5N .....1 liter	Acetic acid 5N.....1 Liter

Store refrigerated at 4°C. Generally, both reactives (A and B) are stable for approximately 3 months.

For the investigation of nitrate reduction, use 3 separate tubes: a positive control (*Escherichia coli*), a negative control (*Acetobacter calcoaceticus*) and a third comparison tube.

Procedures:

1. Inoculate each tube heavily by stabbing.

2. Incubate at 35°C for 8, 12 and 24 hours.

3. Add approximately 5 drops of Solution A plus 5 drops of Solution B.

4. The formation of a red color in 1 - 2 minutes indicates the reduction of nitrates to nitrites (positive test).

5. If no color appears, add a pinch of zinc in powder form (free of nitrates and nitrites) to the tubes.

6. Observe if the red color forms or the culture remains colorless. **a)** If there is no nitrate reduction the zinc will be reduced to nitrite and will form a red color upon reacting with the Griess reagent. The test organism is negative (absence of nitrates). **b)** If there is no appearance of color, this indicates that the organism reduced the nitrate present in the culture medium to nitrite, possibly carrying the reaction to the gaseous nitrogen. The test organism is positive (presence of nitrates).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Nitrite	Indole
Escherichia coli ATCC 25922	Good	+	+
Salmonella typhimurium ATCC 14028	Good	+	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Finegold, S.M., Sutter, V.L.; Ahebery, H.R.; Rosenblatt, J.E.: Isolation of Anaerobic Bacteria. Man. Clin. Micro. Biol. 2nd ed. 1974. 365:375.

Finegold, S.M.; Rosenblatt, J.E.: Practical Aspects of Anaerobic Sepsis Medicine. 1973. 52(4), 311:322

IRON AGAR

CAT. 1458

For the detection of hydrogen sulfide production from microorganisms

FORMULA IN g/l

Tryptone	**20.00**	Sodium Thiosulfate	**0.3**
Sodium Chloride	**5.00**	Ferric Citrate	**0.3**
Beef Extract	**3.00**	Bacteriological Agar	**15.00**
Yeast Extract	**3.00**		
Final pH 6.5 ± 0.2 at 25°C			

PREPARATION

Suspend 46.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

IRON AGAR is used for detection of H_2S production.

Tryptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Sodium sulfide and Ferric citrate are H_2S indicators. Bacteriological agar is the solidifying agent.

When the medium becomes black along the inoculation line or within the butt, this indicates that hydrogen sulphide has been produced.

Inoculate and incubate at 35 ± 2°C during 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	H_2S
Enterobacter aerogenes ATCC 13048	Good	-
Escherichia coli ATCC 25922	Good	-
Proteus vulgaris ATCC 6380	Good	+
Salmonella enteritidis ATCC 13076	Good	+
Shewanella putrefaciens ATCC 8071	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Levine, Vaughn, Epstein and Anderson. 1932. Proc. Soc. Exp. Biol. Med. 29:1022.

Levine, Epstein and Vaughn. 1934. Am. J. Public Health 24:505.

IRGASAN TICARCILLIN AND POTASSIUM CHLORATE BROTH (ITC BROTH) ISO 10273

CAT. 1361

For the selective enrichment of *Yersinia enterocolitica*

FORMULA IN g/l

Anhydrous Magnesium Chloride	**28.10**	Yeast Extract	**1.00**
Enzymatic Casein Digest	**10.00**	Malachite Green	**0.01**
Sodium Chloride	**5.00**		
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 44.0 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add two vials of ITC Supplement (**Cat. 6051**), previously reconstituted in 5 ml of sterile distilled water each vial. Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2 - 8°C. The color is blue-green.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

ITC Supplement (Cat. 6051)

(Composition each vial for 500 ml)

Irgasan .....0.5 mg

Ticarcillin .....0.5 mg

Potassium Chlorate..... 500 mg

USES

ITC BROTH BASE (IRGASAN TICARCILLIN AND POTASSIUM CHLORATE) is recommended by ISO 10273 as a selective enrichment broth for the detection of the human pathogenic strain of *Yersinia enterocolitica* in food and water samples.

Enzymatic Casein Digest provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Magnesium chloride and Malachite Green, make the broth highly selective. Irgasan inhibits Gram-positive bacteria, Ticarcillin has bactericide on Gram-negative and Gram-positive bacteria and Potassium chlorate has a disinfecting property.

From those tubes where turbidity (growth) is observed, inoculate and incubate for 48 hours at 25°C on Salmonella Shigella Agar w/ Sodium desoxycholate & Calcium Chloride (SSDC) (**Cat. 1360**) to obtain isolated colonies from which a confirmation will be carried out.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Yersinia enterocolitica ATCC 23715	Good
Yersinia enterocolitica ATCC 9610	Good
Escherichia coli ATCC 25922	Inhibited
Bacillus cereus ATCC 11778	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 10273:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica

KAA CONFIRMATORY AGAR

CAT. 1027

For the isolation and confirmation of intestinal enteroccoci in foods according to Mossel

FORMULA IN g/l

Tryptone	**20.00**	Ammonium Ferric Citrate	**0.50**
Yeast Extract	**5.00**	Sodium Azide	**0.15**
Sodium Chloride	**5.00**	Kanamycin Sulfate	**0.020**
Sodium Citrate	**1.00**	Bacteriological Agar	**15.00**
Esculin	**1.00**		

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 48 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is tournasol-grey.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

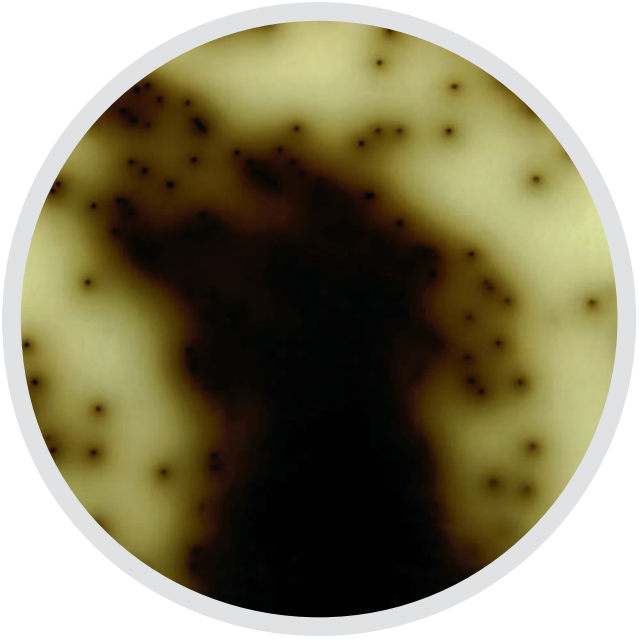
CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

KAA CONFIRMATORY AGAR (Kanamycin, Aesculin, Azide) is a selective medium for the isolation and confirmation of intestinal enteroccoci in foods. KAA Confirmatory Agar is used to confirm positives from KAA Presumptive Broth (**Cat. 1209**) tubes.

Kanamycin, Sodium azide and Sodium citrate have a great inhibitory effect on the accompanying bacterial flora, they inhibit the growth of Gram-positive and Gram-negative bacteria, and the medium is highly selective for esculin-hydrolyzing enterococci. Esculin and Ferric Ammonium citrate are esculin indicators which detect the esculin-hydrolysing bacteria. They hydrolyze the glucoside esculin to give glucose and esculetin. These microorganisms present black zones around the colonies from the reaction of the resulting esculetin with the iron ions. Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The presence of intestinal enterococci, is an indicator for faecal contamination, especially when the contamination occurred long ago and the less resistant coliform bacteria, including *Escherichia coli*, are already dead when the analysis is carried out.



Enterococcus faecalis
ATCC 11700

Streak to obtain isolated colonies and incubate at 35 ± 2°C for 24 - 48 hours. Intestinal enterococci grow forming small, translucent colonies surrounded by a black halo. This medium is recommended by CeNAN for food and drinks analysis.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Color Change	Esculin hydrolisis
Enterococcus faecalis ATCC 11700	Good	Olive green-black	+
Enterococcus faecium ATCC 8043	Good	Olive green-black	+
Staphylococcus aureus ATCC 6538	Moderate		
Escherichia coli ATCC 11775	Inhibited		
Lactococcus lactis ATCC 19435	Slightly inhibited	Olive green-black to colorless	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

M.R. Pascual Anderson. Técnicas para Examen Microbiológico de Alimentos y Bebidas (Centro Nacional de Alimentación y Nutrición CeNAN) Madrid, 1982.Brandl, E. Aspergerger H., Pfleger, F. U-IBEN CH: Zum Vorkomment von D-streptokokken in Käse. 1985..

KAA PRESUMPTIVE BROTH

CAT. 1209

For the presumptive detection of intestinal enterococci in foods according to Mossel

FORMULA IN g/l

Tryptone	**20.00**	Esculin	**1.00**
Yeast Extract	**5.00**	Ferric Ammonium Citrate	**0.50**
Sodium Chloride	**5.00**	Sodium Azide	**0.15**
Sodium Citrate	**1.00**	Kanamycin Sulfate	**0.02**

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 33 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is tournasol.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

KAA PRESUMPTIVE BROTH (Kanamycin, Aesculin, Azide) is a selective medium for the isolation of intestinal enterococci in foods. KAA Presumptive Broth is used in conjunction with KAA Confirmatory Agar (**Cat. 1027**).

Kanamycin, Sodium azide and Sodium citrate have a great inhibitory effect on the accompanying bacterial flora, they inhibit the growth of Gram-positive and Gram -negative bacteria, and the medium is highly selective for esculin-hydrolyzing enterococci. Esculin and Ferric Ammonium citrate are esculin indicators which detect the esculin-hydrolysing bacteria. They hydrolyze the glucoside esculin to give glucose and esculetin. Tubes have a black color due to the reaction of the resulting esculetin with the iron ions. Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

The presence of intestinal enterococci is an indicator for faecal en contamination, especially when the contamination occurred long ago and the less resistant coliform bacteria, including *Escherichia coli*, are already dead when the analysis is carried out.

Inoculate 1 ml of sample in tubes with 9 ml of single-strength medium. Always use 5 tubes for the MPN count technique. Incubate at 35 ± 2°C for 24 - 48 hours. Presumptive positive tubes have a brown-black color. This medium is recommended by CeNAN for food and drinks analysis.

Confirmatory tests, e.g. catalase test, glucose utilisation and growth at 45 °C ± 1 °C, may be carried out.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Esculin hydrolisis
Enterococcus faecalis ATCC 11700	Good	+
Enterococcus faecium ATCC 8043	Good	+
Staphylococcus aureus ATCC 6538	Moderate	
Escherichia coli ATCC 11775	Inhibited	-
Lactococcus lactis ATCC 19435	Moderate-Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

M.R. Pascual Anderson Técnicas para Examen Microbiológico de Alimentos y Bebidas (Centro Nacional de Alimentación y Nutrición CeNAN) Madrid, 1982Corps pag. 76 Aleman

Brandl, E. Aspergerger H., Pfleger, F. U-IBEN CH: Zum Vorkomment von D-streptokokken in Käse. 1985..

KF STREPTOCOCCAL AGAR

CAT. 1034

For the selective isolation and enumeration of fecal enterococci by direct culture or by membrane-filtration

FORMULA IN g/l

Maltose	**20.00**	Sodium Chloride	**5.00**
Peptone Mixture	**10.00**	Lactose	**1.00**
Yeast Extract	**10.00**	Sodium Azide	**0.40**
Sodium Glycerophos-phate	**10.00**	Bacteriological Agar	**20.00**

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 76.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at

121°C for 10 minutes. Cool to 45 - 50°C and aseptically add two vials of TTC 1% supplement (**Cat. 6030**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is clear amber with a pink tint.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

TTC 1% SUPPLEMENT (Cat. 6030)

Triphenyl Tetrazolium Chloride ..... 50 mg

Pour medium into sterile Petri dishes if using the Membrane Filter Technique. If using the Pour Plate Method, hold the liquid medium at 45°C.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

KF STREPTOCOCCAL AGAR Base is a selective medium for the isolation and enumeration of fecal enterococci in water, foodstuffs and other materials, according to the formula developed by Kenner, Clark and Kabler.

It is used for the plate count of enterococci in water samples and for determining the presence of *Enterococcus faecalis* in milk and its derivatives, as well as in other foods. The isolation and enumeration of fecal enterococci is made according to APHA for the examination of water (1998) and foodstuffs (1992).

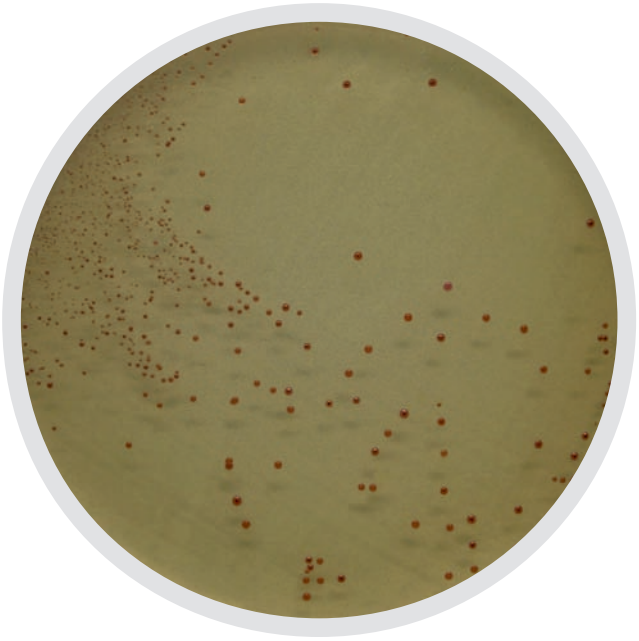
Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Maltose and Lactose are the fermentable carbohydrates as carbon and energy sources. Sodium glycerophosphate is a buffering agent. Sodium azide is a selective agent that inhibits Gram-positive bacteria. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The addition of TTC 1% Supplement (2,3,5 Trypheniltetrazolium chloride) allows fecal enterococci to develop a red color as the result of the reduction of tetrazolium to formazan, an insoluble red pigment, by actively growing microbial cells.

Pour Plate Method: Place selected dilution of sample in Petri Dish. Pour 15 ml of prepared medium at 45°C into each plate. Thoroughly mix and allow agar to solidify. Incubate plates in inverted position at 35 ± 2°C for 46 - 48 hours.

Membrane Filter Technique: Filter suitable volume of sample through sterile membrane. Place membrane filter, inoculum side up, on solidified agar in Petri dish. Incubate inverted plates at 35 ± 2°C for 46 - 48 hours.

The red or pink colonies are counted as fecal enterococci, while colonies with orange, yellow, white or other colors are not counted. The number of fecal enterococci is calculated per 100 ml of water.



Enterococcus faecalis
ATCC 19433

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with supplement added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 46 - 48 hours.

Microorganisms	Growth	Esculin hydrolisis
Enterobacter aerogenes ATCC 13048	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Enterococcus faecalis ATCC 19433	Good	Red
Enterococcus faecalis ATCC 29212	Good	Red

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Donnelly C.W., R.E. Bracket, D.Doores, W.H. Lee, and J. Lovett. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

KF STREPTOCOCCAL BROTH

CAT. 1346

For the isolation of *Enterococcus faecalis*

FORMULA IN g/l

Maltose	**20.00**	Sodium Chloride	**5.00**
Proteose Peptone N°3	**10.00**	Lactose	**1.00**
Yeast Extract	**10.00**	Sodium Azide	**0.40**
Sodium Glycerophosphate	**10.00**	Bromocresol Purple	**0.015**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 56.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 10 minutes. Cool to 45 - 50°C and aseptically add 2 vials of 1% TTC supplement (**Cat. 6030**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2 - 8°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

TTC 1% SUPPLEMENT (Cat. 6030)

(composition each vial for 500 ml of medium)

Triphenyl Tetrazolium Chloride ..... 50 mg

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

KF (Kenner Fecal) STREPTOCOCCAL BROTH was developed by Kenner et al for the detection and enumeration of enterococci in waters. They found that this formulation was superior to other liquid media for the recovery of enterococci in Most Probable Number (MPN) test systems. The medium is not specific for the presumptive identification of group D streptococci. The addition of 1% TTC (2,3,5-Triphenyl Tetrazolium Chloride), in the membrane filter procedure causes the enterococci to have a deep red color as a result of tetrazolium reduction to formazan, an insoluble red pigment, by actively growing microbial cells.

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Maltose and Lactose are the fermentable carbohydrates as carbon and energy sources. Sodium glycerophosphate is a buffering agent. Sodium azide is a selective agent that inhibits Gram-positive bacteria. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bromocresol purple is the pH indicator with a color change from purple to yellow.

The presence of intestinal enterococci is an indicator for faecal contamination, especially when the contamination occurred long before, and the less resistant coliform bacteria, including *Escherichia coli*, are already dead when the analysis is carried out.

Inoculate and incubate at 35 ± 1 °C in a atmosphere saturated with water vapour for 46 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 1 °C in a atmosphere saturated with water vapour for 46 - 48 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Inhibited	
Escherichia coli ATCC 25922	Inhibited	-
Enterococcus faecalis ATCC 19433	Good	Red
Enterococcus faecalis ATCC 29212	Good	Red

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Kenner, Clark and Kabler. 1961. Appl. Microbiol. 9.15.2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md

Facldam and Moody. 1970. Appl. Microbiol. 20.245

KING A MEDIUM (PSEUDOMONAS P AGAR) USP

CAT. 1531

For the identification of *Pseudomonas spp.* based on fluorescein production

FORMULA IN g/l

Gelatin Pancreatic Digest	**20.00**	Magnesium Chloride	**1.40**
Potassium Sulfate	**10.00**	Bacteriological Agar	**13.60**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 45 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15

minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

KING A MEDIUM (Pseudomonas P Agar) is prepared according to the formula described by King et al. for the detection and differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* based on pyocyanin production and fluorescein (pyoverdin) inhibition.

Pseudomonas aeruginosa is a free-living bacterium, present in soil and water. It has become more and more known as an emerging opportunistic pathogen of clinical importance. Various different epidemiological studies track its occurrence as a nosocomial pathogen and claim that antibiotic resistance is increasing in clinical isolates.

This medium contains Gelatin pancreatic digest as a rich nitrogen source, and other nutrients for growth as vitamins, minerals and amino acids. Gelatin peptone is low in phosphorous to reduce the inhibitory action on pyocyanin production. Potassium sulfate and Magnesium chloride provide cations to activate pyocyanin production and enhance pigment production. Glycerol is a carbon source. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

This medium promotes the production of pyocyanin, a blue-green pigment which oxidizes to brown, is water-soluble and, unlike fluorescein, is soluble in chloroform. The pigment diffuses throughout the medium and the blue color is observed. Confirmation of pyocyanin production is by chloroform extraction. Add 2 ml of chloroform to a tube of medium and shake gently to remove pigment.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with glycerol added, after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Pseudomonas aeruginosa ATCC 9027	Good	Blue
Pseudomonas aeruginosa ATCC 25619	Good	Blue-green
Pseudomonas aeruginosa ATCC 27853	Good	Blue

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

US P

King E.O. Ward M.K. Raney D.E.-J. Lab. and Clin Med, 1954. 44. 301-307

Bacteriological Analytical Manual, 8th edition. 1995. AOAC International, Gaithersburg, MD.

The United States Pharmacopoeia. 1995. The United States Pharmacopoeia, 23rd ed. United States Pharmacopoeial Convention, Rockville, MD.

KING B MEDIUM
UNE-EN 12780, EN ISO 16266

CAT. 1154

For the identification and enumeration of *Pseudomonas aeruginosa* by membrane filtration

FORMULA IN g/l

Peptone	**20.00**	Heptahydrated Magnesium Sulfate	**1.50**
Potassium Hydrogen Phosphate	**1.50**	Bacteriological Agar	**15.00**
Final pH 7.2± 0.2 at 25°C			

PREPARATION

Suspend 38 grams of the medium in one liter of distilled water. Add 10 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes, 5 ml in each tube, and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position. The prepared medium should be stored at 2 - 8ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

KING B MEDIUM is prepared according to the UNE-EN 12780. It is a confirmation medium for the detection and differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* based on fluorescein (pyoverdin) production and pyocyanin inhibition.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. It also aids in the production of fluorescein. Potassium hydrogen phosphate is a phosphorus source, and Magnesium sulfate provides cations to activate fluorescein production. Glycerol is a carbon source. Bacteriological agar is the solidifying agent.

Red color colonies from Nutrient Agar (UNE-EN 12780; **Cat. 1156**) that have are oxidase-positive are sown again and incubated for five days maximum (normally 24 hours are enough) at 36 ± 2°C. The growth is examined daily under ultraviolet light and the presence of any fluorescence is registered.

This medium promotes the production of pyoverdin, a yellow-green fluorescent pigment that can be oxidized to yellow. It is water soluble and, unlike pyocyanin, is not soluble in chloroform. The pigment diffuses throughout the medium and the fluorescent yellow-green color is observed by use of a Wood's UV lamp.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with glycerol added, after incubation at a temperature of 36 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Fluores-cein
Pseudomonas aeruginosa ATCC 9027	Good	+
Pseudomonas aeruginosa ATCC 10145	Good	+
Pseudomonas aeruginosa ATCC 25783	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

UNE-EN 1 2780, Quality of water. Identification and enumeration of Pseudomonas aeruginosa by membrane filtration

KING B MEDIUM (PSEUDOMONAS F AGAR) USP

CAT. 1532

For the identification of *Pseudomonas spp.* based on fluorescein production

FORMULA IN g/l

Peptone Mixture	**20.00**	Magnesium Sulfate	**1.50**
Dipotassium Phosphate	**1.50**	Bacteriological Agar	**14.00**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 37 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

KING B MEDIUM (Pseudomonas F Agar Base) is prepared according to the formula described by King *et al*. for the detection and differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* based on fluorescein (pyoverdin) production and pyocyanin inhibition.

Pseudomonas aeruginosa is a free-living bacterium, present in soil and water. It has become more and more known as an emerging opportunistic pathogen of clinical importance. Various different epidemiological studies track its occurrence as a nosocomial pathogen and claim that antibiotic resistance is increasing in clinical isolates.

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. It also aids in the production of pyoverdin. Dipotassium phosphate is a phosphorus source, and Magnesium sulfate provides cations to activate pyoverdin production. Glycerol is a carbon source. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

This medium promotes the production of fluorescein (pyoverdin), a green-yellow fluorescent pigment that oxidizes to yellow. It is water-soluble and, unlike pyocyanin (blue-green pigment), it is not soluble in chloroform. The pigment diffuses throughout the medium and the fluorescent yellow-green color is observed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with glycerol added, after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Pseudomonas aeruginosa ATCC 9027	Good	Yellow-green
Pseudomonas aeruginosa ATCC 10145	Good	Yellow-green
Pseudomonas aeruginosa ATCC 25619	Good	Yellow-green
Pseudomonas aeruginosa ATCC 27853	Good	Yellow-green

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

US P

King E.O. Ward M.K. Raney D.E.-J. Lab. and Clin Med, 1954. 44. 301-307

Bacteriological Analytical Manual, 8th edition. 1995. AOAC International, Gaithersburg, MD.

KING FG AGAR

CAT. 1053

For the enumeration of psychotropic microorganisms in foods

FORMULA IN g/l

Bacteriological Peptone	**20.00**	Potassium Phosphate	**1.50**
Maltose	**10.00**	Magnesium Sulfate	**0.75**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 52.25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 2 ml of sterile-filtered 0.05% crystal violet solution. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

KING FG AGAR is used for the enumeration of psychotropic microorganisms in foods and drinks.

Psychotropic organisms can tolerate low temperatures between 4 - 20°C. Organisms in this group are *Pseudomonas*, *Achromobacter, Alcaligenes, Flavobacterium*, and *Aeromonas*, as well as other species of enterobacteriaceae from the genera: *Escherichia, Proteus, Klebsiella, Enterobacter* and *Hafnia*. These are all Gram-negative bacteria.

Peptone provides provide nitrogen, vitamins, minerals and amino acids essential for growth. Maltose is a fermentable carbohydrate as an energy and carbon source. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Potassium phosphate and Magnesium sulfate provide minerals and ions and act as a buffer. Bacteriological agar is the solidifying agent. Crystal violet inhibits gram-positive bacteria.

The total count per ml of food sample is performed using serial dilutions, placing 1.0 ml of each dilution on the surface of the medium and spreading it with a sterile glass rod. Incubation should last 5 days at 17°C.

Count only larger (not punctiform) colonies and multiply by the dilution factor to obtain the total count.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with Crystal violet added, after incubation at a temperature of 17°C and observed after 4 - 5 days.

Microorganisms	Growth
Pseudomonas spp.	Good
Escherichia coli ATCC 25922	Good
Proteus mirabilis ATCC 14273	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Pascual Anderson – Metodología analítica para alimentación y bebidas - Diaz Santos, 1999.

KLIGLER IRON AGAR

CAT. 1042

For the differentiation of Gram-negative Enterobacteria

FORMULA IN g/l

Peptone Mixture	**20.00**	Ferric Ammonium Citrate	**0.50**
Lactose	**10.00**	Sodium Thiosulfate	**0.50**
Sodium Chloride	**5.00**	Phenol red	**0.025**
Dextrose	**1.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 52 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5 - 2 cm depth. The prepared medium should be stored at 2 - 8°C. The color is pink-

orange. For best results, Kliger Iron Agar should be used on the day of preparation or melted and solidified before use.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

Uninoculated Tube *Salmonella enteritidis* ATCC 13076

USES

KLIGLER IRON AGAR may be used to differentiate Gram-negative Enterobacteria on the basis of carbohydrate fermentation and H_2S production.

The Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose and Lactose are the fermentable carbohydrates, producing acid indicated by the Phenol red indicator. The color changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Sodium sulfide and Ferric ammonium citrate are H_2S indicators. Bacteriological agar is the solidifying agent.

Inoculate the medium with the colony under study by stabbing the butt and streaking the surface of the tube. Incubate at 35 ± 2°C for 24 hours. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid formation caused by the fermentation of dextrose. Once the dextrose supply runs out in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids. The reversion does not occur in the anaerobic environment in the butt, which remains acid (yellow butt). Lactose fermenters produce yellow slants and butts due to the fact that sufficient acid is produced in the slant to maintain an acid pH under aerobic conditions. Organisms incapable of fermenting the carbohydrates produce red slants and butts.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Slant	Base	H_2S	Gas
Escherichia coli ATCC 25922	Good	Yellow	Yellow	-	+
Proteus vulgaris ATCC 6380	Good	Red	Yellow	+	-
Salmonella enteritidis ATCC 13076	Good	Red	Yellow	+	+
Shigella flexneri ATCC 12022	Good	Red	Yellow	-	-
Citrobacter freundii ATCC 8090	Good	Yellow	Yellow	+	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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J. Bact. 13:1 83. 1927. J. Bact. Clin. Med. 25:649, 1940.

KLIGLER IRON AGAR ISO 10273

CAT. 1364

For the presumptive test of *Yersinia enterocolitica*

FORMULA IN g/l

Pancreatic Digest of Casein	**20.00**	Glucose	**1.00**
Lactose	**10.00**	Ferrous Sulfate	**0.2**
Sodium Chloride	**5.00**	Sodium Tiosulfate Anhydrous	**0.2**
Meat Peptone	**3.00**	Phenol red	**0.025**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 57.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5 - 2.0 cm depth. The prepared medium should be stored at 2 - 8ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

KLIGLER IRON AGAR is recommended by the ISO normative 10273 for presumptive tests of *Yersinia enterocolitica*.

Peptone Digest of Casein and Meat Peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose and Lactose are the fermentable carbohydrates producing acid, indicated by the Phenol red indicator. The color changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Sodium Thiosulfate is the H_2S indicator. Ferrous Sulfate provides sources of sulfates and metallic ions. Bacteriological agar is the solidifying agent.

Once the colonies are purified in the Nutrient Agar (**Cat.1060**), spread them on Kligler Iron Agar and incubate at 30°C for 24 - 48 hours.

Interpretation of color changes:

BUTTS:

YELLOW – glucose-positive (fermentation of glucose)

RED or WITHOUT CHANGE – glucose-negative (Glucose doesn't ferment)

BLACK – Formation of H_2S

BUBBLES – Formation of Gas

SLANTED POSITION:

YELLOW – Lactose-positive (use of Lactose).

RED or WITHOUT CHANGE – Lactose-negative (no use of Lactose).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 24 - 48 hours.

Microorganisms	Growth	Slant	Recovery	Base	H_2S	Gas
Yersinia enterocolitica ATCC 27729	Good	Red	Good	Yellow	-	-
Escherichia coli ATCC 25922	Good	Yellow	Good	Yellow	-	+
Proteus vulgaris ATCC 6380	Good	Red	Good	Yellow	+	-
Salmonella enteritidis ATCC 13076	Good	Red	Good	Yellow	+	+
Shigella flexneri ATCC 12022	Good	Red	Good	Yellow	-	-
Citrobacter freundii ATCC 8090	Good	Yellow	Good	Yellow	+	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 10273 Microbiology of Food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica.

KOSER CITRATE BROTH

CAT. 1200

For the differentiation of *Escherichia coli* and *Enterobacter* on the basis of citrate use

FORMULA IN g/l

Sodium Citrate	3.00	Monopotassium Phosphate	1.00
Sodium Ammonium Phosphate	1.50	Magnesium Sulfate	0.20
Final pH 6.7 ± 0.2 at 25°C			

PREPARATION

Suspend 5.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into screw-capped tubes and sterilize in autoclave at 121°C for 15 minutes. Tighten the caps after sterilization. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is colorless.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

KOSER CITRATE BROTH is used to differentiate *Escherichia coli* from the Enterobacter group on the basis of citrate use utilization.

It is used in the same way as Simmons Citrate Agar (**Cat. 1014**), with the advantage of differentiating between coliforms of fecal origin (the majority is citrate-negative) and organisms from dirt that are 90% positive according to Wilson and Miles. These same authors report that only 6.7% of the coliforms isolated from human or animal feces are citrate-positive. *Enterobacter aerogenes* and *Enterobacter cloacae* use Sodium citrate as a source of carbon and the inorganic Ammonium phosphate salt as a source of nitrogen. *Escherichia coli* cannot use Sodium citrate as carbon source and does not grow in this medium. Biochemical identification methods for identifying *E. coli* frequently include Koser citrate.

Magnesium sulphate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication. Monopotassium phosphate is a buffer.

Inoculate and incubate at 35 ± 2°C for 18 – 24 hours. Any bacteria able to use citrate as their carbon source will grow in the medium causing turbidity.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth
Enterobacter aerogenes ATCC 13048	Good
Enterobacter cloacae ATCC 23355	Good
Escherichia coli ATCC 25922	Null

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Koser J. Bact. 8:493. 1973. Wilson G.S. and Miles A.A., "Topley and Wilson's Principles of Bacteriology and Inmunology", 4th Ed., Edward Arnold Ltd., London, Vol. 1. page 760.

LACTOSE AGAR WITH BROMOTHYMOL BLUE AND CRYSTAL VIOLET (DRIGALSKI)

CAT. 1344

Selective medium for Gram-negative Enterobacteria in urine and feces

FORMULA IN g/l

Lactose	**15.00**	Sodium Thiosulfate	**1.00**
Casein Peptone	**7.50**	Sodium Desoxycholate	**1.00**
Meat Peptone	**7.50**	Bromothymol Blue	**0.08**
Beef Extract	**3.00**	Crystal Violet	**0.005**
Yeast Extract	**3.00**	Bacteriological Agar	**12.00**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 115 °C for 20 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is dark green.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LACTOSE AGAR WITH BROMOTHYMOL BLUE AND WITH CRYSTAL VIOLET (DRIGALSKI) is a selective medium used for the isolation of Gram-negatives bacteria from urine, feces and other clinical specimens.

Casein peptone, Meat peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Lactose is the fermentable carbohydrate providing carbon and energy. Sodium thiosulfate, Sodium desoxycholate and Crystal violet inhibit Gram-positive organisms. Bromothymol blue is the pH indicator. Bacteriological agar is the solidifying agent.

The growth of Gram-negative bacteria depends on their ability to ferment lactose. Coliform organisms (*E. coli, Klebsiella, Citrobacter, Enterobacter*) ferment lactose with acid production, resulting in a colour change of the pH indicator bromothymol blue that turns yellow when acid is produced. Gram-negative lactose non-fermenting bacteria (*Salmonella, Shigella, Proteus, Alkaligenes, Pseudomonas*, etc) grow with blue-green colonies.

Incubate at temperature of 35 ± 2°C and observe after 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Yellow
Klebsiella pneumoniae ATCC 13883	Good	Yellow
Salmonella typhimurium ATCC 14028	Good	Blue – Green
Alcaligenes faecalis ATCC 8750	Good	Blue – Green

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

V. Drigalski, H. Conrad, Z. Hyg. Infektionskr., 39, 298 (1902)

Wurtz, Technique Bacteriologique, Paris, Masson (1897)

Williams and Wilkins, Baltimore (1985)

LACTOSE BROTH EUROPEAN PHARMACOPOEIA

CAT. 1206

For the cultivation of *coliforms* and *Salmonella* in water, foods, dairy products and other materials

FORMULA IN g/l

Pancreatic Digest of Gelatin	**5.00**	Beef Extract	**3.00**
Lactose Monohydrate	**5.00**		
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 13 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Dispense into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 121°C for 15 minutes. Cool as quickly as possible. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Escherichia coli ATCC 25922 — Uninoculated Tube

USES

LACTOSE BROTH is included in many Standard Methods for testing foods, dairy products and other materials for Enterobacteria and other Gram-negative microorganisms. It is also widely used for testing for coliforms in water and foods.

It is used as preenrichment for testing foods and dairy products for *Salmonella*, where conservation processes may have injured or reduced their numbers. Preenrichment in a non-selective medium allows for repair of cell damage, diluting toxic or inhibitory substances, and provides a nutritional base for *Salmonella*. A preenrichment medium provides a higher ratio of *Salmonella* to non-*Salmonella* organisms after incubation. Most non-*Salmonella* bacteria ferment lactose whilst *Salmonella* does not. As lactose is metabolized, the pH decreases creating a bacteriostatic effect on competing organisms.

Gelatin and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is a complex carbohydrate energy source.

Check the sterilization of the medium by incubating the tubes at 35°C for 24 hours prior to inoculation. Ensure that the fermentation tubes are free from air bubbles before inoculation. Seed aliquots of 1, 10 or 100 ml of the sample liquid in adequate containers according to the quantity of the medium. Incubate at 35 ± 2°C for 18 - 24 hours and check for the presence of gas, which constitutes a presumptive test. Subculture to VRBGL (**Cat. 1144**) for detection and quantification tests.

Large water samples may require a double-strength of Lactose Broth to maintain correct concentration of the medium.

The European Pharmacopoeia recommends in Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" this medium in the detection test of Enterobacteriaceae, recognizing recovery of other microorganisms such as *Aeromonas* and *Pseudomonas*, for example. Lactose Broth is used to prepare the non-sterile product to be examined in place of Buffered Peptone Water (**Cat. 1401**), incubated at 35 - 37°C for 2 - 5 hours, a time sufficient to revive bacteria but not to multiply. 1 g or 1 ml of the contents are then transferred to 100ml of enrichment Mossel EE Broth (**Cat. 1202**), incubated at 35 ± 2°C for 18 - 48 hours, subculturing to VRBGL (**Cat. 1144**). For more details consult the Standard Methods for water, milk, and food analysis texts.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Gas production
Escherichia coli ATCC 25922	Good	+
Klebsiella pneumoniae ATCC 13883	Good	+
Salmonella typhimurium ATCC 14028	Good	-
Proteus vulgaris ATCC 13315	Good	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

European Pharmacopoeia, 6th Edition 2007

American Public Health Association. Standard Methods of the Examination of Dairy Products, 12th Edition APHA, New York, 12th,

1967. American Public Health Association. Standard Methods for the Examination of Water and Wastewater Edition APHA, Inc. New York, 1966.

LACTOSE SULFITE BROTH BASE EUROPEAN PHARMACOPOEIA

CAT. 1009

Selective medium recommended for the detection and enumeration of spores of *Clostridium perfringens* in food products

FORMULA IN g/l

Lactose Monohydrate	**10.00**	Yeast Extract	**2.50**
Pancreatic Digest of Casein	**5.00**	Cysteine Hydrochloride	**0.30**
Sodium Chloride	**2.50**		
Final pH 7.1 ± 0.2 at 25°C			

PREPARATION

Suspend 20.3 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense 8 ml per tube with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. Before using, add to each tube 0.5 ml of a 12 g/liter solution of Sodium Metabisulfite and 0.5 ml of a solution of 10 g/liter of Ferric Ammonium Citrate. Both solutions have to be freshly prepared and sterilized. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LACTOSE SULFITE BROTH BASE is a selective medium used to detect and enumerate spores of *Clostridium perfringens* based on lactose fermentation and production of hydrogen sulfide.

The nutrient base provides optimal conditions for the development of Clostridia. Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Lactose is a complex carbohydrate energy source. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Cysteine hydrochloride is the reducing agent.

European Pharmacopoeia recommends to prepare samples using 1:100 and 1:1000 dilutions with Buffered Peptone Water (**Cat. 1401**). Determine the most probable number of bacteria (MPN) in tubes or other suitable containers with small Durham tubes. Mix the sample and the medium with minimum shaking and incubate at 45.5 - 46.5°C for 24 - 48 hours.

Colonies producing hydrogen sulfide are characterized by a blackening due to the reaction of Sodium bisulfite and the Ferric ammonium citrate salt. The containers showing a blackening and abundant formation of gas in the Durham tube (at least 1/10 of the volume) indicate the presence of *C. perfringens*. Estimate the most probable number using the appropriate table (MPN Table).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, from type cultures, with additives added, after incubation at a temperature of 45.5 - 46.5°C and observed after 24 - 48 hours. According to European Pharmacopoeia 7.0.

Microorganisms	Growth	Gas production	Blackening
Clostridium prefringens ATCC 13124	Good	+	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

European Pharmacopoeia 6th Edition 2007

LAURYL SULFATE AGAR (FOR MEMBRANE FILTRATION)

CAT. 1309

Selective isolation and enumeration of coliforms

FORMULA IN g/l

Casein Peptone	**40.00**	Sodium Lauryl Sulfate	**1.00**
Lactose	**30.00**	Phenol red	**0.20**
Yeast Extract	**6.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 92.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and beige pink in color. If there are any physical changes, discard the medium.

USES

LAURYL SULFATE AGAR is a selective medium used in the presumptive coliforms detection Method in waters, dairy products, seafood and foods, according to APHA Standard Methods.

The coliform group is both aerobic and anaerobic facultative, Gram-negative, non-spore forming rods which ferment lactose producing acid and gas at 35°C within 48 hours.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Lactose is a fermentable complex carbohydrate energy source. Sodium lauryl sulfate is the selective agent used to inhibit organisms other than coliforms. Sporulating aerobic bacteria are completely inhibited. Phenol red is a pH indicator. Bacteriological agar is the solidifying agent.

Membrane Filter method: Filter a suitable volume of sample through a sterile membrane. Place membrane filter, inoculum side up, on solidified agar in the Petri dish. Incubate inverted plates at 35 ± 2°C for 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Good	Yellow
Escherichia coli ATCC 25922	Good	Yellow
Salmonella enteritidis ATCC 13076	Good	Colorless
Staphylococcus aureus ATCC 25923	Inhibited	
Enterococcus faecalis ATCC 19433	Inhibited	
Pseudomonas aeruginosa ATCC 10145	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

APHA 1998 Standard Methods for the examination of water and wastewater, 20th edition.

LAURYL SULFATE BROTH (LAURYL TRYPTOSE BROTH – LTB) ISO 4831

CAT. 1310

For the detection of coliforms in waters

FORMULA IN g/l

Tryptose	**20.00**	Dipotassium Phosphate	**2.75**
Sodium Chloride	**5.00**	Monopotassium Phosphate	**2.75**
Lactose	**5.00**	Sodium Lauryl Sulfate	**0.10**
Final pH 6.8± 0.2 at 25°C			

PREPARATION

Suspend 35.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and pale beige in color. If there are any physical changes, discard the medium.

NOTE: Refrigerated broth is cloudy, but clears considerably at room or incubator temperatures. Clarity is not required for the performance of the medium because only gas formation is considered significant.

USES

LAURYL SULFATE BROTH is a selective medium recommended for the enumeration of coliforms in water and dairy products as well as for confirmatory tests of lactose fermentation with gas production by coliforms in foods. Another advantage of this medium is that the indole test can be performed directly in the tube.

APHA recommends the use of Lauryl Tryptose Broth for the Most Probable Number Presumptive Test of coliforms in waters, effluent or sewage, also as a confirmatory test of lactose fermentation with gas production for milk samples, and for the detection of coliforms in foods. This broth was elaborated to promote a rich growth and high gas production from small inocula of coliform organisms.

The coliform group is both aerobic and anaerobic, Gram-negative, non-pore forming, and ferment lactose producing acid and gas at 35°C within 48 hours.

Tryptose in a 2% concentration improves the early growth phase of coliforms when compared to Casein peptone. The buffered broth allows slow lactose fermenters to increase gas production in a shorter time.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is a fermentable complex carbohydrate energy source. Potassium phosphates are the buffering agents, and Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium lauryl

sulfate is the selective agent used to inhibit organisms other than coliforms. Sporulating aerobic bacteria are completely inhibited.

Inoculate sample and incubate at 35 ± 2°C for 24 - 48 hours. After 24 hours, measure gas production in the Durham fermentation tubes. If no gas has been formed and trapped in the inverted tube, reincubate and re-examine after a further 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Gas production	Inoculum (cfu/ml)	Recovery Rate %
Enterobacter aerogenes ATCC 13048	Good	+	10^3-10^5	≥30
Escherichia coli ATCC 25922	Good	+	10^3-10^5	≥30
Escherichia coli ATCC 8739	Good	+	10^3-10^5	≥30
Salmonella typhimurium ATCC 14028	Good	-	10^3-10^5	≥30
Staphylococcus aureus ATCC 25923	Markedly Inhibited	-	10^3	≤ 0.01

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 4831 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique

APHA 1998. Standard Methods for the examination of water and wastewater, 20th Edition.

Association of Official Analitical Chemist. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD. Association of Official Analytical Chemists. 1995. Oficial methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA..

LAURYL SULPHATE TRYPTOSE BROTH MODIFIED (mLST) ISO 22964

CAT. 1445

For the selective enrichment of *Enterobacter sakazakii*

FORMULA IN g/l

Sodium Chloride	**34.00**	Monopotassium Phosphate	**2.75**
Enzymatic Digest of Plants & Animal Tissue	**20.00**	Dipotassium Phosphate	**2.75**
Lactose	**5.00**	Sodium Lauryl Sulfate	**0.10**
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 64.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 0.1 ml of Vancomycin Supplement (10mg/10ml) to the 10ml of Lauryl Sulphate Tryptose Broth Modified (final concentration 10 µg/ml of m LST Broth). Homogenize gently and dispense into 10ml tubes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LAURYL SULPHATE TRYPTOSE BROTH MODIFIED (mLST) is recommended for the selective enrichment of *Enterobacter sakazakii* in milk powder and powered infant formula. *E. sakazakii* is currently considered an emerging pathogen responsible for unweaned babies, risking severe meningitis and necrotic enterocolitis that can be the cause of a mortality rate between 40 and 80%.

The pathogenicity of *Enterobacteri sakazakki* for unweaned babies makes it necessary to review the manufacturing process of the products intended for babies, guaranteeing the absence of the bacteria in the final product. Additional prevention measures at hospitals include the sanitary hygiene of the prepared media, reducing the time between the preparation and its administration, to impede the multiplication of microorganisms.

Enzymatic Digest of plants & animal tissue provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Potassium phosphates act as a buffer system Lactose is the fermentable carbohydrate providing carbon and energy. Sodium lauryl sulfate is the selective agent, inhibiting many organisms except coliforms.

The ISO normative 22964 recommends this medium as a selective enrichment with the addition of Vancomycin and incubation at 44°C. All the tubes must be subcultured in the chromogenic agar for the isolation of *Enterobacter sakazakii* ESIA Cat. 1446.

Incubate at 44 ± 0.5°C and observe for 24 ± 2 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 44 ± 0.5°C and observed after 24 ± 2 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Escherichia coli ATCC 8739	Good
Enterobacter sakazakii ATCC 29544	Good
Staphylococcus aureus ATCC 25923	Inhibited
Enterococcus faecalis ATCC 29212	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO/TS 22964 Milk and milk products- Detection of Enterobacter sakazakii

GUILLAUME-Gentil, O., Sonnard, V. Kandahai, M.C., Mauragg, J.D. and Jootsen, H. A simple and Rapad Cultural Method for Detection of Enterobacter sakazakii in environmental samples. Journal of Food.

Protection, 68 (1), 2005, pp. 64-69

LEGIONELLA CYE AGAR BASE ISO 11731-2

CAT. 1311

Selective medium for the cultivation of *Legionella*

FORMULA IN g/l

Yeast Extract	**10.00**	Bacteriological Agar	**13.00**
Activated Charcoal	**2.00**		
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 2.5 grams of the medium in 90 ml of distilled water. Heat until boiling and until the medium is completely dissolved. Distribute into appropriate containers and sterilize in autoclave at 121 °C for 15 minutes. Cool to 45 - 50°C and aseptically add 1 vial of Legionella BCYE Growth Supplement (**Cat. 6022**), previously reconstituted in 10 ml of sterile distilled water and, if desired, also add 1 ml of 1 vial of LEGIONELLA GVPC SUPPLEMENT (**Cat. 6025**), previously reconstituted with 10 ml of sterile 1:1 water/acetone (see Preparation of **Cat. 6025**). Mix well and distribute into appropriate containers. The prepared medium should be stored at 8 - 15°C. The color is black.

The dehydrated medium should be homogeneous, free-flowing and black in color. If there are any physical changes, discard the medium.

Legionella BCYE Growth Supplement (Cat. 6022)
(For 100 ml of base medium)

Potassium Hydroxide ..... 200 mg

L-Cysteine HCl ..... 40 mg

Ferric Pyrophosphate 25mg

Buffer ACES ..... 1.0 g

Alpha Ketoglutarate 0.1g

Legionella GVPC Growth Supplement (Cat. 6025)
(For 500 ml of base medium)

Polymyxin ..... 39600 IU

Glycine 1.5g

Cycloheximide 40mg

Vancomycin 0.5mg

CAUTION: Legionella GVPC Supplement contains cycloheximide and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

LEGIONELLA CYE AGAR BASE and its supplements have been proven to be optimum for the cultivation of *Legionella* with shorter incubation periods from environmental and clinical samples.

Yeast extract provides vitamins, particularly of the B-group, and other growth co-factors. L-Cysteine provides the required nutrional source. Activated charcoal is a protective agent neutralizing and absorbing toxic metabolites produced by bacterial growth. It decomposes hydrogen peroxide, a toxic metabolic product, and can also collect CO_2 and modify surface tension.

Inoculate sample and incubate at 35 ± 2°C for 24 - 72 hours and up to 10 days. Colonies suspected of being *Legionella* are subcultured to Tryptone Soy Agar (**Cat. 1138**) with 5% sheep blood.

Isolates that grow on CYE Agar but fail to grow on Tryptone Soy Agar with blood may be presumed to be *Legionella*. Confirmation to be made by biochemical and serological tests. The following criteria are suggested:

1. Colonies which have characteristic morphology
2. Isolates should not grow on Tryptone Soy Agar with blood
3. Organisms show gram morphology

Colony Morphology after incubation at 35°C after 48 - 60 hours:

L. pneumophila: 1 - 2 mm diameter (increases after further incubation). White, glistening, circular, smooth, raised with entire edge.

L. gormanii: 1 - 2 mm diameter. Cream, mucoid, slightly raised.

Other Legionellae: 1 - 2 mm diameter (increases after further incubation). White, glistening, circular, smooth, raised with entire edge.

The International standard ISO 11731 recommends the following steps for the confirmation of presumptive *Legionella* colonies: Subculture to BCYE without Cysteine at least five colonies characteristic of *Legionella* at random for each sample for subculture onto plates of BCYE and BCYE without Cysteine medium. If different types of presumptive *Legionella* colonies are present, make sure to select at least two colonies from each type. Subculture each colony onto plates of both media. Incubate at 36 ± 2°C for at least 2 days. Regard those colonies which grow on BCYE but fail to grow on BCYE without Cysteine medium as *Legionella*. Record the results for each plate.

Nutrient agar or blood agar medium may be used instead of BCYE without Cysteine medium.

Legionella pneumophila
ATCC 33153

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with both supplements added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 72 hours and up to 10 days.

Microorganisms	Growth	Colony Color
Legionella pneumophila ATCC 33153	Good	White
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus epidermidis ATCC 12228	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Feeley J.C., Groman G.W.Weaver R.E., Mackel D.C..

International standard ISO 11731 water quality- Detection and enumeration of Legionella

LETHEEN AGAR MODIFIED

CAT. 1111

For the microbiological analysis of cosmetics

FORMULA IN g/l

Casein Peptone	**10.00**	Yeast Extract	**2.00**
Meat Peptone	**10.00**	Glucose	**1.00**
Polisorbate 80	**7.00**	Lecithin	**1.00**
Sodium Chloride	**5.00**	Sodium Bisulfite	**0.1**
Beef Extract	**3.00**	Bacteriological Agar	**20.00**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 59.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LETHEEN AGAR MODIFIED is based on the formula described in the FDA Bacteriological Analytical Manual, is a modification of Letheen Broth Base. It is a highly nutritious medium recommended for use in the microbiological testing of cosmetics. It can be used to inactivate quarternary ammonium

compounds and other preservatives when establishing the number of bacteria present in cosmetics and other materials.

Beef extract, Casein peptone and Meat peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Lecithin, Polisorbate 80 and Sodium bisulfite neutralize quaternary ammonium compounds and partially neutralize the preservative system commonly found in cosmetics. Bacteriological Agar is the solidifying agent.

The medium is also used for microbiological samples from surfaces that have been treated with disinfectants.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed alter 18 - 24 hours.

Microorganisms	Growth	Inoculum (cfu/ml)	Recovery Rate (%)
Staphylococcus pneumoniae ATCC 25923	Good	10^2–10^3	≥ 70
Streptococcus pyogenes ATCC 19615	Good	10^2–10^3	≥ 70
Staphylococcus aureus ATCC 25923	Good	10^2–10^3	≥ 70
Staphylococcus epidermidis ATCC 12228	Good	10^2–10^3	≥ 70

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

FIJA Bacteriological Analytical Manual (BMA) 1995. Microbiological Methods for cosmetics, Letheen Agar (modified). Letheen Broth (modified)

LETHEEN BROTH MODIFIED

CAT. 1244

For the microbiological analysis of cosmetics

FORMULA IN g/l

Casein Peptone	**5.00**	Yeast Extract	**2.00**
Meat Peptone	**20.00**	Glucose	**1.00**
Polisorbate 80	**5.00**	Lecithin	**0.70**
Sodium Chloride	**5.00**	Sodium Bisulfite	**0.10**
Beef Extract	**5.00**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 43.8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LETHEEN BROTH MODIFIED is based on the formula described in FDA Bacteriological Analytical Manual, and is a modification of Letheen Broth Base. It is a highly nutritious liquid medium recommended for use in the microbiological testing of cosmetics.

Beef extract and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Glucose is the fermentable carbohydrate providing carbon and energy. Lecithin, Polisorbate 80 and Sodium bisulfite neutralize quaternary ammonium compounds and partially neutralize the preservative system commonly found in cosmetics.

The medium is also used for microbiological samples from surfaces that have been treated with disinfectants.

Inoculate and incubate at 35°C ± 2°C during 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C ± 2°C and observed alter 18 - 24 hours.

Microorganisms	Growth	Inoculum (cfu/ml)	Recovery
Escherichia coli ATCC 25922	Good	10^2-10^3	≥70
Pseudomonas aeruginosa ATCC 27853	Good	10^2-10^3	≥70

Microorganisms	Growth	Inoculum (cfu/ml)	Recovery
Staphylococcus aureus ATCC 25923	Good	10^2-10^3	≥70
Staphylococcus epidermidis ATCC 12228	Good	10^2-10^3	≥70
Salmonella typhimurium ATCC 14028	Markedly Inhibited	10^2-10^3	≥70

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

FDA Bacteriological Analytical Manual (BMA) 1995. Microbiological Methods for cosmetics, Letheen Agar (modified), Letheen Broth (modified).

LEVINE AGAR (EMB)

CAT. 1050

For the isolation and differentiation of Enterobacteria from clinical samples

FORMULA IN g/l

Gelatin Peptone	**10.00**	Eosin Y	**0.40**
Lactose	**10.00**	Methylene Blue	**0.065**
Dipotassium Phosphate	**2.00**	Bacteriological Agar	**15.00**

Final pH 7.1 ± 0.2 at 25°C

PREPARATION

Suspend 37.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is purple-blue.

The dehydrated medium should be homogeneous, free-flowing and pink-reddish in color. If there are any physical changes, discard the medium.

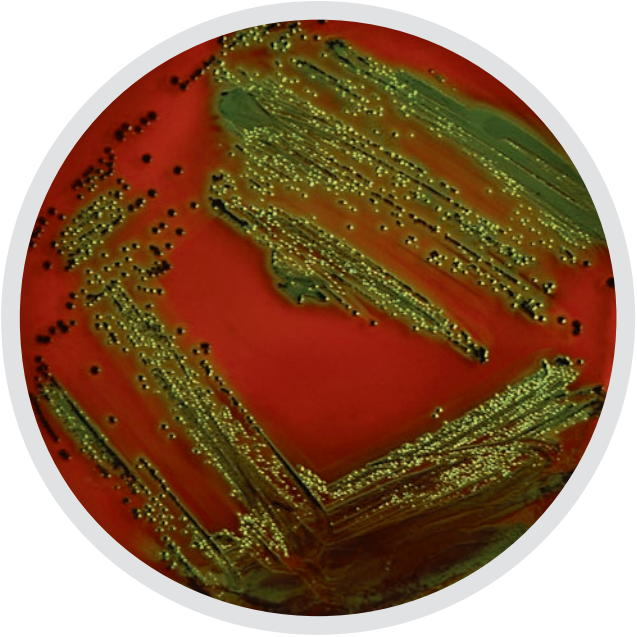
USES

LEVINE AGAR (EMB) is a slightly selective medium for the investigation and differentiation of lactose-fermenting and lactose non-fermenting Enterobacteria in foods, dairy products and clinical samples. It is used for the examination of samples of sanitary importance for the presence of coliforms.

Gelatin Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Eosin Y and Methylene blue are inhibitors of Gram-positive bacteria. Bacteriological agar is the solidifying agent.

Coliforms, being lactose-fermenting organisms, are identified as blue-black colonies, and colonies of *Salmonella* and *Shigella*, being lactose non-fermenters, are colorless, transparent or amber.

It is also used for the isolation and identification of *Candida albicans*. Inoculate medium with sample and incubate at 35 ± 2°C for 18 - 48 hours. See Table of Colony Morphology. The suspect clinical material such as sputum, expectorations, oral or vaginal secretions, and skin and nail scrapings are streaked on the surface of the Levine Agar (EMB), which contains added Tetracycline. After 24 - 48 hours of incubation at 35°C in an atmosphere of approximately 10% CO_2 colonies appear feathery or similar to a "spider web". As the method is not always uniform, check for the production of chlamydospores in special media at the same time, such as Biggy Agar (**Cat. 1006**) and Czapek-Dox Agar (**Cat. 1015**), and conduct rapid tests for sugar fermentations.



Escherichia coli
ATCC 25922

CHARACTERISTICS OF THE COLONIES

Escherichia coli: 2 - 3 mm in diameter. Blue-black in the center, with edges clear to transmitted light, often with a metallic green sheen with reflected light.	*Enterobacter aerogenes:* Large, 4 - 6 mm in diameter. Elevated and mucoid. Grayish-brown in the center to transmitted light. Generally does not have a metallic sheen.
Salmonella and Shigella: Transparent, amber to colorless.	*Proteus:* When there is no swarming, similar to *Salmonella* or *Shigella*.

Staphylococcus:	*Candida albicans:*
(coagulase-positive): Punctiform, colorless	After 24 - 48 hours at 35°C in 10% CO_2. Feathery or in the form of a spider web.
Other *Candidas:*	
Flat, round, yeast-like colonies. From time to time *Nocardia* can be isolated.	

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Good	Pink
Proteus mirabilis ATCC 14273	Good	Colorless
Salmonella typhimurium ATCC 14028	Good	Colorless
Escherichia coli ATCC 25922	Good	Blue-black with green metallic sheen, black center
Staphylococcus aureus ATCC 25923	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Levine, J. Inf. Dis. 22:43. 1981. J. Bact. 45:471. 1943. Vogel, R.A. and Moses, R.M. Weld's Method for the Rapid Identification of Candida albicans in Clinical Materials. Am. J. Clin. Path. 28:103-106. 1957.

LISTERIA AGAR BASE OXFORD ISO 11290-1

CAT. 1133

Selective medium for the detection of *Listeria monocytogenes*

FORMULA IN g/l

Columbia Agar Base	**39.00**	Esculin	**1.00**
Lithium Chloride	**15.00**	Ferric Ammonium Citrate	**0.50**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 27.8 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Oxford Listeria Selective Supplement (**Cat. 6003**), previously reconstituted in 5 ml of sterile distilled water and acetone. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Oxford Listeria Selective Supplement (Cat. 6003)
(Composition: each vial for 500ml)

Cycloheximide ..... 200 mg

Colistin Sulfate ..... 10 mg

Fosfomycin ..... 5 mg

Acryflavine ..... 2.5 mg

Cefotetan ..... 1 mg

CAUTION: Oxford Listeria Selective Supplement contains cycloheximide and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

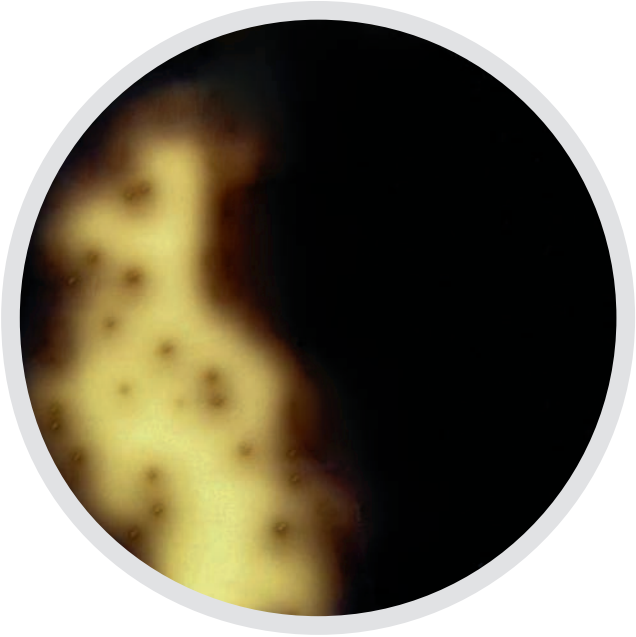
USES

LISTERIA AGAR BASE OXFORD is a selective medium for *Listeria* according to the Oxford formula and it is recommended for the detection of *Listeria monocytogenes* from clinical samples and food products. It is used directly or for confirmation after using Listeria Enrichment Broth Base Fraser (**Cat.1120**).

All *Listeria* species hydrolyze the esculin to esculetin that reacts with the iron ions producing black colonies and a blackening of the medium. Another advantage of this medium is that Columbia Agar Base provides a rich nutrient base for growth and the addition of Ferric ammonium citrate improves the growth of *L. monocytogenes*. Lithium chloride is an inhibiting agent, together with the other antibiotics from the supplement, which inhibit the growth of Gram-negative bacteria and a large part

of Gram-positive ones. Cycloheximide inhibits yeasts. Inoculate sample and incubate at 35 ± 2°C for 24 - 48 hours. Confirmation of *Listeria* is done by biochemical and serological identifications tests.

Although typical *L. monocytogenes* colonies are almost always visible after 24 hours incubation, incubation should be prolonged a further 24 hours in order to detect slower growing strains.



Listeria monocytogenes
ATCC 19111

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with Oxford Listeria Selective Supplement (**Cat.6003**) added, after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Listeria monocytogenes ATCC 19111	Good	Brown-gray colonies with black centerand black halo
Staphylococcus aureus ATCC 25923	Inhibited	White Colonies
Escherichia coli ATCC 25922	Null	
Enterococcus faecalis ATCC 29212	Null	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO NORMATIVE 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

Curtis, G.D.W. Mitchell, R.G., King, A.F., Griffin E.J.A selective medium for the isolation of Listeria monocytogenes. Letters in Appl.Microbiol.8.95-98

LISTERIA AGAR BASE PALCAM ISO 11290-2

CAT. 1141

Selective and differential medium for the detection of *Listeria spp.*, particularly *Listeria monocytogenes*

FORMULA IN g/l

Columbia Agar Base	**39.00**	Esculin	**0.80**
Lithium Chloride	**15.00**	Glucose	**0.50**
Mannitol	**10.00**	Ferric Ammonium Citrate	**0.50**
Yeast Extract	**3.00**	Phenol red	**0.08**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 34.4 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Palcam Listeria Selective Supplement (**Cat. 6004**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Palcam Listeria Selective Supplement (Cat. 6004)
(1 vial for 500 ml of the medium)

Ceftazidime ..... 10 mg

Polymyxin B Sulfate ..... 5 mg

Acryflavin..... 2,5 mg

CAUTION: Listeria Chromogenic Selective Supplement contains Ceftazidime and it is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye face protection.

USES

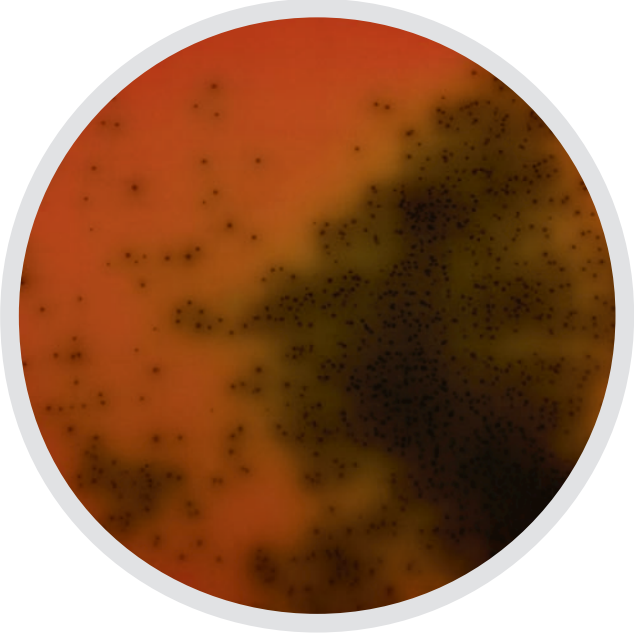
LISTERIA AGAR BASE PALCAM, used with supplements, is a selective and differential medium for *Listeria spp.* It is recommended by ISO 11290 for the detection and enumeration of *Listeria monocytogenes* in food products and clinical samples, and can also be used for environmental samples.

It is used directly or for confirmation after using Listeria Enrichment Broth Base Fraser (**Cat. 1120**). It allows the easy differential diagnosis of *Listeria monocytogenes* using a double-system indicator: Esculin/Iron and Mannitol/Phenol red. Inoculate sample and incubate at a temperature of 35 ± 2°C and observe after 24 - 48 hours.

All *Listeria* species hydrolyze the esculin to esculetin, which reacts with iron ions producing a blackening of the medium.

Lithium chloride included in the medium, along with Ceftazidime, Polymyxin B Sulfate and Acryflavine from the supplement, inhibit the growth of the non-Listeria accompanying bacteria present in foods, which can hydrolyze the esculin. Columbia Agar Base provides a rich nutrient base for growth. Yeast extract is the source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate. Ferric ammonium citrate improves the growth of *L. monocytogenes*.

The Mannitol / Phenol red differentiation system is used to differentiate *Listeria spp.* that do not ferment mannitol from other species that occasionally grow in the medium such as enterococci or staphylococci. Differentiation is achieved by the acid increase in pH causing the Phenol red indicator to change the color of the colonies or medium from red or gray to yellow. Confirmation of *Listeria* is done by biochemical and serological identifications tests.



Listeria monocytogenes
ATCC 19111

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 35± 2°C after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Listeria monocytogenes ATCC 19111	Good	Green-gray colonies with black center and black halo
Listeria innocua ATCC 33090	Good	Green-gray colonies with black center and black halo
Enterococcus faecalis ATCC 29212	Inhibited	-
Staphylococcus aureus ATCC 29212	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO NORMATIVE 11290-2:Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 2: Enumeration method.

Van Netten, P., I. Perales A. Van de Moosalijk G.D.W. Curtis and DAA Mossel 1989 Liquid and solid selective differential media for the detection and enumeration of L. monocytogenes and other Listeria spp. Int. J. of Food Microbiol 8: 299-317.

Farber JMDW Warburton and T. Babiuk, 1994 Isolation of Listeria monocytogenes from all food and environmental samples.

LISTERIA CHROMOGENIC AGAR BASE ISO 11290-1

CAT. 1345

Selective medium for the detection and enumeration of *Listeria monocytogenes*

FORMULA IN g/l

Meat Peptone	**18.00**	Glucose	**2.00**
Lithium Chloride	**10.00**	Sodium Pyruvate	**2.00**
Yeast Extract	**10.00**	Magnesium Glycerophosphate	**1.00**
Tryptone	**6.00**	Magnesium Sulfate	**0.50**
Sodium Chloride	**5.00**	X-glucoside	**0.05**
Disodium Hydrogen Phosphate	**2.50**	Bacteriological Agar	**13.50**

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 35.275 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. To prepare more quantity of

500 ml, it is recommended to sterilize at 115 ° C for 10 minutes. Cool to 45 - 50°C and aseptically add one vial of Listeria Lipase C Supplement (**Cat. 6031**), and one vial of Listeria Chromogenic Selective Supplement (**Cat. 6040**), previously reconstituted in 5 ml of sterile distilled water/acetone or water/ethanol 1:1. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

LISTERIA LIPASE C SUPPLEMENT (Cat. 6031)
(1 vial for 500 ml of the medium)

Lipase C Substrate ..... 10 ml

LISTERIA CHROMOGENIC SELECTIVE SUPPLEMENT (Cat. 6040)
(1 vial for 500 ml of the medium)

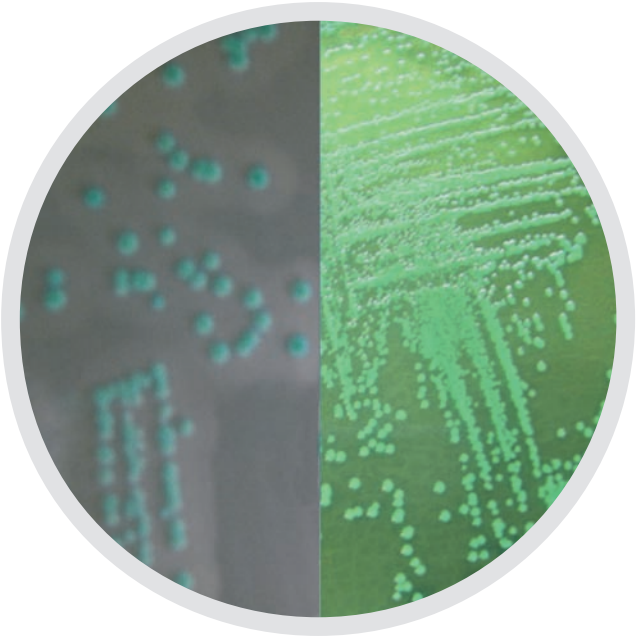
Cycloheximide ..... 50 mg

Ceftazidime ..... 10 mg

Nalidixic acid ..... 10 mg

Polymyxin B ..... 38350 IU

CAUTION: Listeria Chromogenic Selective Supplement contains Cycloheximide and it is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye face protection.



Listeria monocytogenes ATCC 19111 *Listeria innocua* ATCC 33090

USES

LISTERIA CHROMOGENIC AGAR BASE is a selective medium for the presumptive isolation and identification of *Listeria monocytogenes* and *Listeria spp.* in food and clinical samples. It is used for confirmation after using Listeria Enrichment Broth Base Fraser (**Cat. 1120**). This medium is also recommended by ISO 11290-1 for the detection and enumeration for *Listeria monocytogenes*.

Meat peptone and Tryptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium pyruvate is a source of energy for bacterial metabolism and aids in resuscitation of stressed organisms. Glucose is the fermentable carbohydrate providing carbon and energy. Magnesium glycerophosphate is a buffering compound. Magnesium sulphate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication. The differential activity of the medium is due to two factors. Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Bacteriological agar is the solidifying agent.

The presence of the chromogenic component X-glucoside, a substrate for the detection of the enzyme ß-glucosidase, is common to all *Listeria* species giving the colonies their blue colour. Other organisms that possess this enzyme, for example enterococci, are inhibited by the selective agents within the medium and by the selective supplement. The differential activity is also obtained by lipase C substrate, upon which the specific enzyme for *L. monocytogenes acts*. The lipase is responsible for the opaque white halo which surrounds *L. monocytogenes*.

The combination of both substrates allows us to differentiate the colonies of *Listeria monocytogenes* from the rest of *Listeria spp.* since, although all are blue in colour, *L. monocytogenes* present an opaque white halo surrounding them.

It has been observed that some strains of *Listeria ivanovii*, mostly pathogenic to animals although some have caused infections in humans, also possess lipase activity.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with Listeria Chromogenic Selective Supplement (**Cat. 6040**) & Listeria Chromogenic Lipase C Supplement (**Cat. 6031**) added, from type cultures after incubation at a temperature of 37°C and observed after 24 ± 2 hours. Incubate the negative strains for an extra 24 ± 2 hours.

Microorganisms	Growth	Colony Color	Halo
Listeria monocytogenes ATCC 19111	Good	Blue	+
Listeria monocytogenes ATCC 13932	Good	Blue	+
Listeria innocua ATCC 33090	Good	Blue	-
Enterococcus faecalis ATCC 19433	Inhibited		-
Escherichia coli ATCC 25922	Inhibited		-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June

ISO 11290-1:2004 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method.

LISTERIA ENRICHMENT BROTH BASE FRASER ISO 11290-1

CAT. 1120

Enrichment medium for the detection and enumeration of Listeria in food and environmental samples

FORMULA IN g/l

Sodium Chloride	**20.00**	Beef Extract	**5.00**
Disodium Hydrogen Phosphate	**12.00**	Lithium Chloride	**3.00**
Tryptone	**5.00**	Monopotassium Phosphate	**1.35**
Proteose Peptone	**5.00**	Esculin	**1.00**
Yeast Extract	**5.00**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 28.7 grams of the medium in 500 ml. of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Fraser Listeria Selective Supplement (**Cat. 6001**) for preparing Fraser or Half Fraser Listeria Selective Supplement (**Cat. 6002**) reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Fraser Listeria Selective Supplement (Cat. 6001)
(Composition: each vial for 500 ml)

Vial A: Ferric Ammonium Citrate..... 250 mg

Vial B: Acryflavine ..... 12.5 mg

Nalidixic Acid ..... 10 mg

Half Fraser Listeria Selective Supplement (Cat. 6002)
(Composition: each vial for 500 ml)

Vial A: Ferric Ammonium Citrate..... 250 mg

Vial B: Acryflavine ..... 6.25 mg

Nalidixic Acid ..... 5 mg

USES

LISTERIA ENRICHMENT BROTH BASE FRASER is an appropriate medium for the selective enrichment of *Listeria* in the two-step method according to ISO 11290-1, for the preparation of Fraser or Half Fraser Broth by adding the respective supplements.

It is recommended for the detection of *Listeria spp.* in food products and in samples from the environment. All *Listeria* species hydrolyze the Esculin to esculetin, which reacts with iron ions producing a blackening of the medium. Another advantage of this medium is that the addition of Ferric ammonium citrate improves the growth of *L. monocytogenes*. Lithium chloride included in the medium, along with Nalidixic acid and Acryflavine from the supplement, inhibit the growth of the accompanying flora, which can hydrolyze the esculin. The high amount of Sodium chloride inhibits the growth of enterococci. Tryptone, Proteose Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Phosphate Salts act as a buffer system.

Primary enrichment of Half Fraser medium and mix thoroughly. Incubate for 24 ± 2 hours at 30°C. Secondary enrichment: transfer 0.1 ml of incubated Half Fraser medium to 10 ml Fraser Broth. Incubate at 35 - 37°C for 48 ± 2 hours. Compare each inoculated tube with a non-inoculated control tube with a white background. After incubation of the primary and secondary enrichment, inoculate the tubes in Agar Oxford **Cat. 1133** (with supplement **Cat. 6003**) and Agar Palcam **Cat. 1141** (with supplement **Cat. 6004**). Confirm the suspicious colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures with Listeria Enrichment Broth base Fraser with Half Fraser Listeria selective supplement (**Cat. 6002**) added, after incubation at a temperature of 30 ± 1°C, in aerobic conditions and observed after 24 ± 3 hours, and with Listeria Enrichment Broth base Fraser with Fraser Listeria selective supplement (**Cat. 6001**) added, after incubation at temperature of 35 - 37°C after 48 ± 3 hours.

Microorganisms	Growth	Esculin Reaction
Listeria monocytogenes ATCC 19111	Good	+
Enterococcus faecalis ATCC 29212	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO NORMATIVE 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

Fraser J.A. and Sperber W.H (1988) McClain D. and Lee W.H(1988)

LISTERIA FRASER BROTH BASE ISO 11290-1

CAT. 1182

Enrichment medium for the detection and isolation of *Listeria* in food and environmental samples

FORMULA IN g/l

Sodium Chloride	**20.00**	Lithium Chloride	**3.00**
Disodium Phosphate	**12.00**	Monopotassium Phosphate	**1.35**
Tryptone	**5.00**	Esculin	**1.00**
Meat Peptone	**5.00**	Acriflavine	**0.025**
Beef Extract	**5.00**	Nalidixic Acid	**0.02**
Yeast Extract	**5.00**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 28.7 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Ferric Ammonium Citrate Supplement (**[illegible]**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

[illegible]

(Composition: each vial for 500 ml)

Ferric Ammonium Citrate..... 0.25 g

USES

LISTERIA FRASER BROTH BASE is used in the rapid detection of *Listeria* from food and environmental samples. The antibiotics are already included in the formula so it is only necessary to add the Ferric Ammonium Citrate Supplement.

The medium is used for the selective enrichment and enumeration of *Listeria monocytogenes* and other *Listeria* species in all food types, including milk and dairy products, and environmental samples. This formula is adheres to ISO 11290-1.

Tryptone, Meat peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Potassium phosphates act as a buffer system. All *Listeria* species hydrolyze esculin, which reacts with ferric ions producing a blackening of the medium. The addition of ferric ammonium citrate improves the growth of *Listeria monocytogenes*. Lithium chloride inhibits the growth of enterococci that can hydrolyze the esculin.

[illegible]

Weigh 25 g (or 25 ml) of the sample and add 225 ml of Half Fraser Broth (**[illegible]**). Homogenize and incubate at 30 ± 1°C for 24 ± 3 hours.

[illegible]

Inoculate 0.1 ml of incubated Half Fraser Broth culture into 10 ml of Fraser Broth (**[illegible]**).

Incubate at 35 - 37°C for 48 ± 3 hours in aerobic conditions.

[illegible]

The tubes that present a blackening should be subcultured in Listeria Oxford Agar (**[illegible]**) or Listeria Palcam Agar (**[illegible]**), and Listeria Chromogenic Agar (**[illegible]**). The tubes that preserve the original color are considered negative.

[illegible]

Identify as *Listeria spp.* or *Listeria monocytogenes* using biochemical tests.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 35 - 37°C in aerobic conditions and observed after 48 ± 3 hours.

Microorganisms	Growth	Esculin Reaction
Listeria monocytogenes ATCC 19111	Good	+
Enterococcus faecalis ATCC 29212	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Fraser. J.A and Sperber W.H (1988) McClain D. and Lee W.H (1988)

ISO NORMATIVE 11290-1 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method

LISTERIA HALF-FRASER BROTH BASE ISO 11290-1

CAT. 1183

Enrichment medium for the detection and isolation of *Listeria* in food and environmental samples

FORMULA IN g/l

Sodium Chloride	20.00	Lithium Chloride	3.00
Disodium Phosphate	12.00	Monopotassium Phosphate	1.30
Tryptone	5.00	Esculin	1.00
Meat Peptone	5.00	Acriflavine	0.0125
Beef Extract	5.00	Nalidixic Acid	0.01
Yeast Extract	5.00		

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 28.7 grams of the medium in 500 ml. of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add one vial of Ferric Ammonium Citrate Supplement (fififfifififi) previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

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(Composition: each vial for 500 ml)

Ferric Ammonium Citrate.. 0.25 g

USES

LISTERIA HALF-FRASER BROTH BASE is a modification of Listeria Fraser Broth Base (fififfififififi)fi which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/L and 12.5 mg/L respectively. The antibiotics are already included in the formula so it is only necessary to add the Ferric Ammonium Citrate Supplement.

The medium is used for the selective enrichment medium and enumeration of *Listeria monocytogenes* and other Listeria species in all food types, including milk and dairy products, and environmental samples. This formula adheres to ISO 11290-1.

Tryptone, Meat Peptone and Beef Extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Potassium phosphates act as a buffer system. All *Listeria* species hydrolyze esculin, which reacts with ferric ions producing a blackening of the medium. The addition of ferric ammonium citrate improves the growth of *Listeria monocytogenes*. Lithium chloride inhibits the growth of enterococci that can hydrolyze the esculin.

Listeria Half Fraser Broth is used as a selective preenrichment medium.

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Weigh 25 g (or 25 ml) of the sample and add 225 ml of Half Fraser Broth (fififffifififi Homogenize and incubate at 30 ± 1°C for 24 ± 3 hours.

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Inoculate 0.1 ml of incubated Half Fraser Broth culture into 10 ml of Fraser Broth (fififfifififi)fi

Incubate at 35 - 37°C for 48 ± 3 hours in aerobic conditions.

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The tubes that present a blackening should be subcultured in Listeria Oxford Agar (fififfifififi)fior Listeria Palcam Agar (fififi fififi), and Listeria Chromogenic Agar (fififfifififi)fi The tubes that preserve the original color are considered negative.

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Identify as *Listeria spp.* or *Listeria monocytogenes* using biochemical tests.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 30 ± 1°C in aerobic conditions and observed after 24 ± 3 hours.

Microorganisms	Growth	Esculin Reaction
Listeria monocytogenes ATCC 19111	Good	+
Enterococcus faecalis ATCC 29212	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Fraser. J.A and Sperber W.H (1988) McClain D. and Lee W.H (1988)

ISO NORMATIVE 11290-1 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method

LIVER AGAR

CAT. 1142

For the cultivation of *Brucella* and other pathogenic organisms

FORMULA IN g/l

Liver Base	**30.00**	Dextrose	**0.20**
Starch	**0.20**	Bacteriological Agar	**11.00**
Final pH 7.6± 0.2 at 25°C			

PREPARATION

Suspend 41.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LIVER AGAR is used to grow *Brucella* and other fastidious pathogens in foods. Whilst most strains of *Brucella* will grow on chocolate or blood agar, this medium is preferred due to the high nutrient base permitting an extensive growth of *Brucella* and other fastidious pathogens.

The growth of most of anaerobic bacteria is promoted by the growth nutrients and stimulants such as nitrogen, vitamins, minerals, amino acids contained in the Liver agar. Dextrose is the source of fermentable carbohydrate for carbon and energy. In low concentrations, Starch absorbs any toxic metabolites produced. Bacteriological agar is the solidifying agent.

Brucellosis is a zoonotic disease with a domestic animal reservoir. The usual route of transmission is by milk, milk products, meat and direct contact with infected animals.

Inoculate and incubate medium at 35 ± 2°C under 5 - 10% CO_2 for 18 - 48 hours and up to 72 hours if necessary.

For isolating *Brucella* from contaminated milk, Crystal violet can be added to suppress Gram-positive organisms. Addition of 5% heated horse or rabbit serum enhances the growth of *Brucella*.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures after incubation at a temperature of 35 ± 2°C under 5 - 10% of CO_2 and observed after 18 - 48 hours.

Microorganisms	Growth
Brucella abortus ATCC 4315	Good
Brucella melitensis ATCC 4309	Good
Brucella suis ATCC 4314	Good
Streptococcus mitis ATCC 9895	Good
Clostridium sporogenes ATCC 11439	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Scarr M. Pamela (1958) DSIR Proc. Symp Found Microbiol 1957 HMSO London pp 29-33

LIVER BROTH

CAT. 1242

For the cultivation of a wide variety of microorganisms, particularly *Brucella* and anaerobes

FORMULA IN g/l

Liver Base	**50.00**	Meat Peptone	**5.00**
Dextrose	**10.00**	Disodium Phosphate	**2.00**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 67 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

LIVER BROTH is used to grow *Brucella* and other fastidious pathogens in foods and clinical samples. It is well suited to support the growth of anaerobic microorganisms, especially *Clostridium spp.*

The growth of most anaerobic bacteria is promoted by growth nutrients and stimulants such as nitrogen, vitamins, minerals and amino acids contained in the Liver base and the Meat peptone. Dextrose is the source of fermentable carbohydrate. Potassium phosphate is a buffer to maintain the pH. Liver Broth maintains

an adequate degree of anaerobiosis for the growth of anaerobic microorganisms, especially *Clostridium* species.

Brucellosis is a zoonotic disease with a domestic animal reservoir. Transmission is usually by milk, milk products, meat and direct contact with infected animals.

Inoculate and incubate medium at 35 ± 2°C, under 5 - 10% CO_2, for 18 - 48 hours and up to 72 hours if necessary. Incubate *Clostridium* under anaerobic conditions.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% of CO_2, and observed after 18 - 48 hours. Clostridium is grown under anaerobic conditions.

Microorganisms	Growth
Brucella abortus ATCC 4315	Good
Brucella melitensis ATCC 4309	Good
Brucella suis ATCC 4314	Good
Streptococcus mitis ATCC 9895	Good
Clostridium sporogenes ATCC 11437	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Scarr M. Pamela (1958) DSIR Proc. Symp Found Microbiol 1957 HMSO London pp 29-33.

LOWENSTEIN - JENSEN MEDIUM BASE

CAT. 1116

For the cultivation of *Mycobacterium tuberculosis* and other *Mycobacteria*

FORMULA IN g/l

Potato Flour	**30.00**	Magnesium Citrate	**0.60**
Asparagine	**3.60**	Malachite Green	**0.40**
Monopotassium Phosphate	**2.50**	Magnesium Sulfate	**0.24**
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 37.3 grams of the medium in 600 ml of distilled water with 12 ml of Glycerol (do not add Glycerol if bovine bacilli or other glycerophobic organisms are going to be cultivated). Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C. Meanwhile, prepare one liter of whole eggs, aseptically obtained and swirled to mix without introducing air bubbles. Add the egg slowly to the base to obtain a homogeneous mixture without bubbles. Distribute into screw-capped tubes. Place the tubes in a slanted position. Inspissate at 85 - 90°C for 45 minutes to coagulate the medium. The prepared medium should be stored at 2 - 8°C. The color is blue-green.

The dehydrated medium should be homogeneous, free-flowing and blue-green in color. If there are any physical changes, discard the medium.

USES

LOWENSTEIN-JENSEN MEDIUM BASE can be used, with whole egg and glycerol, to cultivate and isolate a wide variety of *Mycobacteria* other than *M. leprae* from clinical samples. The growth of *Mycobacteria* on egg media can be used for niacin testing.

Glycerol and egg mixture provide fatty acids and protein necessary for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization gives a solid medium for inoculation purposes. Monopotassium phosphate acts as a buffer system. Magnesium sulphate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication. Malachite green suppresses the growth of contaminating bacteria.

With 5% sodium chloride, Lowenstein-Jensen Medium can be used as an aid in the differentiation of rapid-growing *Mycobacteria* from slow growers on the basis of salt tolerance.

M. fortuitum, M. triviale, M. chelonei and some strains of *M. flavescens* grow on this medium while most other mycobacteria strains are inhibited.

Lowenstein-Jensen Medium in a deep-butt tube may be used to aid the differentiation of *Mycobacteria* on the basis of the catalase test. Lowenstein-Jensen Medium with antibiotics can be used to selectively isolate *Mycobacteria* and inhibit contaminating flora. The addition of ribonucleic acid to the Lowenstein-Jensen Medium may increase percentage of tubercle bacilli recovered from clinical specimens compared to recovery on the standard Lowenstein-Jensen Medium.

M. bovis will not grow on Lowenstein Jensen Medium containing glycerol.

Inoculate and incubate at 35 ± 2°C for up to 28 days. Confirmation should be made with biochemical test.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with glycerol and egg added, after incubation at a temperature of 35 ± 2°C and observed during 28 days.

Microorganisms	Growth
Mycobacterium tuberculosis H37RV	Good
Mycobacterium fortuitum ATCC 6841	Good
Mycobacterium kansasii ATCC 12478	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bailey and Scott. Diagnostic Microbiology. The C.V. Mosby Company, Saint Louis, 1978. Diagnostic Procedures and Reagents., APHA. Fifth Ed. 1970. New York. Raiza Nikolajuk of Irurzum and A.J.F., Irurzum. The Laboratory in the Diagnostics of Tuberculosis. Ed. Medical Panamericana, Buenos Aires, 1972.

LPT DILUTION BROTH

CAT. 1399

Recommended as a solvent for the homogenization of samples in cosmetic products analysis

FORMULA IN g/l

Disodium Phosphate	**9.00**	Monopotassium Phosphate	**1.50**
Sodium Chloride	**8.50**	L- Histidine	**1.00**
Sodium Thiosulfate	**5.00**	Tryptone	**1.00**
Lecithin	**3.00**		
Final pH 7.0 ± 0.2 at 25°C			

PREPARATION

Suspend 29 grams of the medium in one liter of distilled water. Mix well and add 30 ml of Tween 80. Dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige with a hint of pink in color. If there are any physical changes, discard the medium.

USES

LPT DILUTION BROTH BASE is recommended by UNIPRO Publication for the homogenization of the samples in the analysis of cosmetic products.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Potassium phosphates act as a buffer system. Lecithin and Tween 80 neutralize quaternary ammonium compounds and inhibit the action of the preservatives. Antimicrobial preservatives are commonly used in cosmetic products in order to reduce their microbial contamination. The cosmetics, due to their nature, are sensitive to microbial contamination and should be preserved as they are used in eyes, damaged skin or in long period times.

Incubate at 35 ± 2 °C and observe after 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with Tween 80 added, after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Salmonella enteritidis ATCC 13076	Good
Salmonella typhi ATCC 19430	Good
Salmonella thyphimurium ATCC 14028	Good
Staphylococcus aureus ATCC 6538	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Microbiologia nell'Industria Cosmetica. UNIPRO, aprile 1990, vol. 1. Ricerche e Tecnologie Cosmetiche

LYSINE DECARBOXYLASE BROTH

CAT. 1208

For the identification of microorganisms, especially enteric bacilli, based on the decarboxylation of lysine

FORMULA IN g/l

Gelatin Peptone	**5.00**	Dextrose	**1.00**
L-Lysine	**5.00**	Bromocresol Purple	**0.02**
Yeast Extract	**3.00**		
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 14 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense quantities of 5 ml into screw-capped tubes. Sterilize in autoclave at 121°C for 15 minutes. Leave caps loose to allow gas exchange. Close well after

sterilization. The prepared medium should be stored at 2 - 8°C. The color is violet.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

Escherichia coli ATCC 25922 | *Proteus vulgaris* ATCC 13315

USES

LYSINE DECARBOXYLASE BROTH is used to detect and differentiate Enterobacteria from other microorganisms, based on lysine decarboxylation.

Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate. Bromocresol purple is the pH indicator. Lysine is added to detect the production of the specific enzyme.

When the medium is inoculated with a bacterium that is able to ferment dextrose, the acid produced lowers the pH of the medium and changes the color of the indicator from purple to yellow. The acidic condition also stimulates decarboxylase activity. The bacteria that decarboxylate the L-Lysine to cadaverine are identified by the presence of a purple-red color. The production of these amines elevates the pH of the medium. A yellow color after 24 hours indicates a negative result.

The tubes are inoculated with the microorganism samples and incubated at 35 ± 2°C. for 24 hours By substituting L-Lysine with Arginine or Ornithine, the new resulting medium (Falkow Broth Base) can be used to study the decarboxylation of these amino acids.

The following table indicates the typical reactions of the important groups of the Enterobacteria.

Positive Reaction Purple	*Escherichia Klebsiella Salmonella*, except *S.parathyphi* A	Negative Reaction Yellow	*Proteus Providencia S.parathypi* A
	Arizona *Alkalescens* Dispar *Serratia*		*Shigella Aeromonas Citrobacter*

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C for 18 - 48 hours.

Microorganisms	Lysine Decarboxylation
Escherichia coli ATCC 25922	+
Salmonella typhi ATCC 6539	+
Salmonella paratyphi ATCC 9150	-
Proteus vulgaris ATCC 13315	-
Serratia liquifaciens ATCC 27592	+ (slow)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Falkow A. S. Clin. Path. 28:598, 1958.

Ewing Davis and Deaves, Studies in the Serratia Group. U.S. Dept. H.E.W.C.D.C. Atlanta, 1972. Edwards and Ewing. Identification of Enterobacteriaceae, Burgess Publ. Co. Minneapolis, Minn., 1961.

LYSINE DECARBOXYLASE MEDIUM ISO 6579 & ISO 10273

CAT. 1176

For the biochemical confirmation of *Salmonella*

FORMULA IN g/l

Lysine Monohydrate	**5.00**	Dextrose	**1.00**
Yeast Extract	**3.00**	Bromocresol Purple	**0.015**
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 9 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense quantities of 5

ml into screw-capped tubes. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is violet.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

LYSINE DECARBOXYLASE BROTH is recommended by ISO 6579 for the biochemical confirmation of *Salmonella* based on lysine decarboxylation. It is also recommended by ISO 10273 for the biochemical confirmation of *Yersinia*.

When the medium is inoculated with a bacterium that is able to ferment dextrose, the acid produced lowers the pH of the medium and changes the color of the indicator from purple to yellow. The acidic condition also stimulates decarboxylase activity. The bacteria that decarboxylate the L-Lysine to cadaverine are identified by the presence of a purple-red color. The production of these amines elevates the pH of the medium. A yellow color after 24 hours indicates a negative result.

Yeast extract is the source of vitamins, of particularly the B-group, essential for growth. Dextrose is the fermentable carbohydrate. Bromocresol purple is the pH indicator. Lysine is added to detect the production of the specific enzyme.

The tubes are inoculated with the microorganism samples and incubated for 24 ± 3 hours at 37 ± 1°C.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1 °C and observed after 24 ± 3 hours.

Microorganisms	Lysine Decarboxylation
Salmonella typhi ATCC 6539	+
Salmonella paratyphi ATCC 9150	-
Proteus vulgaris ATCC 13315	-
Salmonella gallinarum NCTC 9240	+
**Yersinia enterocolitica* ATCC 27729	-

**Incubate at 30°C during 24 hours according to ISO 10273.*

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.

ISO 10273: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presuntive pathogenic Yersinia enterocolitica.

Falkow A. S. Clin. Path. 28:598, 1958.

Ewing Davis and Deaves, Studies in the Serratia Group. U.S. Dept. H.E.W.C.D.C. Atlanta, 1972.

Edwards and Ewing. Identification of Enterobacteriaceae, Burgess Publ. Co. Minneapolis, Minn., 1961.

LYSINE IRON AGAR

CAT. 1044

For studies of the decarboxylation of lysine for the rapid differentiation of *Salmonella arizonae*

FORMULA IN g/l

L-Lysine	**10.00**	Ferric Ammonium Citrate	**0.50**
Gelatin Peptone	**5.00**	Sodium Thiosulfate	**0.04**
Yeast Extract	**3.00**	Bromocresol Purple	**0.02**
Dextrose	**1.00**	Bacteriological Agar	**13.50**
Final pH 6.7 ± 0.2 at25°C			

PREPARATION

Suspend 33 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 12 minutes. Allow to cool in a slanted position. The prepared medium should be stored at 8 - 15°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LYSINE IRON AGAR is used for the rapid differentiation of Enterobacteriaceae, especially *Salmonella arizonae*, on the basis of lysine decarboxylation and deamination, and H_2S production. This medium is very useful for the rapid differentiation of *Salmonella arizonae* from *Citrobacter* and *Proteus spp.*

The strains that rapidly ferment the lactose produce a large quantity of acid, changing the original purple color of the medium to yellow. Some strains of *S. arizonae* can rapidly ferment lactose and form colonies that are colorless or pink to red on media such as MacConkey Agar (**Cat. 1052**) or Desoxycholate Agar (**Cat. 1020**). Lysine Iron Agar is especially formulated to avoid this confusion.

Gelatin peptone and Yeast extract provide the nutrient sources for growth: nitrogen, vitamins, minerals and amino acids. One reaction is the degradation of the fermentable carbohydrate Dextrose, with the production of acid, manifested in the color change from red to yellow. Sodium thiosulfate provides Sulphur and Ferric ammonium citrate is the indicator for H_2S production under alkaline conditions. The bacteria that decarboxylate

the L-Lysine to cadaverine, such as *Salmonella arizonae*, are identified by the presence of a purple-red color due to the elevation of the pH. Bromocresol purple is the pH indicator. Bacteriological agar is the solidifying agent.

Cultures rapidly producing lysine decarboxylase cause an alkaline reaction (purple colour) throughout the medium. Those organisms that do not decarboxylate lysine produce an alkaline slant and an acid butt (yellow colour). *Proteus* and *Providencia* produce a characteristic orange-red color on the slant while the butt is yellow from the production of acid from the deamination of lysine.

Inoculate and incubate at 35 ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Slant Lysine deamination	Butt Lysine decarboxylation	H_2S Production
Salmonella arizonae ATCC 13314	Good	Red-purple	Red-purple	+
Salmonella typhimurium ATCC 14028	Good	Red-purple	Red-purple	+
Escherichia coli ATCC 25922	Good	Red-purple	Red-purple	-
Citrobacter freundii ATCC 8090	Good	Red-purple	Yellow	+
Proteus mirabilis ATCC 25933	Good	Deep red	Yellow	-
Shigella flexneri ATCC 12022	Good	Red-purple	Yellow	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Edwards and Fite Applied Microbiol. 9:478, 1961. Edwards and Ewing. Identification of Enterobacteriaceae. Burgess Publishing Co. Minneapolis, Minn., 1962.

M17 AGAR

CAT. 1318

For the cultivation and enumeration of lactic streptococci in milk and dairy products

FORMULA IN g/l

Sodium Glycerophosphate	**19.00**	Casein Peptone	**2.50**
Soy Peptone	**5.00**	Yeast Extract	**2.50**
Beef Extract	**5.00**	Ascorbic Acid	**0.50**
Lactose	**5.00**	Magnesium Sulfate	**0.25**
Meat Peptone	**2.50**	Bacteriological Agar	**12.75**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 55 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

M17 AGAR is a nutritionally rich medium used for the cultivation and enumeration of fastidious lactic streptococci. It is recommended to isolate *Streptococcus thermophilus* from yogurt. This medium is also suitable for growing and maintaining starter cultures for cheese and yogurt manufacture.

These homo fermentative organisms produce large amounts of acid and need a good buffer to maintain the pH above 5.7, thereby guaranteeing the growth conditions of the organisms. This maintenance of the pH is important since a lower pH may cause injury and a reduced recovery of lactic streptococci.

Soy, Meat and Casein peptones, and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Lactose provides the carbohydrate as an energy source. Sodium glycerophosphate increases the buffering capacity of the medium and maintains the pH. Ascorbic acid stimulates the growth of lactic streptococci. Magnesium sulfate provides essential ions for growth. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 28 - 30°C for 48 hours, or up to 2 weeks if required.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 28 - 30°C and observed after 48 hours.

Microorganisms	Growth
Streptococcus lactis ATCC 19435	Good
Lactobacillus casei ATCC 393	Moderate

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

American Public Health Association, Standard Methods for the examination of water and wastewater Terzaghi, B.E: a, Sandine, WE: Improved medium for lactic streptococci and their bacteriophages

MACCONKEY AGAR EUROPEAN PHARMACOPOEIA

CAT. 1052

For the isolation and identification of Enterobacteriaceae from feces, urine wastewater and foods

FORMULA IN g/l

Pancreatic Digest of Gelatin	**17.00**	Bile Salts	**1.50**
Lactose Monohydrate	**10.00**	Neutral Red	**0.03**
Sodium Chloride	**5.00**	Crystal Violet	**0.001**
Peptones (Meat & Casein)	**3.00**	Bacteriological Agar	**13.50**
Final pH 7.1 ± 0.2 at 25°C			

PREPARATION

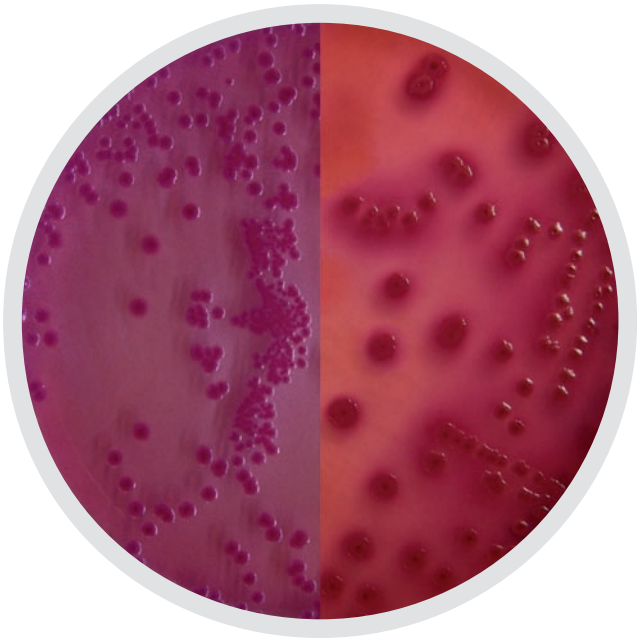
Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. Allow the plates to solidify and place them upside down to avoid excessive moisture on the surface of the medium. The prepared medium should be stored at 8 - 15°C. The color is violet-red.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

USES

MACCONKEY AGAR is used for the selective isolation and identification of Enterobacteriaceae from feces, urine, wastewater and foods. It is also a selective and differential medium for the isolation of enteric Gram-negative bacteria.

The specimen can be streaked directly on the medium or inoculated first into an enrichment broth such a Tetrathionate Broth (**Cat. 1114**), Selenite Cystine Broth (**Cat. 1220**), GN Enrichment Broth (**Cat. 1248**) or MacConkey Broth (**Cat. 1210**).



Enterobacter aerogenes ATCC 13048 | *Escherichia coli* ATCC 25922

Subculture the broth tubes on MacConkey Agar and incubate at 30 - 35°C for 18 - 72 hours, as indicated by the European Pharmacopoeia.

Pancreatic Digest of Gelatin and Peptones (Meat & Casein) provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bile salts and crystal violet are the selective agents, and inhibit Gram-positive organisms. Neutral red is the pH indicator. When lactose is fermented, the pH of the medium decreases, changing the color of neutral red to pink. Bacteriological agar is the solidifying agent.

The European Pharmacopoeia in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms", recommends to inoculate and incubate in casein soya been digest broth TSB Broth (**Cat. 1224**), at 30 - 35°C for 18 - 24 hours, to subculture in MacConkey Broth (**Cat. 1210**) and incubate at 42 - 44 °C for 24 - 48 hours after subculturing on a plate of MacConkey Agar at 30 - 35 °C for 18 - 72 hours.

Interpretation: Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification test. The product complies with the test if no colonies are present or if the identification tests are negative.

Isolated colonies of *Escherichia coli* are brick red in color and are surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation.

It is recommended to streak samples onto other selective media such as Eosin Methylene Blue Agar (**Cat. 1039**), SS Agar (**Cat. 1064**), XLD Agar (**Cat. 1080**), Hektoen Enteric Agar (**Cat. 1030**), Bismuth Sulfite Agar (**Cat. 1011**), especially for *Salmonella typhi*,

and/or Brillant Green Agar (**Cat. 1078**), especially for *Salmonella*. See the listings in this manual for these formulations.

CHARACTERISTICS OF COLONIES

Organisms	Colony Characteristic
Escherichia coli	Red or Pink; Not mucoid; Round; Opaque precipitate of bile salts
Klebsiella	Large, Red, Mucoid
Enterobacter aerogenes	Pink to Red
Serratia	Red to Pink, Not Mucoid
Arizona and Citrobacter	Colorless, Transparent; Red if lactose is fermented
Proteus	Colorless and Transparent
Salmonella	Colorless, Transparent or amber
Shigella	Colorless, Transparent or faintly Pink

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Colony Color	Inoculum (cfu/ml)	Recovery Rate %
Enterobacter aerogenes ATCC 13048	Good	Pink-red	10^3-10^5	≥30
**Escherichia coli* ATCC 25922	Good	Pink-red (bile precipitate)	10^3-10^5	≥30
**Escherichia coli* ATCC 8739	Good	Pink-red (bile precipitate)	10^3-10^5	≥30
Proteus vulgaris ATCC 13315	Good	Colorless	10^3-10^5	≥30
Salmonella enteritidis ATCC 13076	Good	Colorless	10^3-10^5	≥30
Shigella dysenteriae ATCC 13313	Good	Colorless	10^3-10^5	≥30
Staphylococcus aureus ATCC 6538	Inhibited	Colorless	>10^5	≤ 0.01

*According to European Pharmacopeia Incubate at 30 - 35°C for 18 - 72 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD EP

MacConkey J. H. 5:33. 1905. Joseph Md. State. Dept. Health. Procedures, 1960.

European Pharmacopoeia 7.0

MACCONKEY AGAR N° 2

CAT. 1035

For the identification of enterococci in the presence of coliforms and non-fermenting organisms in water, foods and clinical samples

FORMULA IN g/l

Bacteriological Peptone	**20.00**	Neutral Red	**0.05**
Lactose	**10.00**	Crystal Violet	**0.001**
Sodium Chloride	**5.00**	Bacteriological Agar	**13.50**
Bile Salts N ° 2	**1.50**		
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 50 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is red-violet.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

USES

MACCONKEY AGAR N°2 is a slightly selective and differential medium especially useful for the recognition of enterococci in the presence of coliforms and non-lactose fermenters from water, sewage, food and clinical samples.

Bacteriological peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate energy source, causing a pH drop and a color change in the pH indicator (Neutral red), and bile precipitation. Bile salts n° 2 and Crystal violet are the selective agents, inhibiting Gram-positive, bile-tolerant microorganisms such as staphylococci and non-fecal streptococci. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The presence of intestinal enterococci, is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

Enterococci grow as intensely red, small colonies surrounded by a zone of pale red precipitate. Non-lactose fermenting bacteria form colorless colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Pink-red (bile precipitate)
Enterococcus faecalis ATCC 29212	Good	Red
Salmonella enteritidis ATCC 13076	Good	Colorless
Staphylococcus aureus ATCC 25923	Inhibited	—

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Mac Geachie J. and Kennedy A.C. J. Clin. Path. 16. 32-38, 1963

MACCONKEY AGAR w/o CRYSTAL VIOLET

CAT. 1037

For the growth of staphylococci and enterococci for the differentiation of enteric microorganisms in water, dairy products and feces samples

FORMULA IN g/l

Gelatin Peptone	**17.00**	Peptone Mixture	**3.00**
Lactose	**10.00**	Neutral Red	**0.03**
Bile Salts N°3	**5.00**	Bacteriological Agar	**12.00**
Sodium Chloride	**5.00**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 52 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The medium should be stored at 8 - 15°C. The color is orange-red.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

USES

MACCONKEY AGAR w/o CRYSTAL VIOLET is based on the MacConkey Bile Salt–Neutral Red Lactose Agar (**Cat.1052**) and is used for the investigation of enteric microorganisms, especially enterococci from water, feces and other materials.

MacConkey Agar w/o Crystal violet is less selective, lacking the inhibitory Crystal violet and thereby allowing the growth of *Staphylococcus* and *Enterococcus*. Staphylococci produce pale pink to red colonies and enterococci produce tiny red compact colonies on or beneath the surface of the medium.

Pancreatic digest of gelatin and Meat and Casein peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bile salts are the selective agents and inhibit Gram-positive organisms. Neutral red is the pH indicator and when lactose is fermented, the pH of the medium decreases, changing the color of neutral red into pink. Bacteriological agar is the solidifying agent.

MacConkey Agar w/o Crystal Violet is plated directly with the suspected sample. For suspected pathogens from feces and other materials, also inoculate in other parallel selective media such as Desoxycholate Agar (**Cat. 1020**) or DCLS Agar (**Cat. 1045**).

Plates are inoculated and incubated at 35 ± 2°C and observed after 18 - 24 hours.

CHARACTERISTICS OF COLONIES

Organisms	Colony Characteristic
Escherichia coli	Red or Pink
Enterobacter aerogenes	Pink, Mucoid
Enterococci	Red, Small, Discrete
Staphylococci	Red to Pink
Salmonela Shigella & Pseudomonas	Colorless, Lactose-negative

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Red-pink
Enterobacter aerogenes ATCC 13048	Good	Pink
Salmonella enteritidis ATCC 13076	Good	Colorless
Staphylococcus aureus ATCC 25923	Good	Pink
Staphylococcus aureus ATCC 12228	Good	Pink

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Gray, L.D. 1995. Escherichia, Salmonella, Shigella and Yersinia, p. 450-456. In P.R. Murray, E.J. Baron, M.A. Pfaller. F.C. Tenover, and

R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Eaton, A.D., L.S. Clesceri, and A.E. Greenberg (ed.) 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

MACCONKEY AGAR w/o CRYSTAL VIOLET AND w/o SODIUM CHLORIDE

CAT. 1098

Differential medium for the detection and isolation of Enterobacteria, and inhibition of *Proteus* swarming. Recomended for urine analysis

FORMULA IN g/l

Gelatin Peptone	**17.00**	Peptone Mixture	**3.00**
Lactose	**10.00**	Neutral Red	**0.075**
Bile Salts N°3	**5.00**	Bacteriological Agar	**12.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 47 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

USES

MACCONKEY AGAR w/o CRYSTAL VIOLET AND w/o SODIUM CHLORIDE is a differential medium used for the detection and isolation of enteric microorganisms. The lack of Sodium chloride provides an electrolyte deficient medium preventing *Proteus spp.* from spreading (swarming), aiding the detection and isolation of enteric microorganisms. Also, as this medium does not contain Crystal violet, staphylococci, and enterococci species are able to grow well.

Gelatin Peptone and Peptone mixture provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Bile salts are the selective agents and inhibit Gram-positive organisms. Neutral red is the pH indicator. When lactose is fermented, the pH of the medium decreases, changing the color of neutral red into pink. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Red-pink
Enterobacter aerogenes ATCC 13048	Good	Pink
Proteus vulgaris ATCC 13315	Good	Colorless Inhibited swarming
Staphylococcus aureus ATCC 25923	Good	Pale pink
Enterococcus faecalis ATCC 19433	Good	Pink pinpoint

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

MacConkey, A. 1905 Lactose-fermenting bacteria in feces J. Hyg 5:333-379

Murray, P.R., E.J. Baron, M.A. Pfaller, F,C, Tenover, and R.H. Yolken (eds) Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Mazura-Reets, G.T. Neblett, and J.M. Galperin, 1979 MacConkey Agar: Co2 vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

MACCONKEY AGAR WITH SORBITOL

CAT. 1099

Selective and differential medium for *Escherichia coli* O157:H7

FORMULA IN g/l

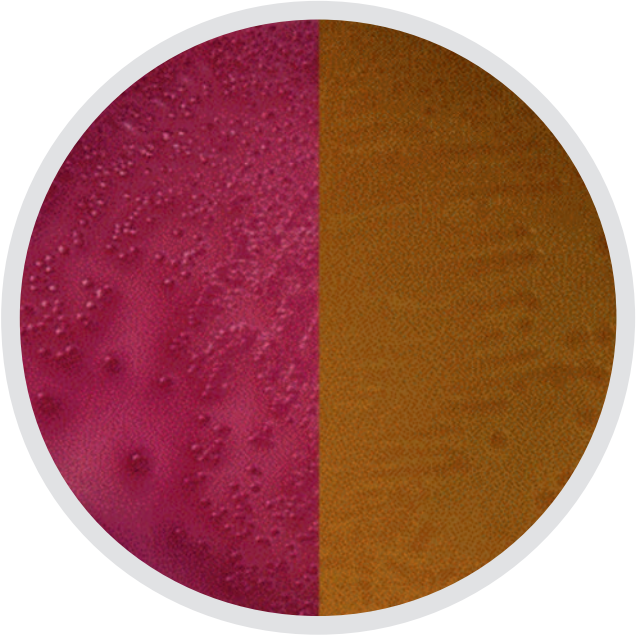
Gelatin Peptone	**20.00**	Sodium Chloride	**5.00**
Neutral Red	**0.03**	Crystal Violet	**0.001**
Sorbitol	**10.00**	Bacteriological Agar	**15.00**
Bile Salts N ° 3	**1.50**		
Final pH 7.1 ± 0.2 at 25°C			

PREPARATION

Suspend 51.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense

into plates. The prepared medium should be stored at 8 - 15°C. The color is violet-red.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.



Escherichia coli ATCC 25922 | *Escherichia coli* 0157:H7

USES

MACCONKEY AGAR WITH SORBITOL is based on the formula developed by Rappaport & Hening. This medium is recommended for the research of *E. coli* 0157:H7 in clinical and food testing. The composition is similar to MacConkey Agar but the lactose has been substituted with sorbitol for differentiating enteropathogenic *E. coli* serotypes. These strains are typically sorbitol-negative. On standard MacConkey Agar containing lactose, this strain cannot be distinguished from other lactose fermenting *E. coli*.

Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sorbitol is the carbohydrate energy source. Bile salts n°3 and Crystal violet are inhibitors of Gram-positive organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Neutral red is the pH indicator, when sorbitol is fermented, the pH of the medium decreases, changing the color from neutral red to pink. Bacteriological agar is the solidifying agent.

E. coli 0157:H7 does not ferment Sorbitol and therefore produces colorless colonies. As most of the other *E. coli* do ferment it, their colonies are pink.

E. coli 0157:H7 has become a widespread public health issue as it is responsible for hemorrhagic colitis, characterized by a bleeding diarrhea with acute abdominal pain. Incorrect antibiotic treatment may increase the risk of haemolytic uraemic syndrome development, a potentially fatal complication of this form of colitis. Optimal incubation temperature for *E. coli* 0157: H7 is 35 ± 2°C for 18 – 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Good	Pink-red (bile precipitate)
Escherichia coli 0157:H7	Good	Colorless

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Rappaport F. and Hening E. (1952), J.Clin.Path, 5.361. Karmali M.A. (1988), Culture, 9,2. Doyle M.P. and Schoeni S.L (1984), Appl. and Envir. Microbiol., 48, 855-856.

MAcCONKEY BROTH EUROPEAN PHARMACOPOEIA

CAT. 1210

For the detection of coliforms in water, milk and other materials of sanitary importance

FORMULA IN g/l

Pancreatic Digest of Gelatin	**20.00**	Dehydrated Ox Bile	**5.00**
Lactose Monohydrate	**10.00**	Bromocresol Purple	**0.01**
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 35 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. To analyze 10 ml samples, prepare a double-concentration medium. Dispense 10 ml in tubes with Durham gas collecting tubes for gas detection for samples of 1 ml or less, and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

MACCONKEY BROTH is used as a presumptive test medium for the presence of coliforms in water and other materials of sanitary importance. It is also used for cultivating Gram-negative, lactose-fermenting bacilli in water and foods.

The formation of gas and acid confirms the presence of coliforms, as demonstrated by the change of the medium color from purple to yellow.

Pancreatic digest of gelatin provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is a fermentable carbohydrate causing a drop in the pH and subsequently a color change of the pH indicator (Bromocresol purple) and bile precipitation. Ox bile is a selective agent to inhibit the growth of Gram-positive organisms.

Inoculate and incubate the medium at 35°C ± 2°C for 18 - 24 hours.

The European Pharmacopoeia recommends in Paragraph 2.6.13 Microbiological examination of non-Sterile products: to test for specified microorganisms, transfer 1 ml of Casein soy bean digest broth, where the sample was previously diluted, to 100-ml of MacConkey Broth and incubate at 42 - 44°C for 24 - 48 hours. Subculture on a plate of MacConkey agar at 30 - 35°C for 18 - 72 hours.

Interpretation:

Growth of colonies indicates the possible presence of *Escherichia coli*. This is confirmed by identification tests.

The product complies with the test if no colonies are present or if the identification tests are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Acid	Gas
Enterobacter aerogenes ATCC 13048	Good	+	+
Escherichia coli ATCC 25922	Good	+	+
**Escherichia coli* ATCC 8739	Good	+	+
Salmonella cholerasuis ATCC 12011	Acceptable	-	-
Salmonella typhimurium ATCC 14028	Acceptable	-	-
Staphylococcus aureus ATCC 25923	Null	-	-
**Staphylococcus aureus* ATCC 6538	Null	-	-

* Incubate at 42 - 44°C for 24 - 48 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

MacConkey, A. 1905. Lactose-fermenting bacteria in faeces. J. Hyg 5:333-379.

MacConkey, A. 1908. Bile salt media and their advantage in some bacteriological examinations. J. Hyg. 8:322-334.

Chils, E., and L. A. Allen. 1953. Improved methods for determining the most probable number of Bacterium coli and of Enterococcus faecalis. J. Hyg.Camb. 51:468-477.

European Pharmacopoeia. 7.0

MALT EXTRACT AGAR

CAT. 1038

For the cultivation, isolation, and enumeration of fungi and yeast

FORMULA IN g/l

Maltose Certified	**12.75**	Peptone	**0.78**
Dextrin	**2.75**	Bacteriological Agar	**15.00**
Glycerol	**2.35**		
Final pH 4.7 ± 0.2 at 25°C			

PREPARATION

Suspend 33.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. DO NOT OVERHEAT. Sterilize in autoclave at 118°C for 10 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

NOTE: If the medium is overheated the agar loses its capacity to solidify and will remain soft.

USES

MALT EXTRACT AGAR is used for the isolation, cultivation and enumeration of yeasts and molds from foods.

Malt extract provides the carbon, protein and nutrient sources required for the growth of microorganisms. It is particularly suitable for yeasts and molds as it contains a high concentration of maltose and other saccharides as energy sources. Dextrin and Glycerin are the carbon sources, and Peptone is a nitrogen source. Bacteriological agar is the solidifying agent. The acidic pH of the Malt Extract Agar is optimum for the growth of yeasts and molds whilst restricting other bacterial growth.

Malt Extract Agar has been used for years to cultivate fungi and yeast cultures in the sugar industry, in the manufacturing of syrups, soft drinks, and other drinks.

Inoculate and incubate at 30 ± 2°C for 18 - 48 or 72 hours.

It is also recommended for use in conjunction with other specific media such as Orange Serum Agar (**Cat. 1307**), Yeast Extract Agar (**Cat. 1312**) or other media for yeasts and fungi.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 2°C and observed after 18 - 48 or 72 hours.

Microorganisms	Growth
Saccharomyces cerevisiae ATCC 9763	Good
Sacharomyces uvarum ATCC 9080	Good
Candida albicans ATCC 10231	Good
Aspergillus brasiliensis ATCC 16404	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Thom and Raper, Manual of the Aspergili 39:1945

MALT EXTRACT BROTH

CAT. 1245

For the isolation and enumeration of molds and yeast

FORMULA IN g/l

Malt Extract	**13.00**	Yeast Extract	**0.50**
Gelatin Peptone	**5.50**		
Final pH 4.7 ± 0.2 at 25°C			

PREPARATION

Suspend 19 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MALT EXTRACT BROTH is used for the isolation, cultivation and enumeration of yeasts and molds from foods.

Malt extract provides the carbon, protein and nutrient sources required for the growth of microorganisms. It is particularly suitable for yeasts and molds as it contains a high concentration of maltose and other saccharides as energy sources. Maltose is added as an energy source. Dextrose is included as a source of fermentable carbohydrate. Yeast extract is the source of vitamins, particularly of the B-group. The acidic pH of the Malt Extract Broth is optimum for the growth of yeasts and molds whilst restricting other bacterial growth.

Inoculate and incubate at 30 ± 2°C for 18 -72 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 2°C and observed after 18 - 48 or 72 hours.

Microorganisms	Growth
Saccharomyces cerevisiae ATCC 9763	Good
Sacharomyces uvarum ATCC 9080	Good
Candida albicans ATCC 10231	Good
Asperigullus brasiliensis ATCC 16404	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Thom and Raper, Manual of the Aspergili 39:1945

MANNITOL NITRATE MOTILITY MEDIUM

CAT. 1509

For the rapid differentiation of Enterobacteria from clinical samples

FORMULA IN g/l

Casein Peptone	**10.00**	Phenol red	**0.04**
Mannitol	**7.50**	Bacteriological Agar	**3.50**
Potassium Nitrate	**1.00**		
Final pH 7.6 ± 0.2 at 25°C			

PREPARATION

Suspend 22 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes to obtain a butt depth of 6 – 7 cm and sterilize in autoclave at

121°C for 15 minutes. The prepared medium should be stored at 2 - 8ºC. The color is pink.

The dehydrated medium should be homogeneous, free-flowing and pink-orange in color. If there are any physical changes, discard the medium.

USES

MANNITOL NITRATE MOTILITY MEDIUM is a semisolid medium that permits the rapid identification of Enterobacteria on the basis of motility, mannitol utilization and nitrate reduction to nitrite.

Casein peptone provides the nitrogen, minerals, amino acids and nutrients essential for bacterial growth. Mannitol is a fermentable carbohydrate for energy source. Potassium nitrate provides additional nutrients and organisms capable of reducing nitrate show increased motility. Phenol red is a pH indicator. Bacteriological agar is the solidifying agent.

The medium is inoculated by stabbing the center of the tube to its base and incubating at 35 ± 2°C for 18 - 24 hours.

Motile bacteria show a diffuse turbidity away from the inoculation line, while non-motile organisms only grow along the stab line. If mannitol is fermented, the medium changes color from red to yellow.

Nitrate reduction tests are conducted adding Griess reagent (2 drops of solution A, and then 2 drops of the solution B) to the surface of the medium. Nitrate-negative organisms are unable to reduce nitrates and they yield no color after adding the reagent.

Nitrate-positive: The appearance of a pink or red coloration indicates that the nitrates have been reduced to nitrites.

Griess reagent consists of solutions:

Solution A:	**Solution B:**
Sulfanilic Acid..... 8g	Dimetil-α-naphtylamine...10 mg
Acetic Acid 5N.....1 liter	Acetic Acid 5N.....1 liter

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Motility	Mannitol	Nitrate
Escherichia coli ATCC 25922	+	+	+
Klebsiella pneumoniae ATCC 13883	-	+	+
Proteus mirabilis ATCC 25933	+	-	+
Acinetobacter anitratum ATCC 17924	-	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Titters R.R. and L.A. Sancholzer 1936. The use of semi-solid agar for the detection of bacterial motility, J. Bacteriol 31: 575-580. Snell and Wright; 1941. J. Biolog. Chem. 13: 675.

Compendium of methods for the microbiological examination of foods. Am. Public. Health Association.

MANNITOL SALT AGAR (MSA) (CHAPMAN MEDIUM) EUROPEAN PHARMACOPOEIA

CAT. 1062

For the isolation and enumeration of pathogenic staphylococci from clinical samples and other materials

FORMULA IN g/l

Sodium Chloride	**75.00**	Beef Extract	**1.00**
D-Mannitol	**10.00**	Phenol red	**0.025**
Pancreatic Digest of Casein	**5.00**	Bacteriological Agar	**15.00**
Peptic Digest of Animal Tissue	**5.00**		
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 111 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

USES

MANNITOL SALT AGAR (MSA) is a selective medium prepared according to the recommendations of Chapman for the isolation of presumptive pathogenic staphylococci. Most of the other bacteria are inhibited by the high concentration of Sodium chloride.

The Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Mannitol is the carbohydrate energy source and Phenol red is the pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The degradation of mannitol by bacteria produces acidic products that change the color of the medium from pink to yellow. Due to its high content of sodium chloride, a heavy inoculum of the material in study can be used.

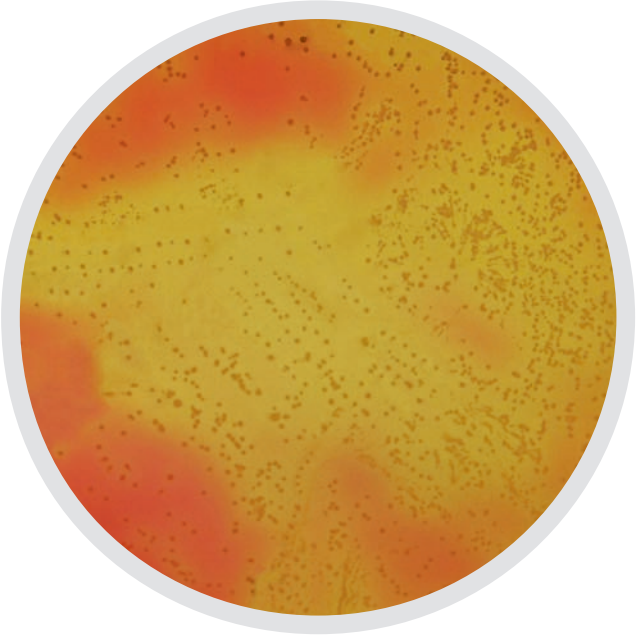
The European Pharmacopeia recommends in the Paragraph 2.6.13 "Microbiological examination of non-sterile products: Test for specified microorganisms". After incubation in Casein Soya Bean Digest Broth (TSB **Cat. 1224**) at 30 - 35°C for 18 - 24 hours, subculture on a plate of Mannitol Salt Agar (MSA), the incubation of the plates at 30 - 35°C for 18 - 72 hours for growing promotion test and also to inoculate and incubate *Escherichia coli* ATCC 8739 as negative control. The mannitol fermenting pathogenic staphylococci are large and are surrounded by a yellow zone, colonies of non-pathogenic staphylococci appear as small colonies surrounded by a red or purple zone.

Interpretation: The possible presence of *S. aureus* is indicated by the growth of yellow /white colonies surrounded by a yellow zone. This is confirmed by identification test.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

The addition of 5% Egg Yolk Emulsion (**Cat. 5152**) allows to detect the lipase activity of staphylococci, as well as mannitol fermentation. The high concentration of salt in the medium clears the egg yolk emulsion, and lipase production is detected as a yellow opaque zone around the colonies of staphylococci producing this enzyme. This phenomenon, together with a positive coagulase test, confirms the organism as a pathogenic *Staphylococcus*.

Inoculate and incubate at 35 ± 2°C and observe after 18 - 24 hours and after 48 hours.



Staphylococcus aureus
ATCC 25923

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours and after 48 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Inhibited	
**Escherichia coli* ATCC 8739	Inhibited	
Enterobacter aerogenes ATCC 13048	Inhibited	
Staphylococcus aureus ATCC 25923	Good	Yellow
**Staphylococcus aureus* ATCC 6538	Good	Yellow
Staphylococcus epidermidis ATCC 12228	Acceptable	Red
Staphylococcus epidermidis ATCC 14990	Good	Red

* According to European Pharmacopeia 7.0 incubate at 30 - 35°C during 18 - 72

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD EP

McColloch Am. J. Vet. Research, 8:173. 1947. Velilla, Faber, and Pelczar Am. J. Vet. Research, 8:275. 1947.

Chapman, G.H. 1945 J. Bact. 50:201-203

European Pharmacopoeia. 7.0.

MARINE AGAR

CAT. 1059

For the isolation and enumeration of heterotrophic marine bacteria

FORMULA IN g/l

Sodium Chloride	**19.40**	Potassium Bromide	**0.08**
Magnesium Chloride	**8.80**	Strontium Chloride	**0.034**
Bacteriological Peptone	**5.00**	Boric Acid	**0.022**
Sodium Sulfate	**3.24**	Disodium Phosphate	**0.008**
Calcium Chloride	**1.80**	Sodium Silicate	**0.004**
Yeast Extract	**1.00**	Sodium Fluoride	**0.0024**
Potassium Chloride	**0.55**	Ammonium Nitrate	**0.0016**
Sodium Bicarbonate	**0.16**	Bacteriological Agar	**15.00**
Ferric Citrate	**0.10**		
Final pH 7.6 ± 0.2 at 25°C			

PREPARATION

Suspend 55.20 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent, and may present a light precipitation. It is recommended to homogenize the medium in its container before pouring into plates.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MARINE AGAR is a medium containing all the nutrients necessary to cultivate the majority of marine bacteria.

Since the marine environment is characterized by unique environmental conditions, its microflora is also unique. Marine microorganisms have the ability to survive at very low temperatures and at high salinity levels.

Both Marine Agar (**Cat. 1059**) and Marine Broth are prepared according to ZoBell, containing almost double the mineral content of seawater. The high salt content helps to simulate seawater. Bacteriological peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a is source of vitamins, particularly of the B-group. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 20 - 25°C for 24 - 72 hours.

Using the conventional plate count technique or streaking the surface of the plate yields good results. In the spread plate technique, the agar is poured while hot and allowed to cool before inoculation. However, precaution must be taken in the pour plate method to cool the medium to 42°C before pouring, as the majority of marine organisms are heat-sensitive.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 20 - 25°C and observed after 24 - 72 hours.

Microorganisms	Growth
Vibrio fischeri ATCC 7744	Good
Vibrio harveyi ATCC 14126	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

J. Marine Research N:42. 1941. Limnology and Oceanography 5:78, 1960.

ZoBell, C.E. 1941. Studies on Marine Bacteria. I. The cultural requirements of heterotrophic aerobes. J.Mar.Res. 4:42-75. Buck, J.D., and R.C. Cleverdon. 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. Weiner, R.M., A.M. Segall, and R.R. Colwell. 1985.

MARINE BROTH

CAT. 1217

For the isolation and enumeration of heterotrophic marine bacteria

FORMULA IN g/l

Sodium Chloride	**19.40**	Ferric Citrate	**0.10**
Magnesium Chloride	**8.80**	Potassium Bromide	**0.08**
Bacteriological Peptone	**5.00**	Strontium Chloride	**0.034**
Sodium Sulfate	**3.24**	Boric Acid	**0.022**
Calcium Chloride	**1.80**	Disodium Phosphate	**0.008**
Yeast Extract	**1.00**	Sodium Silicate	**0.004**
Potassium Chloride	**0.55**	Sodium Fluoride	**0.0024**
Sodium Bicarbonate	**0.16**	Ammonium Nitrate	**0.0016**
Final pH 7.6 ± 0.2 at 25°C			

PREPARATION

Suspend 40.20 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent. It may present a light precipitation.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MARINE BROTH is similar to Marine Agar (**Cat. 1059**), lacking the agar, but containing all the nutrients necessary to cultivate the majority of marine bacteria.

Since the marine environment has environmental conditions completely different to those of other environments, its microflora is also very different. Marine Microorganisms are capable of surviving at very low temperatures and in high salinity levels.

Both Marine Agar (**Cat. 1059**) and Marine Broth are prepared according to ZoBell, containing almost double the mineral content of sea water. The high salt content helps to simulate seawater. Bacteriological peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Bacteriological agar is the solidifying agent.

Dispense 50 ml of the broth in 250 ml Erlenmeyer flasks. Inoculate and incubate at 20 - 25°C for 24 - 72 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 20 - 25°C and observed after 24 - 72 hours.

Microorganisms	Growth
Vibrio fischeri ATCC 7744	Good
Vibrio harveyi ATCC 14126	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

J. Marine Research N:42. 1941. Limnology and Oceanography 5:78, 1960.

ZoBell, C.E. 1941. Studies on Marine Bacteria. I. The cultural requirements of heterotrophic aerobes. J.Mar.Res. 4:42-75. Buck, J.D., and R.C. Cleverdon. 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. Weiner, R.M., A.M. Segall, and R.R. Colwell. 1985.

m-EI CHROMOGENIC AGAR BASE

CAT. 1412

For the detection and enumeration of *Enterococcus* in water through the single step membrane filtration technique

FORMULA IN g/l

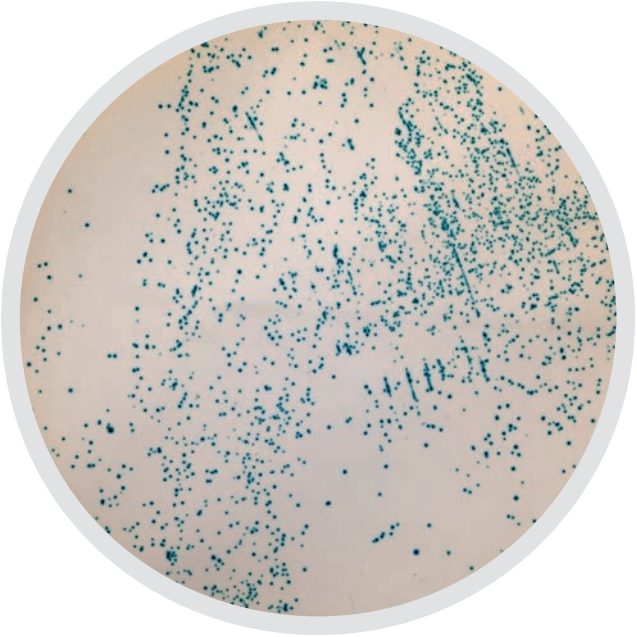
Yeast Extract	**30.00**	X-Glucoside	**0.75**
Sodium Chloride	**15.00**	Sodium Azide	**0.15**
Peptone	**10.00**	Cycloheximide	**0.05**
Esculine	**1.00**	Bacteriological Agar	**15.00**
Final pH 7.1 ± 0.2 at 25°C			

PREPARATION

Suspend 71.95 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. For a more selective medium, prepare a solution of 0.24 grams of nalidixic acid in 5 ml of sterile distilled water with a few drops of sodium hydroxide 0.1N (for a better dissolution), and aseptically add to one liter of medium. If desired, 15 ml per liter of a 1% TTC solution can be added. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium contains Sodium azide and Cycloheximide and it is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye face protection.



Enterococcus faecalis
ATCC 19433

USES

m-EI CHROMOGENIC AGAR BASE is recommended for the detection and enumeration of enterococci in water by the membrane filter technique.

The medium was developed as a single-step procedure that does not require the transfer of the membrane filter to another substrate. Observation of blue color colonies confirms the presence of enterococci.

A wide range of sample volumes or dilutions can be tested by this single-step membrane filtration procedure for the detection and enumeration of enterococci in potable, fresh, estuarine, marine and shellfish-growing waters.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract provides trace elements, vitamins and amino acids. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. Cycloheximide inhibits most fungi, and the sodium azide inhibits Gram-negative bacteria. X-Glucoside is the substrate of the glucosidase-positive enterococci and the agar is added into the medium as a solidifying agent.

Inoculate and incubate to 41 ± 0.5 °C and observe alter 18 - 24 hours. *Enterococcus* species will growth as blue colonies. If TTC is added, then the colonies will grow red.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the nalidixic acid added and without TTC, after incubation at a temperature of 41 ± 0.5°C and observed alter 18 - 24 hours.

Microorganisms	Growth	Colony Color
Enterococcus faecium ATCC 9790	Good	Blue
Enterococcus faecalis ATCC 19433	Good	Blue
Escherichia coli ATCC 25922	Inhibited	–

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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U.S. Environmental Protection Agency. 2002. Method 1600: Enterococci in water by membrane filtration using membrane enterococcus indoxyl –D- glucoside agar (mEI). Publication EPA-821- R-02-022. USEPA Office of Water, Office of Science and Technology, USEPA, Washington, DC.

MINERALS MODIFIED GLUTAMATE BROTH (MMGB) ISO 16649-3

CAT. 1365

For the enumeration of coliforms in water

FORMULA IN g/l

Lactose	**10.00**	L-Cystine	**0.02**
Sodium Glutamate	**6.35**	Ferric Ammonium Citrate	**0.01**
Dipotassium Phosphate	**0.90**	Calcium Chloride Dehydrate	**0.01**
Sodium Formate	**0.25**	Bromocresol Purple	**0.01**
Heptahydrate Magnesium Sulfate	**0.10**	Thiamine	**0.001**
L (-) Aspartic Acid	**0.024**	Panthothenic Acid	**0.001**
L (+) Arginine	**0.02**	Nicotinic Acid	**0.001**
Final pH 6.7 ± 0.1 at 25°C			

PREPARATION

Suspend 17.7 grams of the medium in one liter of distilled water. Add 2.5 grams of Ammonium Chloride. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 116°C for 10 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MINERALS MODIFIED GLUTAMATE BROTH Base (MMGB) is recommended by ISO 16649-3 for enumeration of *E. coli* using the most probable number Method (MPN). MMGB Broth is also an alternative broth used for the presumptive identification of coliforms in water.

ISO 16649-3 recommends to inoculate three tubes of a single strength and three tubes of a double strength medium. The tubes of double- and single-strength medium are inoculated at 37 °C for 24 hours. The tubes are examined for acid production, indicating lactose fermentation. Each tube of selective enrichment medium showing acid production is subcultured to TBX Chromogenic Agar (**Cat. 1151**).

In comparison to other media, this broth is better as it gives fewer false positive results. Sodium Glutamate and Sodium formate are the basis of the medium for the enumeration of coliform organisms in water. Lactose is the source of carbohydrates. The addition of vitamins, aminoacids and heptahidrate magnesium sulfate increase fermentation, whereas the addition of ferric ammonium citrate permits increase of gas production. The Bromocresol purple is a pH indicator.

Incubate at 35°C ± 2°C and observe after 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium (having added 2.5 grams of ammonium chloride per liter) from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Gas and Acid Production (yellow)
Enterobacter aerogenes ATCC 13048	Good	+
Enterococcus faecalis ATCC 19433	Inhibited	-
**Escherichia coli* ATCC 25922	Good	+
**Escherichia coli* ATCC 8739	Good	+
Salmonella typhimurium ATCC 14028	Good	-

* Incubate at 37°C for 24 ± 2 hours According to ISO 16649

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 16649-3 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of _-glucuronidase-positive Escherichia coli- Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-_-D-glucuronide.

Departments of the Environment, Health & Social Security, and P.H.L.S. 1982. The bacteriological examination of drinking water supplies. Report on public Health and Medical Subjects No. 71., H.M.S.O., London, England.

MIO MEDIUM (MOTILITY - INDOLE - ORNITHINE)

CAT. 1510

For the differentiation of Enterobacteriaceae

FORMULA IN g/l

Gelatin Peptone	10.00	Dextrose	1.00
Casein Peptone	10.00	Bromocresol Purple	0.02
L-Ornithine	5.00	Bacteriological Agar	2.00
Yeast Extract	3.00		
Final pH 6.5 ± 0.2 at 25°C			

PREPARATION

Suspend 31 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in screw-capped tubes and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MIO MEDIUM (Motility - Indole - Ornithine) is a semisolid medium used for the differentiation of the Enterobacteriaceae group by motility, ornithine decarboxylase activity and indole production.

Gelatin and Casein peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. They also provide tryptophan, needed for the creation of indole. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. L-ornithine is added to test the presence of the enzyme ornithine decarboxylase. If the organisms posses such enzyme, it will be activated in an acid environment created by the initial fermentation of dextrose. Once the amino acid is decarboxylated, diamine putescine is produced. The result is an alkalinization of the medium, which turns it a dark blue. Organisms without the enzyme, will remain acidic due to the fermentation, resulting in a yellow color in the medium. Bromocresol purple is a pH indicator to indicate decarboxylase activity; the low concentration of Bacteriological agar is for motility.

Inoculate by stabbing the MIO medium and incubate in an aerobic atmosphere for 18 - 24 hours at 35 ± 2°C. If the indole reaction is negative, incubate for an additional 24 hours. Read the motility and ornithine decarboxylase reactions before adding the Kovac's Reagent (**Cat. 5205**) for the indole test. The motility is indicated by cloudiness in the media or growth extending away from the line of inoculation. Ornithine decarboxylation is indicated by a purple color in the medium. A negative ornithine reaction produces a yellow color at the bottom of the tube.

For the indole test, add 3 to 4 drops of Kovac's Reagent (**Cat. 5205**), and shake the tube gently. The appearance of a red or pink color in the reagent layer is a positive indication of indole. Kovacs reagent detects the microorganism capable of cleaving the tryptophan. When these microorganisms are present in the medium they liberate indole that reacts with 4-dimethylaminobenzaldehyde to form a dark red dye.

Compare the results with a non-inoculated test tube.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2 and observed after 18 - 24 hours.

Microorganisms	Growth	Motility	Indole	Ornithine Decarboxylation
Escherichia coli ATCC 25922	Good	+	+	+
Enterobacter aerogenes ATCC 13048	Good	+	-	+
Klebsiella pneumoniae ATCC 13883	Good	-	-	-
Proteus mirabilis ATCC 25933	Good	+	-	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ederer, G.M., and M. Clark. 1970. Motility-Indole-Ornithine medium. Appl. Microbiol. 2:849.

Oberhofer, T.R., and R. Hajkowski. 1970. Evaluation of non-lactose-fermenting members of the Klebsiella-Enterobacter-Serratia Division. I. Biochemical characteristics. Am. J. Clin. Pathol. 54:720.

MOELLER KCN BROTH BASE

CAT. 1112

For the differentiation of enteric bacilli

FORMULA IN g/l

Sodium Phosphate	**5.64**	Peptone Mixture	**3.00**
Sodium Chloride	**5.00**	Potassium Phosphate	**0.225**
Final pH 7.6 ± 0.2 at 25°C			

PREPARATION

Suspend 14 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and aseptically add 15 ml of a 0.5 % potassium cyanide solution (0.5 g per 100 ml of sterile distilled water) to each tube containing 10 ml of medium and close tightly. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: Take extreme care when handling cyanide solution. Do not pipette by mouth.

USES

MOELLER KCN BROTH BASE, supplemented with a solution of potassium cyanide, is used in the differentiation of enteric bacilli based on their ability to grow quickly in the presence of cyanide.

The medium facilitates the recognition and identification of enteric bacilli similar to *Citrobacter freundii*, especially those that are slow to fermentate but develop rapidly in the presence of cyanide. Also, this medium is very useful in differentiating *Salmonella* (including the Arizona group).

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium phosphate and Potassium phosphate provide minerals and ions and act as a buffer system.

Inoculate the medium lightly so that the inoculum cannot be misinterpreted as growth when cultures are examined. This may be accomplished by using a 3 mm loopful of an overnight (24 hours) broth cultura, or by transferring a light inoculum from an agar slant culture with a straight wire. Inoculate and incubate at 35 ± 2°C for 24 - 48 hours.

The following table indicates the growing of the important groups of Enterobacteria.

GROWTH	*Enterobacter* *Klebsiella* *Proteus* *Citrobacter* *Providencia* *Hafnia*	NO GROWTH	*Escherichia* *Arizona* *Salmonella* *Shigella*

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Enterobacter spp.	Good
Citrobacter freundii ATCC 8090	Good
Proteus vulgaris ATCC 6380	Good
Escherichia coli ATCC 25922	Null
Salmonella enteritidis ATCC 13076	Null
Shigella flexneri ATCC 12022	Null

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Moeller. Acta Path. and Microbiol. Scand., 134:11 5. 1954.

Gershmand Cn. J. Mocrobiol, 1. 1960

Edwards and Ewing, Identification of Enterobacteriaceae. Burgess Publ. Co., Minneapolis, Minn., 1972.

MOSSEL EE BROTH EUROPEAN PHARMACOPEIA

CAT. 1202

Selective medium for Enterobacteriaceae in foods, especially *Salmonella* and coliforms

FORMULA IN g/l

Dehydrated Ox Bile	**20.00**	Glucose Monohydrate	**5.00**
Pancreatic Digest of Gelatin	**10.00**	Potassium Dihydrogen Phosphate	**2.00**
Disodium Hydrogen Phosphate Dihydrate	**8.00**	Brilliant Green	**0.015**

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 45 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Heat at 100°C for 30 minutes. Cool immediately. AVOID OVERHEATING. DO NOT AUTOCLAVE. Dispense into appropriate containers. The prepared medium should be stored at 2 - 8°C. The color is green.

The dehydrated medium should be homogeneous, free-flowing and light green in color. If there are any physical changes, discard the medium.

Escherichia coli ATCC 8739 — Uninoculated Tube

USES

MOSSEL EE (ENTEROBACTERIACEAE ENRICHMENT) BROTH is as an enrichment broth, used to promote the growth of the Enterobacteriaceae group, microorganisms which contaminate foods.

The enumeration of Enterobacteriaceae is of great importance when controlling the sanitary condition of food. Microorganisms can be injured in food processing, including exposure to low temperatures, sub marginal heat, drying, radiation, preservatives or sanitizers. Recovery depends on the adequate resuscitation of damaged cells. *Escherichia coli*, even though present in small numbers as a contaminant in foods, grows easily in this medium.

Pancreatic digest of gelatin provides nitrogen, vitamins, minerals and amino acids essential for growth. Glucose is the fermentable carbohydrate providing carbon and energy. Disodium phosphate and Monopotassium phosphate act as a buffer system. Brilliant green and Ox bile are selective agents, inhibiting Gram-positive microorganisms.

Inoculate e incubate at 35 ± 2°C for 18 - 48 hours.

The European Pharmacopoeia in Paragraph 2.6.13: "Microbiological examination of non-Sterile products: test for specified microorganisms" recommends this medium for the testing of products for bile-tolerant Gram-negative bacteria. The sample is prepared using 1 ml in 10 ml of Casein Soya Bean Digest Broth (TSB Broth **Cat. 1224**). For the absence test, use the dilution made previously and the volume corresponding to 1 g of the product to inoculate in Mossel EE Broth. Incubate at 30 - 35°C for 24 - 48 hours. Subculture on plates of Violet Red Bile Agar with Glucose (VRBG) (**Cat. 1092**). Incubate at 30 - 35°C for 18 - 24 hours. The product complies with the test if there is no growth of colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Yellow Color (Acid)
Enterobacter aerogenes ATCC 13048	Good	+
Escherichia coli ATCC 25922	Good	+
**Escherichia coli* ATCC 8739	Good	+
Salmonella enteritidis ATCC 13076	Good	± (could be slow)
Salmonella typhimurium ATCC 14028	Good	± (could be slow)
Staphylococcus aureus ATCC 25923	Inhibited	-
**Staphylococcus aureus* 6538	Inhibited	-
**Pseudomonas aeruginosa* ATCC 9027	Good	-

*According to European Pharmacopoeia incubate at 30 - 35°C for 24 - 48 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

Mossel D.A.A., Visser M. and Cornelissen A.M.R.J App, Bact. 24:444. 1963.

Mossel D.A.A et al. J. BAct. 84:381. 1982

European Pharmacopoeia. 7.0

MR-VP MEDIUM

CAT. 1512

For the differentiation of the *Escherichia-Enterobacter* group (Methyl Red and Voges-Proskauer reactions) from clinical samples

FORMULA IN g/l

Peptone Mixture	**7.00**	Potassium Phosphate	**5.00**
Dextrose	**5.00**		
Final pH 6.9 ± 0.2 at 25°C			

PREPARATION

Suspend 17 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

MR-VP MEDIUM is used in the differentiation of enteric Gram-negative bacilli on the basis of the methyl red and acetylmethylcarbinol (Voges-Proskauer) reactions of the *Escherichia-Enterobacter* group.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphate acts as a buffer system.

In 1915, Clark and Lubs used methyl red as an indicator of acidity in the cultures of the Coli-Enterobacter group. This test is now known as the methyl red test and serves to distinguish between those microorganisms that produce and maintain a high concentration of acid from those that initially produce a small amount of acid and are capable of later attacking those same acids, turning the medium neutral or alkaline, such as *Enterobacter* species.

Voges and Proskauer described in 1898 a fluorescent red coloration that appeared in certain cultures upon adding drops of KOH solution. Later it was supposed that this reaction was due to the oxidation of acetylmethylcarbinol to diacetyl that reacted with the peptone of the medium to give a red color. *Enterobacter* bacteria oxidize the acetylmethylcarbinol and give a red coloration, in contrast to *Escherichia coli* that does not.

Methyl Red test (MR):

Add 5 drops of a 0.4% solution of methyl red to 5 ml of an already incubated tube at a temperature of 35 ± 2°C for 3 to 5 days. A positive reaction will give a red color, and a negative a yellow color. The reaction is immediate.

Voges-Proskauer test (VP):

To a 5 ml of up to 5 days inoculated and incubated medium, add 0.6 ml of 5% alpha-naphthol in absolute ethanol and 0.2 ml of 40% sodium hydroxide and shake from time to time over a 15-minute period. The tube may be held at room temperature or incubated at 35 ± 2°C. It is important to add the reagents in sequence. A positive test is indicated by the development of a faint pink to red color. The test should not be read after one hour because negative VP cultures may develop a copper color after that time.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours or after 5 days. Determine the Methyl Red and Voges-Proskauer test reactions.

Microorganisms	Growth	MR	VP
Enterobacter aerogenes ATCC 13048	Good	- (yellow)	+ (red)
Escherichia coli ATCC 25922	Good	+ (red)	- (without change)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Clark and Lubs. J.: Inf. Dis. 17:160. 1955.

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Edwards and Ewing. Identification of Enterobacteriaceae Burgess Publ. Co. Minneapolis, Minn., 1962.

Voges, O., and B. Proskauer. 1898. Z. Hyg. 28: 20-22.

Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

MRS AGAR

CAT. 1043

Recommended medium for the growth of lactobacilli

FORMULA IN g/l

Dextrose	20.00	Ammonium Citrate	2.00
Bacteriological Peptone	10.00	Tween 80	1.00
Beef Extract	8.00	Magnesium Sulfate	0.20
Sodium Acetate	5.00	Manganase Sulfate	0.05
Yeast Extract	4.00	Bacteriological Agar	10.00
Dipotassium Phosphate	2.00		
Final pH 6.2 ± 0.2 at 25°C			

PREPARATION

Suspend 62 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 12 minutes. Cool to 45 - 50°C, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MRS AGAR is a selective medium, based on the formulation developed by de Man, Rogosa and Sharpe to provide a medium that would support the good growth of lactobacilli in general, but in particular of those strains which showed poor growth in existing media such as *L. brevis* and *L. fermenti*, replacing a variable product (tomato juice).

The medium is apt for the growth of lactic acid bacteria, including *Lactobacillus, Pediococcus* and *Leuconostoc*.

Ammonium citrate, at a low pH, inhibits most microorganisms, but allows the growth of lactobacilli. Dipotassium phosphate and Sodium acetate are buffer agents to maintain a low pH. Tween 80 is an emulsifier. Manganese and Magnesium sulfates are sources of ions and sulfate. Bacteriological peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate. Bacteriological agar is the solidifying agent.

The pour plate method requires 1 ml of the previously diluted sample to be poured into a sterile Petri dish and the cooled (45 - 50°C) medium is then added. After solidification, a second layer is poured. The plates are incubated in 5% CO_2 at 35°C for 3 days or at 30°C for 5 days. It is important to maintain a humid atmosphere because the plates should not dry out during incubation. Lactobacilli are microaerophilic and generally require layered plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white.

The growth of some *Lactobacillus* strains are inhibited at a higher pH of 6.0 and it is necessary to acidify the media to promote the growth. To acidify the media some drops of acetic acid can be added.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C during 3 days, or at 30°C during 5 days, in a CO_2 enriched atmosphere.

Microorganisms	Growth
Lactobacillus acidophilus ATCC 4356	Good
Lactobacillus casei ATCC 393	Good
Escherichia coli ATCC 25922	Moderate-Good
Pseudomonas aeruginosa ATCC 27853	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Briggs M (1.953) "An Improved Medium for Lactobacilli" J. Dairy Res. 20. 36-40.

De Man, J.C. Rogosa, M., Sharpe, Elisabeth (1960) "A Medium for the Cultivation of Lactobacilli". J. Appl. Bact. 23. 130-135

MRS AGAR low pH ISO 15214

CAT. 1433

Recommended medium for the growth of lactobacilli in general

FORMULA IN g/l

Dextrose	20.00	Triammonium Citrate	2.00
Enzymatic Digest of Casein	10.00	Tween 80	1.08
Beef Extract	10.00	Magnesium Sulfate	0.20
Sodium Acetate	5.00	Manganase Sulfate	0.05
Yeast Extract	4.00	Bacteriological Agar	10.00
Dipotassium Phosphate	2.00		
Final pH 5.7 ± 0.1 at 25°C			

PREPARATION

Suspend 64.00 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well

and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MRS AGAR is a selective medium that supports the good growth of lactobacilli in general.

The medium is apt for the growth of lactic acid bacteria, including *Lactobacillus, Pediococcus* and *Leuconostoc*.

Ammonium citrate, at a low pH, inhibits most microorganisms, but allows the growth of lactobacilli. Dipotassium phosphate and Sodium acetate are buffer agents to maintain a low pH. Tween 80 is an emulsifier. Manganese and Magnesium sulfates are sources of ions and sulfate. Bacteriological peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate. Bacteriological agar is the solidifying agent.

The growth of some lactobacillus strains is inhibited at a pH higher than 6.0 and it is necessary to acidify the medium to favor the growth. With a pH of 5.7 ± 0.1, this medium favors the growth of these strains.

Normative ISO 15214 recommends this medium for the enumeration of mesophilic lactic acid bacteria by colony count technique at 30°C. Inoculate and incubate at 30°C for 72 hours, and count the colonies on the plates that contains between 15 and 300 colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C during 3 days, or at 30°C during 5 days, under a CO_2 enriched atmosphere in an anaerobic jar, and observed after 24 hours.

Microorganisms	Growth
**Lactobacillus acidophilus* ATCC 4356	Good
**Lactobacillus casei* ATCC 393	Good
**Lactobacillus fermentum* ATCC 9338	Moderate-Good
Escherichia coli ATCC 25922	Moderate-Good
Pseudomonas aeruginosa ATCC 27853	Inhibited

* According to ISO 15214 incubate at 30°C during 72 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 15214: Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of mesophilic lactic acid bacteria -- Colony-count technique

ROGOSA, M., a. SHARPE, M.E.: An approach to the classification of the lactobacilli. " J. Appl. Bact., 22; 329-340 (1959).

MRS BROTH

CAT. 1215

Medium to facilitate de growth of lactobacilli

FORMULA IN g/l

Dextrose	**20.00**	Dipotassium Phosphate	**2.00**
Bacteriological Peptone	**10.00**	Tween 80	**1.00**
Beef Extract	**8.00**	Magnesium Sulfate	**0.20**
Sodium Acetate	**5.00**	Manganase Sulfate	**0.05**
Yeast Extract	**4.00**	Ammonium Citrate	**2.00**
Final pH 6.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 52.25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 12 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MRS BROTH has the same formulation as MRS Agar (**Cat. 1043**) without the agar as a solidifying agent. It is used when a fluid medium is preferred.

It was developed by de Man, Rogosa and Sharpe to provide a medium that would support the good growth of lactobacilli, but in particular for those strains which showed poor growth in existing media such as *L brevis* and *L. fermenti*, replacing a variable product (tomato juice). The medium is apt for the growth of lactic acid bacteria, including *Lactobacillus, Pediococcus* and *Leuconostoc*.

Ammonium citrate at a low pH inhibits most microorganisms, but allows the growth of lactobacilli. Dipotassium phosphate and Sodium acetate are buffer agents to maintain a low pH. Tween 80 is an emulsifier; Manganese and Magnesium sulfates are sources of ions and sulfate. Bacteriological peptone and Beef extract provide nitrogen, vitamins, minerals and amino

acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Dextrose is the fermentable.

The times and temperatures of incubation are the same as in MRS Agar (35°C for 3 days or better, 30°C for 5 days). Tubes showing growth are subcultured to MRS Agar to confirm the presence of Lactobacilli.

MRS Broth may be used for other tests in the identification of of lactobacilli such as temperature dependence, growth in 4% NaCl, growth in 0.4% Teepol, etc. as recommended by Sharpe, Fryer and Smith.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35°C during 3 days or at 30°C during 5 days.

Microorganisms	Growth
Lactobacillus acidophilus ATCC 4356	Good
Lactobacillus casei ATCC 393	Good
Lactobacillus fermentum ATCC 9338	Moderate-Good
Escherichia coli ATCC 25922	Moderate-Good
Pseudomonas aeruginosa ATCC 27853	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Sharpe M. Elisabeth, Fryer T.F. and Smith D.G. (1966) "Identification of the Lactic Acid Bacteria in Identification Method for Microbiologist Part A" (Gibbs B.M. and Skinner F.A. eds.) London and New York, Academic Press.

Briggs M. (1953) J. dairy Res., 20: 36-40

Reuter G. (1985) Intern. J. Food Microbiol 2: 55-68.

MRSA AGAR CHROMOGENIC

CAT. 1423

For the detection of methicillin resistant *Staphylococcus aureus* from clinical samples

FORMULA IN g/l

Growth Factors	**78.00**	Chromogenic Substrate	**1.90**
Peptone Mixture	**11.00**	Bacteriological Agar	**12.50**

Final pH 7.2 ± 0.2 at 25ºC

PREPARATION

Suspend 51.7 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. Sterilize in autoclave at 121ºC during 15 minutes. Cool to 45 - 50ºC and aseptically add one vial of Cefoxitin Supplement (**Cat. 6069**) reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and grey in color. If there are any physical changes, discard the medium.

Cefoxitin MRSA Supplement (Cat. 6069)
(1 vial for 500 ml of the medium)

Cefoxitin..... 2 mg

USES

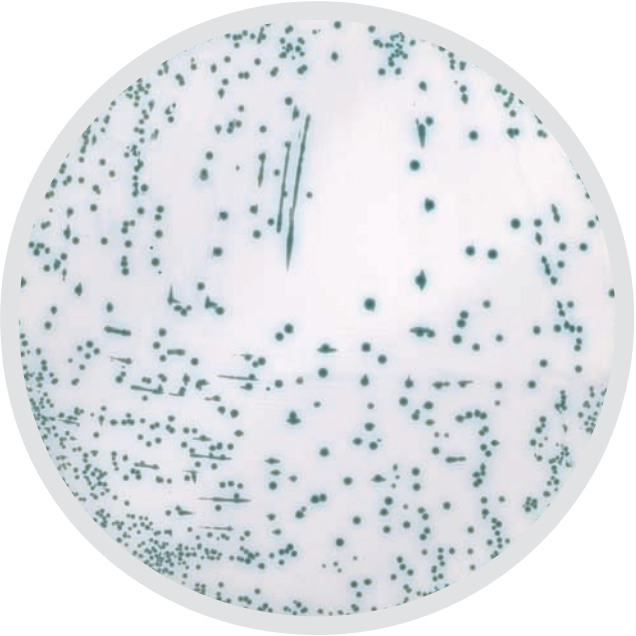
MRSA AGAR BASE is a chromogenic, selective and differential medium for the detection of methicillin resistant *Staphylococccus aureus*.

Methicillin resistant *Staphylococcus aureus*, MRSA, are of particular interest at an international level due to its virulence and resistance to multiple antibiotics. The antimicrobial resistance is a serious threat to public health as it is now regarded as a major hospital acquired disease worldwide. The important changes observed in the epidemiological and microbiological characteristics of the infections caused by *Staphylococcus aureus* are the reason for the increment and prevalence of methicillin-resitant *Staphylococcus aureus* nosocomial (associated to hopitalized patients) and the proliferation of methicillin-resistent *Staphylococcus aureus* acquired by the community. The MRSA continues being a serious problem in many healthcare centres; more than 50% of the *Staphylococcus aureus* obtained are from Intensive Care Units (ICU) and close to 40% are from hospital patients. Effective, rapid laboratory diagnosis and susceptibility testing is critical in treating, managing and preventing MRSA infections.

This chromogenic media has been designed and is adequate for the screening of *Staphylococcus aureus* resistant to methicillin. The α-glucosidase produced by *Staphylococcus aureus* cleaves the chromogenic substrate and gives a blue color to the

Staphylococcus aureus colony. The Cefoxitin inhibits the growth of *Staphylococcus aureus* sensitive to methicillin.

Incubate plates aerobically at 35 ± 2°C for 18 - 24 hours.



Staphylococcus aureus
ATCC 43300

MICROBIOLOGICAL TEST

The following results were obtained of the medium from type cultures, with the supplement added, alter incubation at a temperature of 35 ± 2°C and observed alter 18 - 24 hours.

Microorganisms	Growth	Colony Color
Staphylococcus aureus ATCC 25923	Inhibited	-
Staphylococcus aureus ATCC 43300	Good	Blue
Escherichia coli ATCC 25922	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Hutchison, M.J., Edwards, G.F.S., Morrison, D., Evaluation of chromogenic MRSA Reference Laboratory presented at the 2005 Institute of BioMedical

MRSA AGAR, MODIFIED CHROMOGENIC

CAT. 1498

For the detection and differentiation of methicillin resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*

FORMULA IN g/l

Growth Factors	**56.00**	Chromogenic Mixture	**0.24**
Peptone Mixture	**41.00**	Bacteriological Agar	**12.50**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 110 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50ºC and aseptically add two vials of Cefoxitin Supplement (**Cat. 6069**) reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and grey in color. If there are any physical changes, discard the medium.

Cefoxitin MRSA Supplement (Cat. 6069)
(1 vial for 500 ml of the medium)

Cefoxitin..... 2 mg

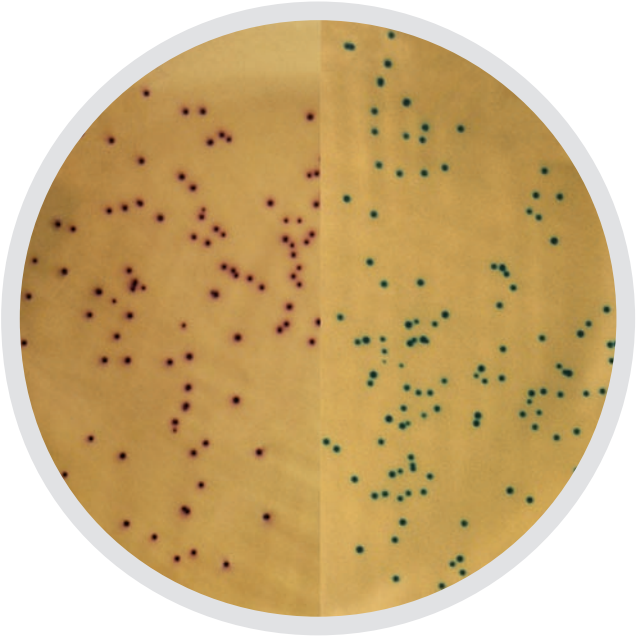
USES

MRSA AGAR MODIFIED is a chromogenic medium for the detection and differentiation of methicillin resitant *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Methicillin resistant *Staphylococcus aureus*, MRSA, are of particular interest at an international level due to its virulence and resistance to multiple antibiotics. The antimicrobial resistance is a serious threat to public health as it is now regarded as a major hospital acquired disease worldwide. The important changes observed in the epidemiological and microbiological characteristics of the infections caused by *Staphylococcus aureus* are the reason for the increment and prevalence of methicillin-resitant *Staphylococcus aureus* nosocomial (associated to hopitalized patients) and the proliferation of methicillin-resistent *Staphylococcus aureus* acquired by the community. The MRSA continues being a serious problem in many healthcare centres; more than 50% of the *Staphylococcus aureus* obtained are from Intensive Care Units (ICU) and close to 40% are from hospital patients. Effective, rapid laboratory diagnosis and susceptibility testing is critical in treating, managing and preventing MRSA infections.

Staphylococcus epidermidis is often present in humans and animals skin and it can be also found in mucous membranes.

Inoculate and incubate at 35 ± 2°C for 24 - 48 hours. Methicillin resistant *Staphylococcus aureus* grow as magenta colonies. Methicillin resistant *Staphylococcus epidermidis* grow as green-blue colonies. The rest of the accompanying flora is inhibited.



Staphylococcus aureus ATCC 43300 *Staphylococcus epidermidis* ATCC 35984

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 35 ± 2°C during 24 - 48 hours.

Microorganisms	Growth	Colony Color
Staphylococcus aureus ATCC 25923	Inhibited	-
Staphylococcus aureus ATCC 43300	Good	Magenta
Staphylococcus epidermidis ATCC 35984	Good	Blue-green
Escherichia coli ATCC 25922	Inhibited	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Hutchison, M.J., Edwards, G.F.S., Morrison, D. Evaluation of chromogenic MRSA Reference Laboratory presented at the 2005 Institute of BioMedicalv

MRSV – MODIFIED SEMISOLID RAPPAPORT VASSILIADIS MEDIUM

CAT. 1376

For the detection of motile *Salmonella* species from food and environment samples

FORMULA IN g/l

Magnesium Chloride (anhydrous)	**10.93**	Potassium Dihydrogen Phosphate	**1.47**
Sodium Chloride	**7.34**	Malachite Green Oxalate	**0.037**
Tryptose	**4.59**	Novobiocin	**0.01**
Acid Casein Peptone	**4.59**	Bacteriological Agar	**2.70**
Final pH 5.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 31.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Dispense into Petri plates. The prepared medium should be stored at 2 - 8°C. The color is blue.

The dehydrated medium should be homogeneous, free-flowing and beige in color If there are any physical changes, discard the medium.

USES

MSRV-MODIFIED SEMISOLID RAPPAPORT VASSILIADIS MEDIUM is a selective medium used for the rapid detection of motile *Salmonella spp.*

This medium is a modification of Rappaport Vassiliadis enrichment broth for detecting motile *Salmonella spp.* in feces, food products and environmental samples. In this medium the main detection is based on the motility and ability of *Salmonella* to migrate through selective medium ahead of competing motile microorganism, therefore producing opaque halos of growth.

The mobility of other microorganisms is inhibited by selective mediums (such as Magnesium Chloride, Malachite Green Oxalate and Novobiocin) as well as by the temperature of incubation at 42°C.

Tryptose and Acid casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Magnesium chloride and Malachite Green Oxalate are inhibitory to organisms other than *Salmonella spp.* Novobiocin is a selective agent that inhibits Gram-positive bacteria and avoids the development of *Proteus*.

This medium is not suitable for the detection of non motile *Salmonella* strains whose presence is very low (≤ 1 %).

After incubating for 20 hours at 37°C and on a Buffered Peptone Water (**Cat. 1401**), transfer three drops to a MRSV Petri dish. Incubate at 42 ± 0.2°C for 16 ± 0.5 hours.)

After incubation check the MRSV dishes for migration halos: motile *Salmonella* show a halo of growth around the original point of inoculation.

It is recommended to conduct serological and biochemical tests for *Salmonella* species confirmation.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures; adding the supplement, after incubation at a temperature of 42 ± 0.5ºC and observed after 16 ± 0.5 hours.

Microorganisms	Growth	Motility
Salmonella typhimurium ATCC 14028	Good	+
Salmonella enteritidis ATCC 13076	Good	+
Citrobacter freundii ATCC 8090	Good	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

De SMEDT et al.; Rapid Salmonella Detection in Foods by Motility Enrichment on a Modified Semi-Solid Rappaport-Vassiliadis Medium. J. Food Protect. VOl. 49, 7; 510-514 (1986)

De SMEDT, a. BOLDERDIJK, R.F.; Dynamics of Salmonella Isolation with Modified Semi-Solid Rappaport-Vassiliadis Medium. J. Food Protect. Vol. 50, 8; 658-661 (1987)

MUELLER HINTON AGAR

CAT. 1058

For sensitivity tests on antibiotics and sulfamides, and for the primary isolation of *Neisseria* and other pathogens from clinical samples

FORMULA IN g/l

Acid Casein Peptone (H)	**17.50**	Starch	**1.50**
Beef Infusion	**2.00**	Bacteriological Agar	**17.00**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 38 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 or 50°C and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80°C for 10 minutes if *Neisseria* development is desired.

DO NOT OVERHEAT. To remelt the cold medium, heat as briefly as possible. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

MUELLER HINTON AGAR, together with Mueller Hinton Broth (**Cat. 1214**), is used for testing the antimicrobial susceptibility of rapidly growing aerobic organisms from clinical samples.

The medium can be used with complete confidence because it is rich in nutrients, able to grow fastidious organisms. The use of a medium with suitable growth characteristics is essential to test the susceptibility of microorganisms to antibiotics. It is also recommended for testing most commonly encountered aerobic and facultative anaerobic bacteria.

Beef infusion and Acid casein peptone (H) provide nitrogen, vitamins, minerals and amino acids essential for growth. The starch absorbs any toxic metabolites produced. Bacteriological agar is the solidifying agent.

Mueller Hinton Agar can be used to cultivate *Neisseria* specimens, the recommended incubation of plates being at a temperature of 35 ± 2°C in a CO_2 atmosphere for 18 - 24 hours.

It has become the standard medium for the Bauer Kirby method and its performance is specified by the NCCLS.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Enterococcus faecalis* ATCC 29212	*Pseudomonas aeruginosa* ATCC 27853
Ampicillin 10 µg	15 - 20	24 - 35		
Tetracycline 30 µg	18 - 25	19 - 27		
Gentamicin 10 µg	19 - 26	19 - 27		16 - 21
Polymyxin B 300 µg	12 - 16	7 - 13		
Sulfamethoxazole 1.25 µg Trimethoprim 23.75 µg	24 - 32	24 - 32	16 - 23	

Diameter halo in mm according to NCCLS

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Mueller and Hinton A. Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus. Proc. Soc. Exp. Biol. and Med. 48:330. 1941. Harris and Coleman Diagnostic.

Procedures and Reagents. 4th Edition APH, Inc. New York, 1963.

National Committee for Clinical Laboratory Standards. 1993.

Atlas, R.M. 1993 Handbook of microbiological media. CRC Press, Boca Raton. Fl..

MUELLER HINTON AGAR II

CAT. 1055

For antibiotic sensitivity tests and for the primary isolation of gonococci and meningococci, and other pathogens from clinical samples

FORMULA IN g/l

Acid Casein Peptone (H)	**17.50**	Starch	**1.50**
Beef Infusion	**2.00**	Bacteriological Agar	**17.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 38 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 or 50°C and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80°C for 10 minutes if Neisseria development is desired. DO NOT OVERHEAT. To remelt the cold medium, heat as briefly as possible. The prepared medium should be stored at 8 - 15°C. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

MUELLER HINTON AGAR II is used for antimicrobial susceptibility testing. It has become the standard medium for the Bauer-Kirby method and its performance is specified by NCCLS (National Committee for Clinical Laboratory Standards). Mueller Hinton Agar II can be used to cultivate gonococci and meningococci specimens. The medium can be used with complete confidence because it is a rich medium able to grow fastidious organisms. The use of a medium with suitable growth characteristics is essential to test susceptibility of microorganisms to antibiotics. It is also recommended for testing most commonly encountered aerobic and facultative anaerobic bacteria.

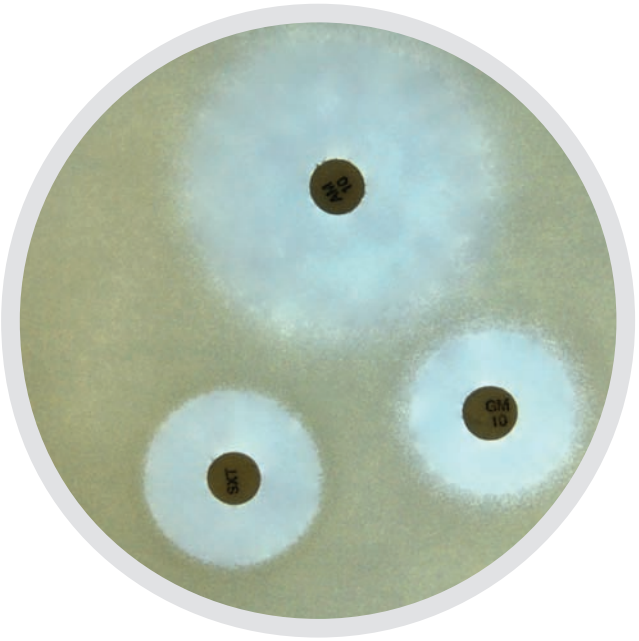
Beef infusion and Acid Casein peptone (H) provide nitrogen, vitamins, minerals and amino acids essential for growth. The starch absorbs any toxic metabolites produced. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

The medium complies with the requirement of NCCLS and is manufactured carefully, choosing the raw materials to contain low concentrations of thymine and thymidine, as well as appropriate levels of calcium and magnesium ions.

Enterococcus faecalis
ATCC 33186

Ampicillin
10 µg



Sulfamethoxazole
1.25 µg

Gentamicin
10 µg

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Enterococcus faecalis* ATCC 29212	*Pseudomonas aeruginosa* ATCC 27853
Ampicillin 10 µg	15 - 20	24 - 35		
Tetracycline 30 µg	18 - 25	19 - 27		
Gentamicin 10 µg	19 - 26	19 - 27		16 - 21
Polymyxin B 300 µg	12 - 16	7 - 13		
Sulfamethoxazole 1.25 µg Trimethoprim 23.75 µg	24 - 32	24 - 32	16 - 23	

Diameter halo in mm according to NCCLS

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Mueller and Hinton A. Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus. Proc. Soc. Exp. Biol. and Med. 48:330. 1941. Harris and Coleman Diagnostic.

Procedures and Reagents. 4th Edition APH, Inc. New York, 1963.

National Committee for Clinical Laboratory Standards. 1993.

Atlas, R.M. 1993 Handbook of microbiological media. CRC Press, Boca Raton. Fl..

MUELLER HINTON BROTH

CAT. 1214

For sensitivity testing to antibiotics in liquid media

FORMULA IN g/l

Acid Casein Peptone (H)	**17.50**	Corn Starch	**1.50**
Beef Infusion	**2.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 21 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. DO NOT OVEARHEAT. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

MUELLER HINTON BROTH is used together with Mueller Hinton Agar to carry out the sensitivity testing of a great number of antimicrobial agents, for the determination of MIC (minimal inhibitory concentrations) of bacteria isolated for example from urine.

It was also developed for the cultivation of pathogenic *Neisseria* and other fastidious microorganisms. It has the same formula as Mueller Hinton Agar (**Cat. 1058**) but can be used when the fluid medium is preferred.

The medium can be used with complete confidence because it is a rich nutrient medium able to grow fastidious organisms. The use of a medium with suitable growth characteristics is essential to test the susceptibility of microorganisms to antibiotics.

Beef infusion and Acid Casein peptone (H) provide nitrogen, vitamins, minerals and amino acids essential for growth. The starch in the medium acts as a growth factor, probably functioning like a colloid protector, and neutralizes toxic products that form during the development of the organisms.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 25923	Good
Escherichia coli ATCC 25922	Good
Enterococcus faecalis ATCC 33186	Good
Pseudomonas aeruginosa ATCC 27853	Good
Streptococcus pyogenes ATCC 19615	Good
Listeria monocytogenes ATCC 11911	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Mueller, J. H. and Hinton J. Proc. Soc. Exp. Biol. and Med. 48:330-333. 1941.

Olsen A.M. and Scott, W.J. Nature, 557; 337. 1946.

Bauer, A.L., W.M. Kirby, J.C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol 45: 493-496.

Wood, G.L. and J.A. Washington, 1995 Antibacterial susceptibility tests, dilution and disk diffusion methods, p. 1327-1341. In Murray, P.R., E.J. Baron, M.A. Pgaller, F.C. Tenover.

MULLER KAUFFMANN BROTH BASE w/BRILLIANT GREEN & NOVOBIOCIN (MKTTN) ISO 6579

CAT. 1173

For the selective enrichment of *Salmonella*

FORMULA IN g/l

Calcium Carbonate	**38.70**	Beef Extract	**4.30**
*Sodium Thiosulfate Anhydrous	**30.50**	Sodium Chloride	**2.60**
Enzymatic Digest of Casein	**8.60**	Novobiocin	**0.04**
Ox Bile	**4.78**	Brilliant Green	**0.0096**
Final pH 8.2 ± 0.2 at 25ºC			
* Equivalent to 47.8 gr of Sodium Thiosulfate pentahydrated			

PREPARATION

Suspend 89.53 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Add 20 ml of a iodine and potassium iodide solution (20 g of iodine and 25 g of potassium iodide in 100 ml of sterile distilled water). Homogenize gently and dispense into sterile containers. The prepared medium should be stored at 2 - 8°C. The color is green-blue.

The dehydrated medium should be homogeneous, free-flowing and white in color. If there are any physical changes, discard the medium.

USES

MULLER-KAUFFMANN BROTH BASE WITH BRILLIANT GREEN & NOVOBIOCIN (MKTTN) is recommended by the ISO 6579 norm to be used as a selective enrichment broth for the detection of *Salmonella spp.* in all food types, including milk and dairy products, molluscan shellfish and other fish products, and in environmental samples.

Beef extract and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Calcium carbonate is a neutralizer which absorbs toxic metabolites. Bile salts, Brilliant green and Novobiocin inhibit organisms other than *Salmonella*. Selectivity is also obtained by both sodium thiosulfate and tetrathionate, suppressing coliforms. Tetrathionate is formed in the medium with the addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will thrive in this medium. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

PREENRICHMENT and SELECTIVE ENRICHMENT

1. Add 25 g of the sample to 225 ml of Buffered Peptone Water ISO 6579 (**Cat.1402**) and incubate at 37 ± 1°C for 18 ± 2 hours.

2. Transfer 0.1 ml of the preenrichment culture to 10 ml of Rappaport Vassiliadis Soy Broth (**Cat. 1174**). Incubate at 41.5ºC for 24 ± 3 hours.

3. Transfer 1 ml of the preenrichment culture to 10 ml of Muller Kauffmann Broth Base (MKTTN). Incubate at 37 ± 1ºC for 24 ± 3 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1 °C and observed after 24 ± 3 hours.

Microorganisms	Inoculum Concentration	Growth	
		6 hours	24 hours
Escherichia coli ATCC 25922	99%	< 30%	< 5%
Salmonella typhimurium ATCC 14028	1%	> 70%	> 95%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.

Kauffmann, F. 1935. Weitere erfahrungen mit dem kombininierten anreicherungsverfahren fur Salmonella bazillen. Ztschr. F. Hyg. 117: 26-32.

MULLER KAUFFMANN TETRATHIONATE BROTH BASE

CAT. 1130

For the selective enrichment of *Salmonella* from meats and other foods

FORMULA IN g/l

Sodium Thiosulfate	**40.70**	Meat Peptone	**4.50**
Calcium Carbonate	**25.00**	Yeast Extract	**1.80**
Ox Bile	**4.75**	Beef Extract	**0.90**
Sodium Chloride	**4.50**		
Final pH 7.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 82 grams of medium in one liter of distilled water. Mix well and dissolve by shortly heating with frequent agitation and cool it quickly. AVOID OVERHEATING. DO NOT AUTOCLAVE. A sediment of Calcium carbonate will remain. Aseptically add 20 ml/l of iodine solution and 10 ml/l of 0.1% Brilliant Green solution. Distribute in tubes or flasks after homogenizing the possible precipitate. Once added, DO NOT REHEAT. Use the medium on the same day it is produced. The prepared medium should be stored at 2 - 8°C. The color is ligh green with white precipitate.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Iodine Solution

Potassium Iodide .....25 g

Iodine .....20 g

Distilled Water .....100 ml

Dissolve the potassium iodide in 5 ml of distilled water, add the iodine and gently warm the solution to dissolve completely. Make the volume reach 100 ml with distilled water.

Brilliant Green Solution

Brilliant Green .....0.1 g

Distilled Water .....100 ml

Add the brilliant green to the distilled water, shake and heat at 100ºC for 30 minutes to ensure the dye has dissolved. Store in brown bottles.

USES

MULLER-KAUFFMANN TETRATHIONATE BROTH BASE

is a recommended selective broth for isolating *Salmonella* from animal feces, polluted sewage water, food, milk, ice cream and pasteurized egg-base products.

Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple sero types. It is also recommended to use Tetrathionate Broth (**Cat. 1114**) for the isolation of *Salmonella*.

Kauffmann modified the formula to include Ox bile and Brilliant green as selective agents to inhibit Gram-positive microorganisms. Sodium thiosulfate plus Iodine result in Tetrathionate formation, inhibiting coliforms and intestinal bacteria. Acidic tetrathionate decomposition products such as sulphuric acid are formed, which are neutralized by calcium carbonate, acting as a buffer. *Salmonella* and *Proteus* are not inhibited as they reduce Tetrathionate. Meat peptone, Beef and Yeast extracts provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Add 10 g of the sample to 100 ml of medium. Shake vigorously and place flasks immediately in a 15°C water-bath for 15 minutes, followed by incubation at 42 - 43°C for 6 - 24 hours.

Subculture to Brilliant Green Agar (**Cat. 1078**) after 18 - 24 hours and again after 48 hours. Incubate plates at 35° ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 42 - 43°C and observed up to 6 - 24 hours.

Microorganisms	Inoculum Concentration	Growth (Revovery)	
		6 hours	24 hours
Escherichia coli ATCC 25922	≈ 99%	< 30%	< 5%
Salmonella typhimurium ATCC 14028	≈ 1%	> 70%	> 95%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Kauffmann, F. 1935. Weitere erfahrungen mit dem kombininierten anreicherungsverfahren fur Salmonella bazillen. Ztschr. F. Hyg. 117: 26-32.

A manual for recommended methods for the microbiological examination of poultry and poultry products. 1982.

MYCOBIOTIC AGAR (FUNGAL SELECTIVE AGAR)

CAT. 1072

For the isolation of fungal pathogens in highly contaminated samples

FORMULA IN g/l

Soy Peptone	**10.00**	Chloramphenicol	**0.05**
Dextrose	**10.00**	Bacteriological Agar	**15.50**
Cycloheximide	**0.40**		
Final pH 6.9 ± 0.2 at 25ºC			

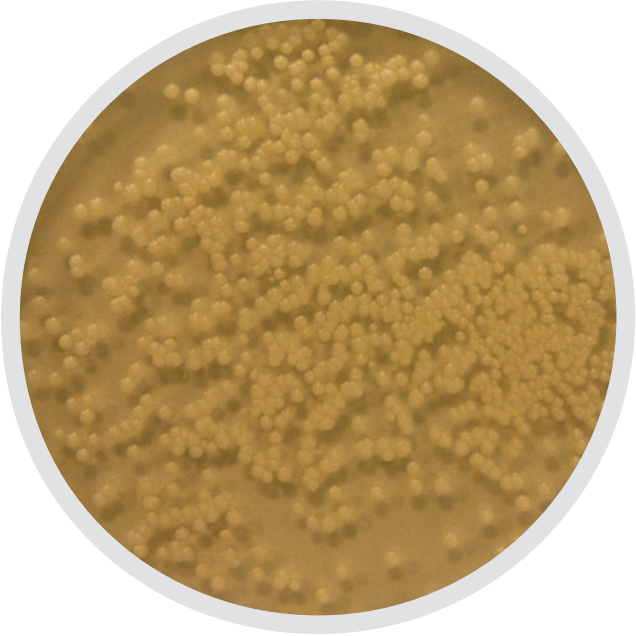
PREPARATION

Suspend 36 grams of the medium in one liter of distilled water. Mix well and soak for 10 - 15 minutes. Dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118ºC for 15 minutes. Cool to 50ºC, mix well and dispense into plates. Use it immediately.

Once cold, remelt only once with the minimum heat. DO NOT OVEARHEAT. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.



Candida albicans
ATCC 2091

USES

MYCOBIOTIC AGAR is a medium for the selective cultivation of fungal pathogens from diverse clinical samples and other materials contaminated with a mixed associated flora.

This medium is basically a Mycology Agar to which Chloramphenicol has been added to inhibit bacterial development, and Cycloheximide to inhibit the growth of saprophytic fungi. Mycobiotic Agar is very useful in isolating pathogenic fungi from diverse types of highly contaminated samples with different types of accompanying flora, such as those of the head, skin scrapings, nails, bronchial lavages, gastric juices, soil, etc.

Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate as a carbon and energy source. Bacteriological agar is the solidifying agent. Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum. Cycloheximide is an antibiotic which inhibits saprophytic fungi but allows for growth of the pathogenic fungi.

It is recommended to inoculate several plates or tubes with the same sample in study and incubate them at 22 - 25°C and at 35°C. The toxic effect of the antimicrobial mixture is greater at 20 - 25°C, reason for which the number of positive isolates is higher at temperatures below 35°C.

It is recommended to inoculate other culture media at the same time such as Biggy Agar (**Cat. 1006**) with the objective to obtain a greater number of isolates. The dermatophytes and other numerous groups of pathogenic fungi grow quickly in the Mycobiotic Agar as it inhibits most of the bacteria and fungal saprophytes or commensal contaminants.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 3 - 7 days.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aureus ATCC 25923	Inhibited
Trichophyton mentagrophytes ATCC 9533	Good
Trichophyton rubrum ATCC 28188	Good
Candida albicans ATCC 2091	Good
Aspergillus brasiliensis ATCC 16404	Inhibited
Penicillium spp.	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Dean and Halley, Public Health Reports, 77:61. 1972. Hupper and Walker, A.J. Clin. Path. 29:291. 1958. McDonough Ajello, Georg, and Brinkman J. Lab. and Clin. Med. 55:116. 1960.

NEUTRALIZING AGAR

CAT. 1449

For the detection and enumeration of microorganisms on surfaces of sanitary interest

FORMULA IN g/l

Dextrose	**10.00**	Yeast Extract	**2.50**
Lecithin	**7.00**	Sodium Bisulfite	**2.50**
Sodium Thiosulfate	**6.00**	Sodium Thioglycollate	**1.00**
Casein Peptone	**5.00**	Bromocresol Purple	**0.02**
Polysorbate 80	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 54 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is violet.

The dehydrated medium should be homogeneous, free-flowing and green-blue in color. If there are any physical changes, discard the medium.

USES

NEUTRALIZING AGAR is used to cultivate a broad range of microorganisms while neutralizing disinfectants and antimicrobials which have inherent bacteriostatic properties.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Five neutralizers inactivate a number of disinfectant and antiseptic chemicals. Sodium bisulfite neutralizes aldehydes. Sodium thioglycollate neutralizes mercurials. Sodium thiosulfate neutralizes iodine and chlorine. Lecithin neutralizes quaternary ammonium compounds. Polysorbate 80, a non- ionic surface active agent, neutralizes substituted phenolics. Bromocresol purple is used as an indicator for dextrose utilization. The organisms that ferment dextrose will turn the medium from a purple to yellow color.

Inoculate and incubate at 35 ± 2°C and observed after 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Bacillus subtilis ATCC 6633	Good
Pseudomonas aeruginosa ATCC 27857	Good
Staphylococcus aureus ATCC 25923	Good
Salmonella typhimurium ATCC 14028	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Downes and Iro(ed), 2001. Compendium of method for the microbilogical examination of foods, 4th ed. American Public Health Association, Washitong, D.C.

Association for the Advancement of Medical Instrumentation. 1984. Process control guidelines for gamma radiation sterilization of medical devices. AAMI, Arlington,VA

NITRATE MOTILITY BASE MEDIUM

CAT. 1565

For the confirmation of *Clostridium perfringens*

FORMULA IN g/l

Casein Peptone	**5.00**	Disodium Phosphate	**2.50**
Galactose	**5.00**	Potassium Nitrate	**1.00**
Beef Extract	**3.00**	Bacteriological Agar	**3.50**
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 20 grams of the medium in one liter of distilled water. Add 5 grams of Glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and dispense into sterile tubes. The prepared medium should be stored at 8 - 15°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NITRATE MOTILITY BASE MEDIUM is a confirmatory test medium for presumptive *Clostridium perfringens* colonies, that measures nitrate reduction and motility by various types of microorganisms. This test is specific for *Clostridium perfringens* in that it is non-motile and reduces nitrates to nitrites.

Nitrate reduction to nitrites, or some other nitrogenous compound such as Nitrogen (N_2), by the nitrate reductase enzyme is a valuable criterion for differentiating and identifying

various types of bacteria. Motility is demonstrated by a diffused growth away from the stab line or inoculation spot. Non-motile organisms grow only amongst the stab line.

Casein peptone and Beef extract provide the nitrogen, minerals and amino acids nutrients essential for bacterial growth. Galactose is the fermentable carbohydrate as an energy source. Disodium phosphate acts as a buffer system. Potassium nitrate provides additional nutrients. Bacteriological agar is the solidifying agent.

Inoculate and incubate at 35 ± 2°C for 24 - 48 hours.

Nitrate reduction tests are conducted adding Griess reagent to the surface of the medium. Nitrate-positive organisms reduce nitrates to nitrites, turning the medium a pink color. Nitrate-negative organisms are unable to reduce nitrates and they yield no color after adding the reagent.

Griess reagent consists of 2 solutions:

Solution A

Sulfanilic Acid ..... 8 g

Acetic Acid 5N ..... 1 liter

Solution B

Dimetil-α-naphtylamine ..... 5 g

Acetic Acid 5N ..... 1 liter

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C during 24 - 48 hours.

Microorganisms	Motility	Nitrate Reduction
Clostridium perfringens ATCC 13124	-	+
Clostridium bifermentans ATCC 638	+	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Titters R.R. and L.A. Sancholzer 1936. The use of semi-solid agar for the detection of bacterial motility, J. Bacteriol 31: 575-580. Snell and Wright; 1941. J. Biolog. Chem. 13: 675. Compendium of methods for the microbiological examination of foods. Am. Public. Health Association.

NOCIVE BREWERS BACTERIA AGAR BASE, MODIFIED

CAT. 1438

Selective medium for the detection of contaminating, spoilage microorganisms in brewery

FORMULA IN g/l

Maltose	**15.00**	Disodium Phosphate	**2.00**
Dextrose	**15.00**	Polisorbate 80	**0.50**
Potassium Acetate	**6.00**	L-Malic Acid	**0.50**
Pancreatic Digest of Casein	**5.00**	L-Cysteine HCl	**0.20**
Yeast Extract	**5.00**	ChlorPhenol red	**0.07**
Beef Extract	**2.00**	Bacteriological Agar	**15.00**

Final pH 5.8 ± 0.2 at 25ºC

PREPARATION

Suspend 66.3 grams of medium in 500 ml of distilled water and 500 ml of beer without gas. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is pink-red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NOCIVE BREWERS BACTERIA AGAR BASE, MODIFIED is a medium used for the detection of contaminating beer spoilage bacteria.

This medium contains a wide variety of nutrients including Pancreatic digest of casein, yeast extract, beef extract, dextrose and maltose. These nutrients favour the growth of spoilage microorganisms in beer and other samples. Potassium acetate (instead of sodium acetate) makes the medium less inhibitory for the growth of spoilage bacteria that deteriorate beer and other samples. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene and formalin. L-Cysteine hydrochloride is the reducing agent. Disodium phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

Incubate at 30 - 35ºC and observe after 4 days. After incubation the isolated acid production colonies, Gram-stained and catalasa test must be carried out. Gram-positive, catalase-negative cocci or rods may tentatively be considered lactic acid bacteria.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30 - 35°C and observed after 4 days.

Microorganisms	Growth	Acid Production
Lactobacillus brevis ATCC 8291	Good	Trace-yellow to yellow
Pediococcus acidilactici ATCC 8042	Good	Trace-yellow to yellow
Pediococcus damnosusi ATCC 29358	Good	Trace-yellow to yellow

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Back, 1980 Brauwelt 1562

Dacha, 1981, Brauwelt 1778

NOCIVE BREWERS BACTERIA BROTH BASE, MODIFIED

CAT. 1440

Selective medium for the detection of contaminating, spoilage microorganisms in brewery

FORMULA IN g/l

Maltose	**15.00**	Disodium Phosphate	**2.00**
Dextrose	**15.00**	Polisorbate 80	**0.50**
Potassium Acetate	**6.00**	L-Malic Acid	**0.50**
Pancreatic Digest of Casein	**5.00**	L-Cysteine HCl	**0.20**
Yeast Extract	**5.00**	ChlorPhenol red	**0.07**
Beef Extract	**2.00**		
Final pH 5.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 51.3 grams of medium in 500 ml of distilled water and 500 ml of beer without gas. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 2 - 8°C. The color is pink-red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NOCIVE BREWERS BACTERIA BROTH BASE, MODIFIED is a medium used for the detection of contaminating beer spoilage bacteria.

This medium contains a wide variety of nutrients including Pancreatic digest of casein, yeast extract, beef extract, dextrose and maltose. These nutrients favour the growth of spoilage microorganisms in beer and other samples. Potassium acetate (instead of sodium acetate) makes the medium less inhibitory for the growth of spoilage bacteria that deteriorate beer and other samples. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene, and formalin. L-Cysteine hydrochloride is the reducing agent. Disodium phosphate acts as a buffer system.

Incubate at 30 - 35ºC and observe after 4 days. After incubation the isolated acid production colonies, Gram-stained and catalasa test must be carried out. Gram-positive, catalase-negative cocci or rods may tentatively be considered lactic acid bacteria.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30 - 35°C and observed after 4 days.

Microorganisms	Growth	Acid Production
Lactobacillus brevis ATCC 8291	Good	Trace-yellow to yellow
Pediococcus acidilactici ATCC 8042	Good	Trace-yellow to yellow
Pediococcus damnosusi ATCC 29358	Good	Trace-yellow to yellow

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Back, 1980 Brauwelt 1562

Dacha, 1981, Brauwelt 1778

NUTRIENT AGAR DEV REGULATIONS

CAT. 1314

For the enumeration of microorganisms in water and other materials

FORMULA IN g/l

Meat Peptone	**10.00**	Sodium Chloride	**5.00**
Beef Extract	**10.00**	Bacteriological Agar	**18.00**
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 43 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NUTRIENT AGAR is a general purpose medium, not selective but suitable for the cultivation of a wide variety of microorganisms.

It is recommended by the German Standard Methods (Deutsche Einheitsverfahren), the German Drinking Water Regulations (Trinkwasser-Verordnung) (1990) and the German Regulation For Food Examination (LMBG).

The American Public Health Association (APHA) suggested this standard culture medium for use in bacterial processing for water analysis. In Standard Methods of Water Analysis and Standard Methods of Milk Analysis, the APHA advocated the use of dehydrated media for the bacterial examination of water and milk.

Meat peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Since this medium contains Sodium chloride it can be used as a base for enrichment with blood or other supplements for cultivating fastidious microorganisms.

Inoculate and incubate the plates at 20 ± 2°C or 35 ± 1°C for 44 ± 4 hours.

Inoculate medium with the test sample and incubate at 20 ± 2°C or 35 ± 1ºC for 44 ± 4 hours. Good growth will appear as translucent colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, as per DEV regulations, after incubation at a temperature of 20 ± 2°C and observed after 44 ± 4 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Salmonella typhimurium ATCC 14028	Good
Proteus vulgaris ATCC 13315	Good
Enterococcus faecalis ATCC 11700	Good
Klebsiella pneumoniae ATCC 13883	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

German Standard Methods (Deutsche Einheitsverfahren), the German Drinking Water Regulations (Trinkwasser-Verordnung) (1990) and the German regulation for food examination (LMBG).

American Public Health Association. 1923. Standard methods of milk analysis, 4th. Ed. American Public Health Association, Washington, D.C.

Association of Official Analytical Chemists. 1995. Official methods of analysis of AOAC International, 1 6th ed. AOAC International, Arlington, VA.

NUTRIENT AGAR ISO 6579 , ISO 10273

CAT. 1060

For the cultivation of non-fastidious microorganisms in water, feces and from clinical samples

FORMULA IN g/l

Gelatin Peptone	**5.00**	Bacteriological Agar	**15.00**
Beef Extract	**3.00**		
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 23 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NUTRIENT AGAR is a general purpose medium, not selective but suitable for the cultivation of a wide variety non-fastidious microorganisms. It can be used as a colony count medium in sanitation, medica and industrial bacteriology.

There are many uses for Nutrient Agar in the bacteriological analysis of drinking water, wastewater, milk and other foods. The American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.

It is also used in the multiplication of microorganisms to produce vaccines and antigens in general, in the tests of sensitivity and resistance, and as a base to prepare an enriched medium by adding ascitic fluid, etc.

It is used to grow microorganisms and for subsequent biochemical tests.

The Gelatin peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent.

ISO 6579 and ISO 10273 recommend this medium to obtain presuntive *Salmonella* and *Yersinia* isolated colonies respectively. A typical or suspicious colony from each selective medium must be seeded, and then the other four if the first one turns out to be negative. In the case of epidemiological studies, it is recommended to identify at least five colonies. Should there be less than five typical or suspicious colonies on a plate, all the typical or suspicious colonies will be used for confirmation.

Inoculate medium with the test sample and incubate at 35 ± 2°C for 18 - 48 hours. Good growth will appear as translucent colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 25923	Good
Escherichia coli ATCC 25922	Good
Salmonella typhimurium ATCC 14028	Good
Streptococcus pyogenes ATCC 12344	Good
Streptococcus pneumoniae ATCC 6301	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Greenberg and Cooper Can. Med. Assn. J. 83:143. 1960. Wetmore and Gochenour J. Bact. 72:79, 1956

Norma UNE-EN-ISO 6579. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Salmonella spp.

ISO 10273-2003-06 Microbiology of Food and animal feeding stuffs- Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica.

NUTRIENT AGAR UNE-EN 12780, EN ISO 16266

CAT. 1156

For the confirmation of *Pseudomonas aeruginosa* by membrane filtration

FORMULA IN g/l

Peptone	**5.00**	Beef Extract	**1.00**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Yeast Extract	**2.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 28 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NUTRIENT AGAR is a confirmation medium to be used with the presumptive positive colonies obtained in Pseudomonas CN Agar Base (**Cat. 1153**) or King B Medium (**Cat. 1154**).

Pseudomonas aeruginosa is an opportunist pathogen for humans, capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water are *Pseudomonas aeruginosa* free at the time of their commercialization. This microorganism can also be found in swimming pool water.

Peptone and Beef extracts provide the nitrogen, vitamins, minerals and amino acids nutrient source; Yeast extract is a vitamins source, particularly of the B-group, essential for bacterial growth; Sodium chloride maintains the osmotic balance and the Bacteriological agar is the solidifying agent.

Subculture positive colonies from the media mentioned and incubate for 22 ± 2 hours at a temperature of 36 ± 2°C.

After incubation, for those cultures which initially did not show fluorescence, the oxidase reduction test is carried out, and fluorescence production and ammonia production capacity from acetamide are investigated in those cultures which are oxidase-positive. For those cultures which initially presented fluorescence, ammonia production capacity from acetamide is studied.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 36 ± 2°C and observed after 22 ± 2 hours.

Microorganisms	Growth	Oxidase
Escherichia coli ATCC 25922	Good	-
Pseudomonas aeruginosa ATCC 25783	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

UNE-EN 12780 Quality of water. Identification and enumeration of Pseudomonas aeruginosa by membrane filtration.

EN ISO 16266 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration

NUTRIENT AGAR WITH SODIUM CHLORIDE ISO 21528

CAT. 1355

For the confirmation of Enterobacteria

FORMULA IN g/l

Peptone	**5.00**	Beef Extract	**3.00**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 28 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NUTRIENT AGAR WITH SODIUM CHLORIDE is a medium recommended by the ISO normative 21528 for the confirmation of Enterobacteriaceae.

Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

This medium is used after enrichment in EE Broth (**Cat. 1362**) and subculture in VRBG Agar (**Cat. 1093**). Select well-isolated characteristic colonies from a 24 hours incubated at 35ºC plate of VRBG AGAR and inoculate in Nutrient Agar with Sodium chloride. If more than one morphology is present in the colonies, select one colony of each morphology to subculture. After 24 ± 2 hours at 37ºC, select a well-isolated colony from each of the incubated plates for the biochemical confirmation tests.

If any of the selected typical colonies is oxidase-negative and glucose-positive, the tube from which the subculture was derived shall be regarded as being positive for Enterobacteriaceae.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C and observed after 24 ± 2 hours.

Microorganisms	Growth	Oxidase
Escherichia coli ATCC 25922	Good	-
Salmonella typhimurium ATCC 14028	Good	-
Pseudomonas aeruginosa ATCC 25783	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 21528: Microbiology of food and animal feeding stuffs- Horizontal methods for the detection and enumeration of Enterobacteriaceae

The Food Hygiene (England) (no.2) Regulations 2005 Draft Statutory Instrument. England: HMSO; 2005.

SANCO 4198/2004 rev.19 (PLSPV/2001/4198/4198R19-EN.doc). Draft Comisión Regulation on Microbiological Criteria for Foodstuffs. 2005

NUTRIENT BROTH

CAT. 1216

For the cultivation of non-fastidious microorganisms in water, feces and other materials

FORMULA IN g/l

Gelatin Peptone	**5.00**	Beef Extract	**3.00**
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NUTRIENT BROTH is used for the general cultivation of a wide variety of microorganisms.

A liquid medium, it is produced according to the formula from APHA and AOAC, and supports the growth of a great variety of microorganisms that are not very nutritionally demanding.

This medium is used in accordance with the official recommended procedures for the bacteriological analyses of water, milk, dairy products and feces of clinical samples, and as a base to prepare media supplemented with other nutrients. Nutrient Broth is used in many laboratory procedures as it is or with added indicators, carbohydrates, organic liquids, salts, etc.

Gelatin peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth.

Inoculate medium with the test sample and incubate at 35 ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Enterobacter aerogenes ATCC 13048	Good
Escherichia coli ATCC 25922	Good
Salmonella typhi ATCC 6539	Good
Staphylococcus epidermidis ATCC 14990	Good
Streptococcus pyogenes ATCC 12344	Moderate

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Walsbren, Carr, and Dunnette A. J. Clin. Path. 21:884. 1951.

American Public Health Association. 1923. Standard methods of water analysis, 5th ed. American Public Health Association, Washington, D.C.

Marshall, R.T. (ed) 1993 Standard methods for the microbiological examination of dairy products, 1 6th ed. American Public Health Association, Washington, D.C.

NUTRIENT BROTH Nº2

CAT. 1251

For the cultivation of fastidious pathogens and other microorganisms

FORMULA IN g/l

Casein Peptone	**10.00**	Sodium Chloride	**5.00**
Beef Extract	**10.00**		
Final pH 7.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NUTRIENT BROTH Nº2 is used for the general cultivation of a wide variety of microorganisms. It is also used as a general use medium, rich in nutrients, that allows the growth of bacteria when there is a low quantity of inocula and fastidious pathogenic microorganisms. It can also be used for sterility testing of aerobic organisms.

The medium is particularly suitable for subculture, especially as a secondary growth medium for staphylococci to be tested for coagulase production.

Gelatin peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Inoculate medium with the test sample and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Enterobacter aerogenes ATCC 13048	Good
Escherichia coli ATCC 25922	Good
Salmonella typhi ATCC 6539	Good
Staphylococcus epidermidis ATCC 14990	Good
Streptococcus pyogenes ATCC 12344	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Walsbren, Carr, and Dunnette A. J. Clin. Path. 21:884. 1951.

American Public Health Association. 1923. Standard methods of water analysis, 5th ed. American Public Health Association, Washington, D.C.

Marshall, R.T. (ed) 1993 Standard methods for the microbiological examination of dairy products, 1 6th ed. American Public Health Association, Washington, D.C.

NUTRIENT GELATIN

CAT. 1300

For testing proteolitic microorganisms that liquefy gelatin

FORMULA IN g/l

Gelatin	**120.00**	Beef Extract	**3.00**
Gelatin Peptone	**5.00**		
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 128 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

NUTRIENT GELATIN is used to investigate the presence of proteolytic microorganisms, as evidenced by the liquefaction of gelatin, especially in the bacteriological analysis of water. The liquefaction rate is important in the characterization of Enterobacteriaceae family groups and other groups of microorganisms.

For the plate count of organisms in water, this medium is replaced by solid media with agar.

Nutrient Gelatin was one of the first solidifying agents used in the beginning of bacteriology and was originally used in the standard method for water and wastewater as a direct plate count technique, replacing the dilution method. As this method required incubation at approximately 20°C, it was not ideal for most organisms. Gelatin peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth.

The tubes are inoculated by stabbing with a needle (straight wire) and incubated at 35 ± 2°C for 7 days, or up to 15 days if required. Refrigerate the test cultures together with an uninoculated Nutrient Gelatin control tube and read the reactions as soon as the control tube has hardened by inverting the tube.

Detection of proteolysis: strong positive remains liquid.

If plates of Nutrient Gelatin are utilized, they can be streaked. Check for the hydrolysis of gelatin on the streaked plate by adding a drop of saturated ammonium sulfate or 20% sulfosacylic acid to an isolated colony. Look for a zone of clearing around the colony (Stone reaction) after 10 minutes.

The Stone reaction is also used on Staphylococcus Medium Nº 110 (**Cat. 1032**).

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C during 1 - 7 days.

Microorganisms	Growth	Gelatinase
Escherichia coli ATCC 25922	Good	-
Staphylococcus aureus ATCC 25923	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ewing Enterobacteriaceae USPHS Publication 734 Washington, 1960.

Edwards and Ewing. Identification of Enterobacteriae, Burgess Publ. Co. Minneapolis, Minn., 1962. Standard Methods for the Examination of Water and Sewage, Ninth Edition APHA Inc. New York, 1960

OF BASAL MEDIUM (HUGH AND LEIFSON)

CAT. 1500

For the identification of non-fermenting bacilli of medical and sanitary importance

FORMULA IN g/l

Sodium Chloride	**5.00**	Bromothymol Blue	**0.03**
Casein Peptone	**2.00**	Bacteriological Agar	**2.50**
Dipotassium Phosphate	**0.30**		
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 9.8 grams of the medium in one liter of distilled water. Heat with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Add 10 ml of 10% glucose (or any suitable sugar) solution, sterilized by filtration, to 100 ml of liquid medium. Mix and aseptically dispense 5 ml per tube. If preferred, add 1.0 grams of carbohydrate directly to 100 ml of medium and sterilize in the autoclave at 118°C for 10 minutes to avoid the degradation of the sugar. The prepared medium should be stored at 2 - 8°C. The color is green-bluish.

The dehydrated medium should be homogeneous, free-flowing and beige with a greenish tint in color. If there are any physical changes, discard the medium.

USES

OF BASAL MEDIUM is a semisolid medium, prepared according to Hugh and Leifson's formula, and is used to determine the metabolism (oxidation, fermentation) of Gram-negative bacteria. It is useful for *Pseudomonas, Salmonella, Shigella* and *Alcaligenes*.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bromothymol blue is the pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dipotassium phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

Inoculate 2 fresh tubes by stabbing with a fresh culture of the organism in study. If the medium has been prepared and stored, remelt in a water bath to expel the dissolved gases.

After inoculation, add a layer of 4 to 5 mm of paraffin oil to one of the tubes. It is not recommended to use mineral oil.

Incubate both tubes at 35°C for 48 hours or more, up to 7 days with the caps loose. To facilitate the identification of Gram-negative non-fermenting bacilli, also use Indole Nitrate Medium (**Cat. 1504**).

Results:

1. Fermentation: Yellow color in both tubes with or without gas.

2. Oxidation: Yellow color only in tube without oil.

3. No oxidation/fermentation: No change in the color of the tubes. The carbohydrates have not been fermented or oxidized. Inert microorganisms, e.g. *Alcaligenes faecalis*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 48 - 72 hours.

Microorganisms	Without sugar		With glucose		With lactose		With Sucrose	
	o	**o**	o	**o**	o	**o**	o	**o**
Alcaligenes faecalis ATCC 8750	K	K	K	K	K	K	K	K
Escherichia coli ATCC 25922	K	K	AG	AG	AG	AG	K	K
Pseudomonas aeruginosa ATCC 27853	K	K	A	K	K	K	K	K
Salmonella enteritidis ATCC 13076	K	K	AG	AG	K	K	K	K
Shigella flexneri ATCC 12022	K	K	A	A	K	K	K	K

o = Opened ● = Closed

K = Alkaline, green (without change)

A = Acid, yellow

G = Gas, sometimes perceptible

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Hugh, R. and Leifson, E.J. Bact. 66:24-26. 1953. Lisenko J. Gen. Microbiol., 35:379, 1961. Edwards y Ewing Identification of Enterobacteriaceae. Burguess Publ. Co. Minneapolis, Minn., 1972.

OGA MEDIUM (OXYTETRACYCLINE GLUCOSE AGAR BASE)

CAT. 1527

For the enumeration and isolation of yeasts and molds in food samples

FORMULA IN g/l

Glucose	**10.00**	Bacteriological Agar	**15.00**
Yeast Extract	**5.00**		
Final pH 6.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 15 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 10 minutes. Cool to 45 - 50ºC and aseptically add one vial of selective OGA Supplement (**Cat. 6018**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is yellowish-white.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

OGA SELECTIVE SUPPLEMENT (CAT. 6018)
(Composition: each vial for 500ml)

Oxytetracycline .....50 mg

* Alternatively, 0.05 grams of Gentamicin may be used as the antibiotic.

USES

O.G.A. MEDIUM (Oxytetracycline Glucose Agar Base) is a selective medium, introduced by MOSSEL *et al*. and recommended for the isolation and enumeration of yeasts and molds in foodstuffs, clinical specimens and cosmetics.

With a neutral pH, the oxytetracycline produces better results than when a low pH medium is used to inhibit bacterial growth. This medium inhibits the acidophilus organisms, *Lactobacillus* included.

Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Glucose is the fermentable carbohydrate as an energy source. Bacteriological agar is the solidifying agent.

The pour plate method is recommended as indicated below:

Inoculate 1 ml 10^{-1} of diluted food sample and incubate at 20 - 25°C. Examine daily from the 2nd to the 6th day for the formation of aerial mycelia.

Count numbers of colonies in plates where there are 50 - 100 colonies after 5 days. Calculate number of yeasts or molds per 1 g or 1 ml by multiplying the number of colonies by the dilution factor.

(*) When examining fecal specimens from patients under tetracycline treatment, *Enterobacteriaceae* are not adequately inhibited. Oxytetracycline should then be replaced by Gentamicin.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 20 - 25°C during 5 - 7 days.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Pseudomonas aeruginosa ATCC 27853	Inhibited
Candida albicans ATCC 10231	Good
Penicillium spp.	Good
Aspergillus brasiliensis ATCC 16404	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

American Public Health Association. Standard Methods for the Examination of Dairy Products, 1 3th Ed. APHA, Inc. New York, 1960. Thom and Raper, Manual of the Aspergili 39:194

MOSSEL, D.A.A., KLEYNEN-SEMMELING, A.M.C., a. VENCENTE, H.M.: Oxytetracycline-Glucose-Yeast Extract Agar for

selective enumeration of moulds and yeasts in foods and clinical material. - J. Appl. Bact., 33; 454-457 (1970).

ORANGE SERUM AGAR

CAT. 1307

For the isolation, cultivation and determination of a great number of acid-tolerant pathogenic germs in fruits juices

FORMULA IN g/l

Casein Peptone	**10.00**	Monopotassium Phosphate	**3.00**
Orange Extract	**5.00**	Yeast Extract	**3.00**
Glucose	**4.00**	Bacteriological Agar	**15.00**
Final pH 5.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118ºC for 15 minutes. DO NOT OVERHEAT. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ORANGE SERUM AGAR is formulated according to Hays, Troy and Beisel. It is recommended for the isolation, cultivation and enumeration of acid-tolerant spoilage microorganisms in fruit juice and fruit juice concentrates, in particular from citrus fruit.

The medium containing Orange extract, is specially indicated for growing the lactic acid micro flora that spoil citric products, such as *Lactobacillus, Leuconostoc* and molds.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group essential. Glucose is the fermentable carbohydrate providing carbon and energy. Monopotassium phosphate acts as a buffer. Bacteriological agar is the solidifying agent. The low pH of citric juices and products limits the growth of microorganisms to acid tolerant pathogens.

Add 1 ml of sample in a sterile Petri dish, add 20 ml of the cooled medium (50°C) and mix.

For lactobacilli, incubate at 35 ± 2°C for 40 - 48 hours. For other microorganisms, at 30 ± 2°C and examine daily for 40 - 48 hours. Report as colony forming units per ml of test material.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30 - 35ºC during 40 - 48 hours.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Lactobacillus fermentum ATCC 9338	Good
Saccharomyces cerevisiae ATCC 9763	Good
Leuconostoc mesenteroides ATCC 23386	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Hays G.L.(1 951), Proc. Florida State Hort. Soc. , 94th Ann. Murdock D.I. and Brokaw C.H.(1 958), Food Tech., 1 2. 573-576. American Public Health Association (1976), Compendium of Methods for the Microbiological Examination of Foods, APHA Inc. Washington DC.

OSMOPHILIC AGAR

CAT. 1057

For the research of osmophilic yeasts in foods

FORMULA IN g/l

Fructose	**60.00**	Bacteriological Agar	**15.00**
Yeast Extract	**5.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 80 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

OSMOPHILIC AGAR is a selective medium as it has a high concentration of fructose and it is recommended to enumerate yeasts that develop in media with a high osmophilic pressure. These yeasts can change or affect fruit concentrates, syrups and honey, etc.

Yeast extract provides vitamins, particularly the B-group essential for bacterial growth. Fructose is a fermentable carbohydrate as an energy source. Bacteriological agar is the solidifying agent.

Yeasts are the most common osmophilic microorganisms found in non-ionic environments of high osmolarity, such as foods containing high concentrations of sugar. Osmophilic yeasts are responsable for the spoilage of high-sugar foods, such as jams, honey, concentrated fruit juices, chocolate candy with soft centers, etc. Osmophilic yeasts are of economic importance to the food industry.

From 1 gram of food sample, make decimal dilutions and place 1 ml aliquots in Petri dishes and add the cooled medium (45 - 50°C). Swirl gently and allow to solidify. Incubate at 20 ± 2°C for 48 - 72 hours.

This medium is formulated according to the standards of the National Center for Foods and Nutrition (CeNAN) for the total counts of osmophilic yeasts.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 20 ± 2°C and observed after 48 - 72 hours.

Microorganisms	Growth
Saccharomyces rouxii ATCC 8383	Good
Saccharomyces mellis ATCC 10685	Good
Zygosaccharomyces spp.	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Pascual Anderson. "Técnicas para el Análisis Microbiológico de Alimentos y Bebidas" (Centro Nacional de Alimentación y Nutrición) (Madrid 1982).

PEPTONE WATER (TRYPTONE WATER)

CAT. 1403

For the detection of indole production by microorganisms

FORMULA IN g/l

Tryptone	**10.00**	Sodium Chloride	**5.00**
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 15 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and white cream to slightly toasted in color. If there are any physical changes, discard the medium.

USES

PEPTONE WATER (TRYPTONE WATER) is recommended for the detection of Enterobacteriaceae, in particularly of *E. coli*, in water and food samples based on indole production.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

This medium is a good substrate for the production of indole because of its high content of tryptophan. The ability of certain organisms to break down the aminoacid tryptophan with indole formation is an important property which is used for the classification and identification of bacteria.

Inoculate and incubate at 35 ± 2ºC for 18 - 24 hours.

For the indole test, add 3 to 4 drops of Kovac's Reagent (**Cat. 5205**) and shake the tube gently. The appearance of a red or pink color in the reagent layer is a positive indication of indole. Compare the results with a non-inoculated test tube.

This medium is recommended by CeNAN (National Center for Food and Nutrition).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth	Indol
Escherichia coli ATCC 25922	Good	+
Salmonella typhimurium ATCC 14028	Good	-
Staphylococcus aureus ATCC 25923	Good	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

M.R. Pascual Anderson (1982) Técnicas para Análisis Microbiológico de Alimentos y Bebidas, CeNAN.

MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 610-612. Williams & Wilkins, Baltimore, M.D.

Finegold, S.M., and W. martin, 1982. Bailey and Scott's diagnostic microbiology, 6th ed. St. Louis.

PEPTONE WATER with LACTOSE ISO 9308-1

CAT. 1357

For the coliforms presence confirmation in water

FORMULA IN g/l

Peptone	**10.00**	Sodium Chloride	**5.00**
Lactose	**10.00**	Phenol Red	**0.01**
Final pH 7.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

PEPTONE WATER with LACTOSE is used for the confirmation of coliform presence in water.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Phenol red is a pH indicator and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Coliforms ferments lactose with gas and acid production. Acid formation changes the pH of medium with a resulting color change from red to yellow. When lactose is not fermented the color of medium remains red.

Incubate at a temperature of 37 ± 1°C and observe after 24 - 48 hours.

Gas production is demonstrated by the displacement of the medium from the Durham tube. Production of both acid and gas is a presumptive indication of the presence of coliforms.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 37 ± 1°C and observed after 24 - 48 hours.

Microorganisms	Growth	Medium Color	Gas
Escherichia coli ATCC 25922	Good	Yellow	+
Proteus mirabilis ATCC 29906	Good	Red	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 9308-1 standards Detection and enumeration of Escherichia coli and coliform bacteria -- Part 1: Membrane filtration method

PHENOL RED BROTH BASE

CAT. 1115

For the study of carbohydrate fermentations

FORMULA IN g/l

Casein Peptone	**10.00**	Phenol Red	**0.018**
Sodium Chloride	**5.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 15 grams of the medium in one liter of distilled water. Add 5 - 10 grams/liter of the desired carbohydrate. If the medium is for the cultivation of anaerobes, add 0.5 - 1 grams of agar. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 116 - 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

PHENOL RED BROTH BASE is a basal medium for determining the fermentation reactions of microorganisms. It must be capable of supporting the growth of test organisms and be free of fermentable carbohydrates.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth, and allows the abundant growth of a wide variety of fastidious microorganisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Phenol red is the pH indicator. Vera recommended using Casein peptone in fermentation test media since she found that it could be used with the pH indicator Phenol red in fermentation tests with a high degree of accuracy.

Inoculate tubes with test organism and incubate at 35 ± 2°C for 18 - 48 hours. Observe for color change. The appearance of a yellow color is the indication of fermentation, with or without gas production.

Phenol red Broth Base is used for the carbohydrate fermentation studies of many microorganisms. Control tubes of the uninoculated medium should be run parallel with inoculated tubes. Tubes should be examined frequently because different carbohydrates are utilized at variable speeds.

Phenol red Broth Base is an excellent substrate for streptococci, as well for other less fastidious bacteria.

For anaerobes the medium should be used on the day of preparation. If not, the medium must be heated and cooled before use.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Glucose		Lactose	
	Acid	Gas	Acid	Gas
Escherichia coli ATCC 25922	+	+	+	+
Proteus vulgaris ATCC 6380	+	+	-	-
Salmonella thyphimurium ATCC 14028	+	+	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ewing, W.H. 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th edition. Elsevier Science Publishing Co., Inc. New York. Vera H.D. 1950 Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am. J. Public Helath0:1 267.

Mac Faddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

PHENOL RED DEXTROSE AGAR

CAT. 1023

For the differentiation of bacteria based on dextrose fermentation

FORMULA IN g/l

Peptone Mixture	**10.00**	Phenol Red	**0.025**
Dextrose	**10.00**	Bacteriological Agar	**15.00**
Sodium Chloride	**5.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8ºC. The color is red.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

PHENOL RED DEXTROSE AGAR is similar to Dextrose Agar (**Cat. 1021**) with the addition of Phenol red as a pH indicator. It is recommended to determine the ability of various organisms to ferment dextrose. Being a solid medium it has the advantage of allowing fermentation reactions under aerobic and anaerobic reactions.

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth, and allows the abundant growth of a wide variety of fastidious microorganisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose is the fermentable carbohydrate providing carbon and energy. Phenol red is the pH indicator. Bacteriological agar is the solidifying agent.

Phenol red Dextrose Agar is an excellent substrate for streptococci, as well as for other less fastidious bacteria.

Inoculate and incubate at 35 ± 2°C for 18 - 48 hours. A yellow color indicates fermentation, as the acid production reacts with the Phenol red pH indicator. A control of Phenol red Agar without carbohydrates should be used to control false positives.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Acid Production	Gas Production
Alcaligenes faecalis ATCC 8750	Good	-	-

Microorganisms	Growth	Acid Production	Gas Production
Escherichia coli ATCC 25922	Good	+	+
Klebsiella pneumoniae ATCC 13883	Good	+	+
Proteus vulgaris ATCC 6380	Good	+	+
Salmonella typhimurium ATCC 14028	Good	+	+
Shigella flexneri ATCC 12022	Good	+	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Diagnostic Procedures and Reagents 3rd Edition p. 107. 1950

Association of Official Analytical Chemists. 1995 official methods of analysis of AOAC Arlington, VA:

Baron EJ LR Peterson and S.M. Finegold 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc. St. Louis, MO. Murray, PR., E.J. Baron M.A. Pfaller F.C. Tenover and R.H. Yolken (ed) 1995. Manual of clinical microbiology, 6th edition. American Society for Microbiology, Washington DC.

PHENOL RED DEXTROSE BROTH

CAT. 1235

For the differentiation of bacteria based on dextrose fermentation

FORMULA IN g/l

Casein Peptone	**10.00**	Sodium Chloride	**5.00**
Dextrose	**5.00**	Phenol Red	**0.018**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 20 grams of the medium in one liter of distilled water. If the medium is for the cultivation of anaerobes, add 0.5 - 1 grams of agar. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in amounts of 5 ml into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

PHENOL RED DEXTROSE BROTH is the same as Phenol red Broth Base (**Cat. 1115**) with the addition of dextrose for fermentation studies.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth and allows the abundant growth of a wide variety of fastidious microorganisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Phenol red is the pH indicador and changes to yellow in acid conditions as a result of bacterial fermentation. Durham tubes trap any gases produced during fermentation. Dextrose is the fermentable carbohydrate providing carbon and energy. Vera recommended using Casein peptone in fermentation test media since she found that it could be used with the pH indicator Phenol red in fermentation tests with a high degree of accuracy.

Inoculate tubes with test organisms and incubate at 35 ± 2°C for 18 - 48 hours. Observe for color change. The appearance of a yellow color is the indication of fermentation, with or without gas production.

Control tubes of the uninoculated medium should be run parallel with inoculated tubes. Tubes should be examined frequently because different carbohydrates are utilized at variable speeds.

For anaerobes, the medium should be used on the day of preparation. If not, the medium must be heated and cooled before use.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Dextrose	
	Acid	Gas
Escherichia coli ATCC 25922	+	+ -
Proteus vulgaris ATCC 6380	+	+
Salmonella thyphimurium ATCC 14028	+	+
Alcaligenes faecalis ATCC 8750	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Rogers, Ryan and Severans. Antibiotic and Chemother 5:382. 1955

Association of Official Analytical Chemists. 1995 official methods of analysis of AOAC Arlington, VA:

Baron EJ LR Peterson and S.M. Finegold 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc. St. Louis, MO. Murray, PR., E.J. Baron M.A. Pfaller F.C. Tenover and R.H. Yolken (ed) 1995. Manual of clinical microbiology, 6th edition. American Society for Microbiology, Washington DC.

PHENOL RED SUCROSE BROTH

CAT. 1239

For the differentiation of bacteria based on sucrose fermentation

FORMULA IN g/l

Casein Peptone	**10.00**	Sodium Chloride	**5.00**
Sucrose	**5.00**	Phenol Red	**0.018**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 20 grams of the medium in one liter of distilled water. If the medium is for the cultivation of anaerobes, add 0.5 - 1 grams of agar. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in amounts of 5 ml into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

PHENOL RED SUCROSE BROTH is the same as Phenol red Broth Base (**Cat. 1115**) with the addition of sucrose for fermentation studies.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth, and allows the abundant growth of a wide variety of fastidious microorganisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Phenol red is the pH indicator. Sucrose is the fermentable carbohydrate providing carbon and energy. Vera recommended using Casein peptone in fermentation test media since she found that it could be used with the pH indicator Phenol red in fermentation tests with a high degree of accuracy.

Inoculate tubes with test organism and incubate at 35 ± 2°C for 18 - 48 hours. Observe for color change. The appearance of a yellow color is the indication of fermentation, with or without gas production.

Control tubes of the uninoculated medium should be run parallel with inoculated tubes. Tubes should be examined frequently because different carbohydrates are utilized at variable speeds.

For anaerobes the medium should be used on the day of preparation. If not, the medium must be heated and cooled before use.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Sucrose	
	Acid	Gas
Escherichia coli ATCC 25922	-	-
Proteus vulgaris ATCC 6380	+	+
Salmonella thyphimurium ATCC 14028	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Rogers, Ryan and Severans. Antibiotic and Chemother 5:382. 1955

Association of Official Analytical Chemists. 1995 official methods of analysis of AOAC Arlington, VA:

Baron EJ LR Peterson and S.M. Finegold 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc. St. Louis, MO. Murray, PR., E.J. Baron M.A. Pfaller F.C. Tenover and R.H. Yolken (ed) 1995. Manual of clinical microbiology, 6th edition. American Society for Microbiology, Washington DC.

PHENYLALANINE AGAR

CAT. 1040

For the differentiation of enteric bacilli which deaminate phenylalanine to phenylpyruvic acid

FORMULA IN g/l

Sodium Chloride	**5.00**	Sodium Phosphate	**1.00**
Yeast Extract	**3.00**	Bacteriological Agar	**12.00**
DL-Phenylalanine	**2.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 23 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121ºC for 10 minutes. Allow to cool in a slanted position. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

PHENYLALANINE AGAR is a solid medium used for differentiating *Proteus, Providencia* and *Morganella* species from other Enterobacteriaceae, based on the deamination of phenylalanine to phenylpyruvic acid by enzymatic activity. The formula is prepared according to Ewing *et al*. (1957). Some strains of *Enterobacter* and a few non-fermenting Gram-negative bacilli are also capable of deaminating phenylalanine.

DL-Phenylalanine is deaminated to phenylpyruvic acid. Yeast extract provides vitamins, particularly of the B-group, and other nutrients for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium phosphate is the buffer and Bacteriological agar is the solidifying agent.

Inoculate heavily with the sample organism. Incubate for 18 - 24 hours at 35 ± 2°C.

Add 4 to 5 drops of 10% ferric chloride. The immediate appearance of an intense green color (1 - 5 minutes) indicates the presence of phenylpyruvic acid.

To differentiate *Proteus* and *Providencia*, seed the suspect organisms heavily in Urea Agar Base (Christensen - **Cat. 1110**), or Urea Broth (**Cat. 1226**). Proteus hydrolyzes the urea. *Providencia* is negative for urease production.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Phenyl pyruvic Acid (deamination)
Escherichia coli ATCC 25922	Good	-
Enterobacter aerogenes ATCC 13048	Good	-
Proteus vulgaris ATCC 13315	Good	+
Providencia spp.	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Bailey and Scott. Diagnostic Microbiology. The C.V. Mosby Company. Saint Louis, 1978. Edwards and Ewing. Identification of Enterobacteriaceae. Burgess Publ. Co. Minneapolis, Minn., 1972. Ewing. Enterobacteriaceae. USPH. Publication 734. Washington, 1969. Lennette E.H., Spaulding and S.P. Truant. Manual of Clinical Microbiology, A.S.M.

MaFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 634-636. Williams & Wilkins, Baltimore, MD.

POTATO DEXTROSE AGAR EUROPEAN PHARMACOPOEIA

CAT. 1022

For the identification, cultivation and enumeration of yeast and molds in foods

FORMULA IN g/l

Dextrose	**20.00**	Bacteriological Agar	**15.00**
Infusion From Potatoes (200 g)	**4.00**		
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 39 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118 - 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

POTATO DEXTROSE AGAR is recommended by APHA and FDA for culturing yeast and molds from dairy products and foods. It can also be used in the identification of fungi and yeasts in parallel with their cellular morphology, or in methods of micro cultivation in slides.

This general purpose medium can be supplemented with acid or antibiotics to inhibit bacterial growth. The nutritionally rich base (potato infusion) encourages a very rich fungal and mold growth. Dextrose is the fermentable carbohydrate as a carbon and energy source. Bacteriological Agar is the solidifying agent.

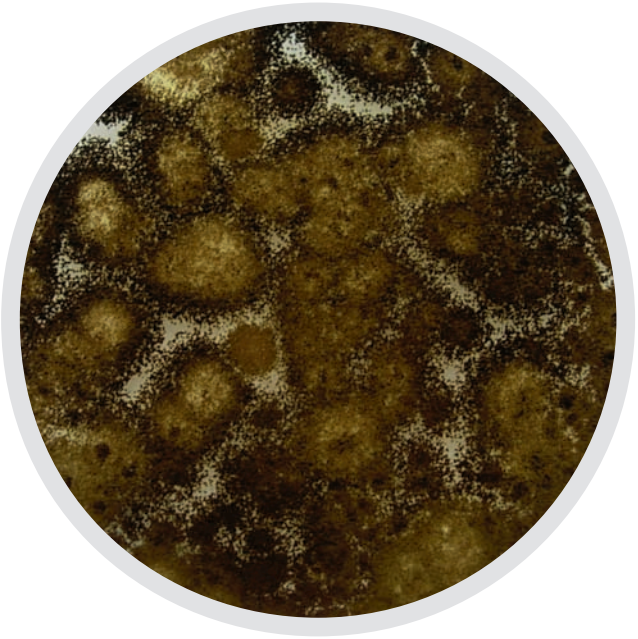
Inoculate the medium with test organisms. Incubate plates at 25 - 30°C for 18 - 48 hours. If the cultivation of *Trichophyton mentagrophytes* is desired, incubate up to 5 - 7 days.

Yeasts will grow as cream to white colonies. Molds will grow as fuzzy colonies of various colors. To differentiate and isolate genus and species, carry out further Microscopic and Biochemical tests.

When the medium is to be used for the enumeration of yeasts and molds, the pH should be lowered to inhibit bacteriological growth. Add to the cooled to 45 - 50°C sterilized medium, approximately 14 ml of a sterilized 10% solution of tartaric acid to obtain a pH of 3.5. Do not reheat the adjusted medium

after adding the acid because the agar may hydrolyze and not solidify.

The European Pharmacopoeia recommends in Paragraph 2.6.12 "Microbiological examination of non-sterile products: Microbial enumeration test. Preparation and use of test microorganisms": inoculation of *Aspergillus brasiliensis* at 20 - 25ºC for 5 - 7 days or until good sporulation is achieved.



Aspergilus brasiliensis
ATCC 16404

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30ºC and observed after 18 - 48 hours.

Microorganisms	Growth
**Aspergilus brasiliensis* ATCC 16404	Good
Candida albicans ATCC 10231	Good
Saccharomyces cerevisiae ATCC 9763	Good
***Trychophyton mentagrophytes* ATCC 9533	Good

*According to European Pharmacopeia Incubation at 20 - 25 ºC 5 - 7 days or until good sporulation is achieved.

** Incubate at 25 - 30ºC during 5 - 7 days.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

American Public Health Association. Standard Methods for the Examination of Dairy Products, 13th Ed. APHA, Inc. New York, 1960.

American Public Health Association. Recommended Methods for the Microbiological Examination of Foods. APHA, New York, 1958.

Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.

European Pharmacopoeia 7.0

POTATO DEXTROSE BROTH

CAT. 1261

For the cultivation of yeasts and molds

FORMULA IN g/l

Dextrose	**20.00**	Infusion from potatoes (Solid)	**6.50**

Final pH 5.6 ± 0.2 at 25ºC

PREPARATION

Suspend 26.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

POTATO DEXTROSE BROTH is a liquid medium used for cultivating yeasts and molds. It can be used for the growth of clinically significant yeasts and molds from foods and dairy products.

This general purpose medium can be supplemented with acid or antibiotics to inhibit bacterial growth. The nutritionally rich base (potato infusion) encourages a very rich fungal and mold growth. Dextrose is the fermentable carbohydrate as carbon and energy source. The low pH of this medium inhibits bacterial growth.

Inoculate the medium and incubate at 25 - 30°C for 48 - 72 hours. Growth is indicated as turbidity.

Inoculation of Potato Dextrose Broth with pure cultures of yeasts can assist in their identification. Observe cultures for surface growth and pellicle formation. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30ºC and observed after 48 - 72 hours.

Microorganisms	Growth
Aspergilus brasiliensis ATCC 16404	Good
Candida albicans ATCC 10231	Good
Saccharomyces cerevisiae ATCC 9763	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.

Frank, J.F. G.L. Christen, and L.B. Bullerman (G.H. Richardson, Tech. Comm.) 1993. Tests for groups of microorganisms. P. 271-286. In Marshall, R.T. (ed.). Standard methods for the microbiological examination of dairy products, 1 6th ed. American Public Health Association, Washington, D.C.

PPLO AGAR BASE w/o CRYSTAL VIOLET

CAT. 1140

For the isolation and culture of PPLO microorganisms: *Mycoplasma* in clinical specimens and mixed cultures

FORMULA IN g/l

Peptone	**10.00**	Sodium Chloride	**5.00**
Beef Heart Infusion	**6.00**	Bacteriological Agar	**14.00**
Final pH 7.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 35 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and, if desired, aseptically add 1% serum fraction or 25% ascetic fluid. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is amber slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

PPLO AGAR BASE w/o CRYSTAL VIOLET is used in the study of the growth requirements of *Mycoplasma*, along with the identification and cultivation of this organism. PPLO stands for Pleuro Pneumonia-Like Organisms and was described by Morton, Smith and Leberman.

Peptone and Beef heart infusion provide the nutrients for growth: nitrogen, vitamins, minerals and amino acids, whilst the Sodium chloride provides the osmotic balance. Crystal violet is not included in this formula since it is inhibitory on some *Mycoplasma*. Bacteriological agar is the solidifying agent.

After growth in PPLO Broth (**Cat. 1262**), subculture to PPLO Agar w/o Crystal Violet and incubate at 35 ± 2°C under 5 - 10% CO_2 for up to 7 days.

Examine microscopically for growth on a daily basis.

PPLO colonies have a round shape and a dense center with a less dense periphery, giving a "fried egg" appearance on PPLO Agar.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2, and observed after 7 days.

Microorganisms	Growth
Mycoplasma bovis ATCC 25523	Good
Mycoplasma pneumoniae ATCC 15531	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Adler, H.E. and AJ Da Massa. 1967 Use of formalinized Mycoplasma gallisepticum antigens and chicken erythrocytes in hemagglutination and hemagglutination-inhibition studies. Appl. Microbiol 15:245-248.

Morton HE and JG Lecce. 1953. Selective action of thallium acetate and crystal violet for pleuropneumonia like organisms of human origin. J. Bacteriol 66:646-649.

PPLO BROTH BASE w/o CRYSTAL VIOLET

CAT. 1262

For the enrichment of microorganisms PPLO microorganisms: *Mycoplasma* in clinical specimens and mixed cultures

FORMULA IN g/l

Peptone	**10.00**	Sodium Chloride	**5.00**
Beef Heart Infusion	**6.00**		
Final pH 7.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 21 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and, if desired, aseptically add supplements and additives. Homogenize gently. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

PPLO BROTH BASE w/o CRYSTAL VIOLET is used in the studies of the growth requirements of *Mycoplasma*, along with the identification and cultivation of this organism. PPLO stands for Pleuro Pneumonia-Like Organisms. PPLO Broth without Crystal violet is prepared according to the formula described by Morton and Lecce.

Peptone and Beef heart infusion provide nitrogen, vitamins, minerals and amino acids essential for growth, whilst the Sodium chloride provides the osmotic balance. Crystal violet is not included in this formula since it is inhibitory on some *Mycoplasma*.

Although some species are normal human respiratory tract flora, *Mycoplasma pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia"). *Mycoplasma hominis, Mycoplasma genitalium* and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract.

Inoculate and incubate at 35 ± 2°C under 5 - 10% CO_2 during 24 - 72 hours. Subculture to PPLO Agar (**Cat. 1140**) and incubate at 35 ± 2°C under 5 - 10% CO_2 for up to 7 days. Examine daily for growth.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the supplemented medium from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2, and observed after 24 - 72 hours.

Microorganisms	Growth
Mycoplasma bovis ATCC 25523	Good
Mycoplasma pneumoniae ATCC 15531	Good
Mycoplasma gallinarum ATCC 19708	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Leland DS, MA Lapworth, RB Jones and MLV French 1982. Comparative evaluation of media for isolation of Ureaplasma urealyticum and genital Mycoplasmas species. J. Clin. Microbiol. 16:709-714.

Kenny GE 1985 Mycoplasmas, p. 407-411 In EH Lennette, A Balows Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington DC.

Morton and Lecce. 1953. J. Bacteriol. 66:646.

PSEUDOMONAS AGAR BASE ISO 13720

CAT. 1356

For the isolation and enumeration of *Pseudomonas spp.* from meat and meat products

FORMULA IN g/l

Gelatin Peptone	**16.00**	Magnesium Chloride	**1.40**
Potassium Sulfate	**10.00**	Bacteriological Agar	**13.00**
Enzymatic Digest of Casein	**10.00**		
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 25.2 grams of the medium in 500 ml of distilled water. Add 5 ml of Glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add one vial of the selective supplement C.F.C (**Cat. 6036**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

C.F.C. SELECTIVE SUPPLEMENT (CAT. 6036)
(Composition: each vial for 500ml)

Cetrimide ..... 5 mg

Fucidin ..... 5 mg

Cephaloridine ..... 25 mg

USES

PSEUDOMONAS AGAR BASE with added C.F.C. Supplement is a selective medium recommended by ISO 13720 for the enumeration of *Pseudomonas spp.* in meat and meat products, including poultry.

Gelatin peptone and Enzymatic digest of casein provide nitrogen, vitamins, minerals and amino acids essential for growth and permits the growth of a great number of *Pseudomonas* species. The quantities of Potassium sulfate and Magnesium chloride favor the formation of pigmentation (production of pyocyanin). The addition of C.F.C supplement (**Cat. 6036**) makes the medium more selective for *Pseudomonas spp.* including *Burkholderia cepacia*, previously known as *Pseudomonas cepacia*. Cetrimide, Fucidin and Cephaloridine inhibit Gram-positive bacteria and support the growth of *Pseudomonas spp.*, (including *P. aeruginosa*), whilst inhibiting most other Gram-negative bacteria.

Inoculate and incubate in aerobic conditions at 25 ± 1ºC and observe after 44 ± 4 hours. All colonies are suspect *Pseudomonas spp.* and are counted as such. All the suspect colonies should be confirmed. Colonies which show a positive oxidase reaction but no glucose fermentation are *Pseudomonas spp.* colonies.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the supplement added, after incubation at a temperature of 25 ± 1°C and observed after 44 ± 4 hours.

Microorganisms	Growth	Recovery
Escherichia coli ATCC 25922	Inhibited	-
Pseudomonas aeruginosa ATCC 27853	Good	≥ 70%
Pseudomonas aeruginosa ATCC 25819	Good	≥ 70%
Pseudomonas aeruginosa ATCC 25608	Good	≥ 70%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Goto, S., and S. Enomoto. 1970. Nalidixic Acid Cetrimide Agar. A New Selective Plating Medium for the Selective Isolation of Pseudomonas aeruginosa. Japan. J. Microbiol. 14: 65 - 72.

Mead, G.C., and B.W. Adams. 1977. A selective medium for the rapid isolation of Pseudomonas associated with poultry meat spoilage. Br. Poult. Sci. 18: 661 670.

ISO 13720. Meat and meat products - Enumeration of Pseudomonas spp.

PSEUDOMONAS CN AGAR BASE UNE-EN 12780, EN ISO 16266

CAT. 1153

For the identification and enumeration of *Pseudomonas aeruginosa* by membrane filtration

FORMULA IN g/l

Gelatin Peptone	**16.00**	Cetrimide	**0.20**
Hydrolyzed Casein	**10.00**	Nalidixic Acid	**0.015**
Anhydrous Potassium Sulfate	**10.00**	Bacteriological Agar	**13.00**
Anhydrous Magnesium Chloride	**1.40**		
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 50.6 grams of the medium in one liter of distilled water. Add 10 ml of Glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates to obtain an agar layer of at least 5 mm thick. Do not remelt the medium. The medium should be stored at 8 - 15ºC. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

PSEUDOMONAS CN AGAR BASE is used for the identification of *Pseudomonas aeruginosa* by membrane filtration technique based on pyocyanin production. It is a modification of Pseudomonas P Agar (King A Medium **- Cat. 1531**). This medium is recommended by UNE-EN 12780 and EN ISO 16266.

Peptone and Casein provide provide nitrogen, vitamins, minerals and amino acids essential for growth. Cetrimide is added as a selective agent, and Nalidixic acid to suppress contaminants of cetrimide media such as *Klebsiella, Proteus* and *Providencia spp.* Potassium sulfate and Magnesium chloride provide cations to activate pyocyanin production and enhance pigment production. Bacteriological agar is the solidifying base.

The membrane is placed on dishes containing the medium and it is incubated at 36 ± 2°C for 40 - 48 hours. The colonies are examined to verify their growth after 20 - 24 and 40 - 48 hours.

The colonies that produce a green-blue color and fluoresce under UV light are considered presumptive *Pseudomonas aeruginosa*.

Presumptive colonies must be confirmed with the biochemical tests.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures, with the glycerol added, in the performance of the medium after incubation at a temperature of 36 ± 2°C during 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Pseudomonas aeruginosa ATCC 27853	Good
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

UNE-EN 12780: 2002, Quality of water. Identification and enumeration of Pseudomonas aeruginosa by membrane filtration.

EN ISO 16266 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration

R2A AGAR EUROPEAN PHARMACOPOEIA

CAT. 1071

For the total aerobe count in treated waters

FORMULA IN g/l

Proteose Peptone	**0.50**	Dipotassium Phosphate	**0.30**
Starch	**0.50**	Sodium Pyruvate	**0.30**
Glucose	**0.50**	Magnesium Sulfate Anhydrous	**0.024**
Yeast Extract	**0.50**	Bacteriological Agar	**15.00**
Casein Hydrolysate	**0.50**		
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 18.12 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

R2A AGAR was developed by Reasoner and Geldreich for bacteriological plate counts of treated potable water, being able to recover the stressed chlorine-treated bacteria. Nutritionally rich mediums suppress these slow growing bacteria, whereas a low nutrient medium, such as R2A Agar, in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria.

Proteose peptone and Casein hydrolysate provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Dextrose is a source of fermentable carbohydrate as an energy source. Starch absorbs toxic metabolic byproducts and thereby aids the recovery of injured organisms. Sodium pyruvate increases the recovery of stressed cells. Magnesium sulfate provides divalent cations and sulfate. Dipotassium phosphate is used to balance the pH and provide phosphate. Bacteriological agar is solidifying base.

Inoculate plates with tap water samples using the streak plate technique and/or membrane filter method. Incubate at 35ºC ± 2ºC for 24 - 72 hours.

R2A Agar is recommended in Standard Methods for the Examination of Water and Wastewater for pour plate, spread plate and membrane filter methods for heterotrophic counts.

The European Pharmacopoeia recommends in paragraph Water for Injections (Aqua ad iniectabilia) this medium for growth promotion. Inoculate plates of R2A Agar separately (with no more than 100 CFU) of *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633 and incubate at 30 - 35°C ≤ 3 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 24 - 72 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Escherichia coli ATCC 11775	Good
Escherichia coli ATCC 8739	Good
Staphylococcus aureus ATCC 25923	Good
Staphylococcus aureus ATCC 6538	Good
Staphylococcus epidermidis ATCC 12228	Good
**Pseudomonas aeruginosa* ATCC 9027	Good

*According European Pharmacopoeia in paragraph Water for Injections (Aqua ad iniectabilia) Inoculate ≤ 100 CFU at 30 - 35ºC ≤ 3 days.

Microorganisms	Growth
**Bacillus subtilis* ATCC 6633	Good

*According European Pharmacopoeia in paragraph Water for Injections (Aqua ad iniectabilia) Inoculate ≤ 100 CFU at 30 - 35°C ≤ 3 days.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

American Public Health Association (1985) Standard Method for the Enumeration of Water and Wasterwater.

European Pharmacopeia 7.0.

RAKA-RAY AGAR BASE

CAT. 1061

Selective medium for the isolation of lactic acid bacteria in beer and beer fermentation

FORMULA IN g/l

Tryptone	**20.00**	Diammonium Hydrogen Citrate	**2.00**
Maltose	**10.00**	Magnesium Sulfate $7H_2O$	**2.00**
Yeast Extract	**5.00**	Potassium Phosphate	**2.00**
Fructose	**5.00**	Liver Extract	**1.00**
Glucose	**5.00**	Manganese Sulfate $4H_2O$	**0.66**
Potassium Aspartate	**2.50**	N-Acetylglucosamine	**0.50**
Potassium Glutamate	**2.50**	Cycloheximide	**0.007**
Betaine Hydrochloride	**2.00**	Bacteriological Agar	**17.00**
Final pH 5.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 77.2 grams of the medium in one liter of distilled water. Add 10 ml of Sorbitan Monooleate. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. DO NOT OVEARTHEAT. Cool to 45 - 50ºC and aseptically add 3 grams of Phenylethanol. Mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is toasted.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

RAKA-RAY AGAR BASE is a selective medium for the isolation of lactic acid bacteria in beer and brewing processes. It yields very good results in the detection of lactobacilli in the fermentation processes of beer.

These organisms can change the organoleptic characteristics of the beer through their metabolites. Detection is complicated because of the nutritional and environmental requirements of these organisms. For these reasons, several formulations have been described to optimize the medium and obtain good growth.

Higher counts of lactobacilli in comparative tests have been obtained with this medium because it contains growth nutrients and stimulants such as Liver extract, Yeast extract, Tryptone, N-acetylglucosamine and Sorbitan monooleate. Maltose and Fructose are added as sources of carbohydrates when certain lactobacilli cannot use glucose. Selectivity is obtained by adding 3 g/l of Phenylethanol, to inhibit Gram-negative bacteria, and Cycloheximide to inhibit yeasts. Sulfate salts provide inorganic ions. Betaine hydrochloride is used as a growth stimulating agent. Diammonium hydrogen citrate and Potassium phosphate are buffering agents. Potassium aspartate and Potassium glutamate are additional sources of amino acids. Bacteriological agar is the solidifying agent.

The inoculation can be performed by the direct streaking of the agar surface or by the double-layer pour-plate method. Incubation is carried out at 25 - 30°C in anaerobic conditions for 4 days. Some organisms grow slower and may require 7 or more days. *Lactobacillus fermentans* grow with white-cream colonies.

If the number of colonies on each plate exceeds 300, dilute sample 1:10 in sterile saline and re-test.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the additives added, after incubation at a temperature of 25 - 30ºC and observed after 4 - 7 days.

Microorganisms	Growth
Lactobacillus fermentans ATCC 9338	Good
Escherichia coli ATCC 25922	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Methods of Analysis of the ASBC (1976) 7th Edition, The Society, St. Paul, Mn. USA.

European Brewing Convention, EBC Analytica Microbiologica: Part II J. Inst. Brewing (1981) 87.303-321.

RAPPAPORT SOY BROTH (VASSILIADIS)

CAT. 1240

Enrichment medium for *Salmonella* from meats, dairy products, feces and sewage polluted water.

FORMULA IN g/l

Magnesium Chloride (anhydrous)	**13.58**	Monopotassium Phosphate	**1.26**
Sodium Chloride	**7.20**	Dipotassium Phosphate	**0.18**
Soy Peptone	**4.50**	Malachite Green	**0.036**
Final pH 5.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 26.75 grams of the medium in one liter of distilled water. Heat with frequent agitation until complete dissolution. Dispense and sterilize at 115°C for 15 minutes.DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is blue.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

RAPPAPORT SOY BROTH (VASSILIADIS) is a medium recommended, after preenrichment, for the selective isolation of *Salmonella* in food or in environmental samples and in fecal samples.

The Rappaport medium was modified by Vassiliadis by reducing Malachite green concentration and increasing incubation temperature offering a better stability of the pH of the prepared medium and the optimization of the concentration of Magnesium chloride, resulting in an improved recovery of Salmonellae.

The Soy peptone provides essential nutrients for growth: nitrogen, vitamins and amino acids. Potassium phosphates balance the low pH of the medium, combined with the presence of Magnesium chloride to raise the osmotic pressure, and Malachite green to inhibit other organisms.

Procedure for the sampling of foods:

- Transfer 0.1 ml of Preenrichment Broth (25 g sample in 225 ml of Buffered Peptone Water (**Cat. 1402**) incubated at 37°C for 20 hours) to 10 ml of Rappaport Soy Broth Vassiliadis.

- Incubate for 24 hours at 42 ± 1°C.

- Subculture to selective agar media, for example: Hektoen Enteric Agar (**Cat. 1030**), Salmonella Shigella Agar (**Cat. 1064**), XLD Agar (**Cat. 1274**) o Chromogenic Salmonella Agar (**Cat. 1122**) and incubate at 35 ± 2°C for 18 - 24 hours.

- Confirm in suitable plates and verify the biochemical and serological characteristics of the suspect colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 42 ± 1°C and observed after 24 hours.

Microorganisms	Medium Concentration	Growth
Escherichia coli ATCC 25922	99%	< 5%
Salmonella typhimurium ATCC 14028	1%	> 95%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Rappaport F., Konforti N. and Navon B. (1956) J. Clin Pathol., 9,261. Peterz M. Wiberg C. and Norberg P. (1989) J. Appl. Bact. 66: 523-528.

RAPPAPORT SOY BROTH (VASSILIADIS) ISO 6579

CAT. 1174

For the selective enrichment of *Salmonella*

FORMULA IN g/1110 ml

*Magnesium Chloride (anhydrous)	**18.73**	Monopotassium Phosphate	**1.40**
Sodium Chloride	**8.00**	Dipotassium Phosphate	**0.20**
Soy Peptone	**5.00**	Malachite Green	**0.04**
* Equivalent to 40.0 g/L Magnesium Chloride Hexahydrate			
Final pH 5.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 33.37 grams of the medium in 1110 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 115°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is blue.

The dehydrated medium should be homogeneous, free-flowing and blue-greenish in color. If there are any physical changes, discard the medium.

USES

RAPPAPORT SOY BROTH (VASSILIADIS) is recommended by ISO 6579, after the preenrichment step, for the selective isolation of *Salmonella spp.*

Rappaport medium was modified by Vassiliadis by reducing Malachite green concentration and increasing incubation temperature , thus offering a better stability of the pH of the prepared medium and optimizing the concentration of Magnesium chloride, resulting in an improved recovery of Salmonellae.

Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Potassium phosphates balance the low pH of the medium, combined with the presence of Magnesium chloride to raise the osmotic pressure, and Malachite green to inhibit other organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Procedure for the sampling of foods:

- Transfer 0.1 ml of Preenrichment Broth (25 g sample in 225 ml of Buffered Peptone Water (**Cat. 1402**) incubated at 37 ± 1°C for 18 ± 2 hours) to 10 ml of Rappaport Soy Broth Vassiliadis.

- Incubate for 24 ± 3 hours at 41.5 ± 1°C.

- Subculture to selective agar media, for example: XLD Agar (**Cat. 1274**) or Salmonella Chromogenic Agar (**Cat. 1122**) and any other medium of your choice, and incubate at 35 ± 2°C for 18 - 24 hours.

- Confirm in suitable plates and verify the biochemical and serological characteristics of the suspect colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 41.5 ± 1°C and observed after 24 ± 3 hours.

Microorganisms	Medium Concentration	Growth
Escherichia coli ATCC 25922	99%	< 5%
Salmonella typhimurium ATCC 14028	1%	> 95%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Rappaport F., Konforti N. and Navon B. (1 956) J. Clin Pathol., 9,261.

Peterz M. Wiberg C. and Norberg P. (1989) J. Appl. Bact. 66: 523-528.

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RAPPAPORT VASSILIADIS BROTH EUROPEAN PHARMACOPEIA

CAT. 1414

Enrichment medium for *Salmonella*

FORMULA IN g/l

*Magnesium Chloride (anhydrous)	**13.58**	Monopotassium Phosphate	**0.60**
Sodium Chloride	**8.00**	Dipotassium Phosphate	**0.40**
Soy Peptone	**4.50**	Malachite Green	**0.036**

* Equivalent to 29.0 g/L Magnesium Chloride Hexahydrate

Final pH 5.2 ± 0.2 at 25ºC

PREPARATION

Suspend 27.11 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 115°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. DO NOT OVERHEAT. The color is blue.

The dehydrated medium should be homogeneous, free-flowing and blue-greenish in color. If there are any physical changes, discard the medium.

USES

RAPPAPORT VASSILIADIS BROTH is recommended as the selective enrichment medium when isolating *Salmonella* species from food and environmental specimens. It can also be used to isolate *Salmonella* from human feces without preenrichment but the inoculum must be small.

This enrichment medium for *Salmonella* is recommended by the European Pharmacopoeia in Paragraph 2.6.13 "Microbiological examination of non-sterile products: test for specified microorganisms".

After preenrichment with Trypticasein Soy Broth (TSB) (**Cat. 1224**), transfer 0.1 ml to 10 ml of Rappaport Vassiliadis Broth and incubate at 30 - 35ºC for 18 - 24 hours. Subcultivate in

plates of XLD Agar (**Cat. 1080**) (according to PHE) and incubate at 30 - 35ºC for 18 - 48 hours.

Interpretation:

The possible presence of *Salmonella* is indicated by the growth of well developed, red colonies with or without black centers. These results can be confirmed with Identification Tests.

The product complies with the test if colonies of the types described are not present, or if the confirmatory identification tests are negative.

This medium has been found to be superior to other *Salmonella* selective enrichment media, especially when small inocula and a preenrichment broth are used.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 35ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922 (conc. 99%)	< 5%
Salmonella typhimurium ATCC 14028 (conc 1%)	> 95%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

European Pharmacopeia 7.0

REINFORCED CLOSTRIDIAL AGAR

CAT. 1087

For the cultivation and enumeration of *Clostridium spp.* and other anaerobes

FORMULA IN g/l

Beef Extract	**10.00**	Sodium Acetate	**3.00**
Peptone	**10.00**	Soluble Starch	**1.00**
Dextrose	**5.00**	L-Cysteine Hydrochloride	**0.50**
Sodium Chloride	**5.00**	Bacteriological Agar	**12.50**
Yeast Extract	**3.00**		
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50ºC and, if desired, add 0.02 g/l of Polymyxin B in a sterile filtered solution. The prepared medium should stored at 8 - 15°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

REINFORCED CLOSTRIDIAL AGAR is recommended for the cultivation and enumeration of anaerobes, particularly *Clostridium*, and other microorganisms in foods and clinical specimens.

Hirsch and Grinstead formulated Reinforced Clostridial Medium (**Cat.1007**) and found that this medium is superior to others in supporting growth and producing high cell counts of Clostridia. When incubated anaerobically, this medium grows various anaerobes and other bacteria. Barnes and Ingram also demonstrated that it can be used to develop vegetative cells in assays of *Clostridium perfringens*.

Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Starch in the medium acts as a growth factor, probably functioning like a colloid protector and neutralizes toxic products that form during the development of the organisms. L-Cysteine hydrochloride is the reducing agent and Sodium acetate is the buffer. Bacteriological agar is the solidifying agent.

Since this medium is a non-selective enrichment one, it allows the growth of various anaerobic microorganisms and facultative bacteria when incubated anaerobically.

Inoculate and incubate under anaerobic conditions at 35 ± 2°C for 40 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the supplement added, after incubation at a temperature of 35 ± 2°C, and observed after 40 - 48 hours.

Microorganisms	Growth
Clostridium bifementans ATCC 19299	Good
Clostridium difficile NCTC 11024	Good
Clostridium perfringens ATCC 13124	Good
Clostridium perfringens ATCC 10543	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Barnes, EMJE Despaul and M. Ingram 1963. The behavior of a food poisoning strain of Clostridium welchii in beef. J. Appl. Bacteriol 26:415.

MacFaddin JF. 1985 Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 660-668. Williams & Wilkins, Baltimore MD.

REINFORCED CLOSTRIDIAL MEDIUM EUROPEAN PHARMACOPOEIA

CAT. 1007

For the cultivation and enumeration of *Clostridium* and other anaerobes

FORMULA IN g/l

Beef Extract	**10.00**	Sodium Acetate	**3.00**
Peptone	**10.00**	Soluble Starch	**1.00**
Glucose Monohydrate	**5.00**	L-Cysteine Hydrochloride	**0.50**
Sodium Chloride	**5.00**	Bacteriological Agar	**0.50**
Yeast Extract	**3.00**		
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 38 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50ºC and, if desired, add 0.02 g/l of Polymyxin B in a sterile filtered solution. The prepared medium should be stored at 2 - 8°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

REINFORCED CLOSTRIDIAL MEDIUM is a semisolid medium. It is recommended for the cultivation and enumeration of anaerobes, particularly *Clostridium* and other microorganisms, in foods and clinical specimens.

It was formulated by Hirsch and Grinstead in 1954. Their work demonstrated that the medium outperformed other media in supporting the growth of *Clostridium* from small inoculum and produced higher viable cell counts.

Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Starch in the medium acts as a growth factor, probably functioning like a colloid protector, and neutralizes toxic products that form during the development of the organisms. L-Cysteine hydrochloride is the reducing agent and Sodium acetate is the buffer.

Since the medium is a non-selective enrichment one, it allows the growth of various anaerobic microorganisms and facultative bacteria when incubated under anaerobic conditions.

European Pharmacopoeia recommends in Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" the following preparations for the sample:

Take two equal portions corresponding to no less than 1 gram or 1 ml of the product to be examined. Heat one portion at 80ºC for 10 minutes and cool rapidly. Do not heat the other portion. Transfer 10 ml of each of the mixed portions to two containers, containing 100 ml of reinforced medium for clostridia. Incubate under anaerobic conditions at 30 - 35ºC for 48 hours. After incubation, make subcultures from each tube on Columbia Agar (**Cat. 1104**) and incubate under anaerobic conditions at 30 - 35ºC for 48 - 72 hours.

Interpretation: The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This is confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 35ºC under anaerobic conditions and observed after 48 - 72 hours According to European Pharmacopoeia 7.0.

Microorganisms	Growth
Clostridium bifementans ATCC 19299	Good
Clostridium difficile NCTC 11024	Good
Clostridium perfringens ATCC 13124	Good
Clostridium perfringens ATCC 10543	Good
Clostridium sporogenes ATCC 19404	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

Andrews, W.H. (ed) 1995. Microbial methods p. 1-119. In Official methods of analysis of AOAC International. 16th ed.

European Pharmacopoeia. 7.0

ROGOSA SL AGAR

CAT. 1096

Selective medium for the cultivation of lactobacilli in medical and food microbiology

FORMULA IN g/l

Sodium Acetate	**15.00**	Ammonium Citrate	**2.00**
Tryptone	**10.00**	Polysorbate 80	**1.00**
Dextrose	**10.00**	Magnesium Sulfate	**0.57**
Monopotassium Phosphate	**6.00**	Manganese Sulfate	**0.12**
Yeast Extract	**5.00**	Ferrous Sulfate	**0.03**
Sucrose	**5.00**	Bacteriological Agar	**15.00**
Arabinose	**5.00**		
Final pH 5.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 75 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Add 1.32 ml of Acetic Acid Glacial and mix well. Heat again at 90 - 100°C for two minutes. DO NOT AUTOCLAVE. Cool the medium to 40 - 45°C and dispense into sterilized appropriate containers. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ROGOSA SL AGAR is used for the isolation, enumeration and identification of lactobacilli in oral bacteriology, feces, vaginal specimens and foodstuffs.

This selective medium, modified by Rogosa to contain high levels of Sodium acetate and Ammonium citrate at a low pH, inhibits most microorganisms, including streptococci and molds and limits swarming but allows the growth of lactobacilli.

Sucrose, Arabinose and Dextrose are fermentable carbohydrates as carbon and energy sources. Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sulfate salts provide inorganic ions. Polysorbate 80 is a surfactant and is incorporated to neutralize phenols, hexachlorophene and formalin. Monopotassium phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

Direct inoculation or plate count methodologies can be used. Inoculate medium and incubate at 35 ± 2°C for 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Lactobacillus casei ATCC 9595	Good
Lactobacillus fermentum ATCC 9338	Good
Lactobacillus plantarum ATCC 8014	Good
Lactobacillus leichmannii ATCC 4797	Good
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Rogosa, M. J. A. Mitchell and R.F. Wiseman. 1951 A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Dental Res. 30: 682.

MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 678-680. Williams & Wilkins, Baltimore, M.D.

ROGOSA SL BROTH

CAT. 1234

Selective medium for the cultivation of lactobacilli in medical and food microbiology

FORMULA IN g/l

Sodium Acetate	**15.00**	Arabionose	**5.00**
Tryptone	**10.00**	Ammonium Citrate	**2.00**
Dextrose	**10.00**	Sorbitan Monooleate	**1.00**
Monopotassium Phosphate	**6.00**	Magnesium Sulfate	**0.57**
Yeast Extract	**5.00**	Manganase Sulfate	**0.12**
Sucrose	**5.00**	Ferrous Sulfate	**0.03**
Final pH 5.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 60 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Add 1.32 ml of Glacial Acetic Acid and mix well. Distribute in tubes and heat again at 90 - 100ºC for 2 - 3 minutes. DO NOT AUTOCLAVE. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ROGOSA SL BROTH is used for the isolation, enumeration and identification of lactobacilli in oral bacteriology, saliva, feces, vaginal specimens and foodstuffs.

Rogosa SL Broth is a modification of media described by Rogosa, Mitchell and Wiseman. Rogosa SL Broth is similar to Rogosa SL Agar (**Cat. 1096**), but lacks the agar and is very selective due to its high Sodium acetate and Ammonium citrate concentrations and its low pH, which is very advantageous for the cultivation of lactobacilli and inhibits most microorganisms including streptococci and molds and limits swarming but allows the growth of lactobacilli.

Sucrose, Arabinose and Dextrose are fermentable carbohydrates as carbon and energy sources. Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sulfate salts provide inorganic ions. Polysorbate 80 is a surfactant and is incorporated to neutralize phenols, hexachlorophene and formalin. Monopotassium phosphate acts as a buffer system.

Inoculate medium and incubate at 35 ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Lactobacillus casei ATCC 9595	Good
Lactobacillus fermentum ATCC 9338	Good
Lactobacillus plantarum ATCC 8014	Good
Lactobacillus leichmannii ATCC 4797	Good
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Rogosa, M. J. A. Mitchell and R.F. Wiseman. 1951 A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Dental Res. 30: 682.

MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 678-680. Williams & Wilkins, Baltimore, M.D.

ROSE BENGAL AGAR + CHLORAMPHENICOL

CAT. 1081

For the cultivation and selective isolation of yeasts and molds

FORMULA IN g/l

Dextrose	**10.00**	Chloramphenicol	**0.10**
Bacteriological Peptone	**5.00**	Rose Bengal	**0.05**
Potassium Phosphate	**1.00**	Bacteriological Agar	**15.00**
Magnesium Sulfate	**0.50**		
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 31.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is intense pink.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

CAUTION: this medium contains Chloramphenicol and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

ROSE BENGAL AGAR + CHLORAMPHENICOL is a neutral selective medium recommended for the enumeration of molds and yeasts in foods, water and environmental materials. Rose-Bengal Chloramphenicol Agar is recommended for fresh proteinaceous foods with flora mostly made up of Gram-negative rod-shaped bacteria. It is also appropriate when higher and longer incubation temperatures, around 35°C, are required.

Bacteriological peptone provides the nitrogen, vitamins, minerals and amino acids source. Dextrose is the fermentable carbohydrate as a carbon and energy source. Potassium phosphate is the buffer. Magnesium sulfate provides sulfur and other trace elements. Rose Bengal is a selective agent that inhibits the growth of bacteria and limits the size and height of faster-growing molds, allowing the development and detection of other slower-growing yeasts - molds that appear pink colored. Chloramphenicol serves as a selective agent, inhibiting bacterial growth. It is a recommended antibiotic for neutral media due

to its heat stability and wide bacterial spectrum. Bacteriological agar is the solidifying agent.

The inoculation can be carried out from a diluted source, either by the extension of 0.1 ml of each dilution into the prepared plates, or by the pouring method, depositing 1 ml of each dilution into the empty plate, pouring the medium immediately after (once it has been cooled to 45°C). Incubate for 7 days at 25 - 30°C.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 25 - 30°C and observed after 7 days.

Microorganisms	Growth	Colony Color
Candida albicans ATCC 10231	Good	Pink, Plane, Bulky
Aspergillus niger ATCC 1015	Good	White mycelium; black spores
Escherichia coli ATCC 25922	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Waksman, S.A. 1922. A method for counting the number of fungi in the soil. J. Bacteriol. 7:339-341

Koburger J.A. 1972. Fungi in foods. Effect of plating medium pH on counts. J. Milk Food Technol. 35:659-660. Papvizas, G.C., and C.B. Davey. 1959. Evaluation of various media and antimicrobial agents for isolation of soil fungi.

Marshall, R.T. (ed) 1993. Standard methods for the examination of dairy products, 1 6th ed. American Public Health assoc., Washington, DC.

ROSE BENGAL AGAR + CHLORAMPHENICOL+ DICHLORAN (DRBC AGAR) ISO 21527-2-1

CAT. 1160

Selective medium for the enumeration of yeasts and molds in foods

FORMULA IN g/l

Glucose	**10.00**	Chloramphenicol	**0.10**
Peptone	**5.00**	Rose Bengal	**0.025**
Monopotassium Phosphate	**1.00**	Dichloran	**0.002**
Magnesium Sulfate	**0.50**	Bacteriological Agar	**15.00**
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 31.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is intense pink.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

CAUTION: this medium contains Chloramphenicol and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

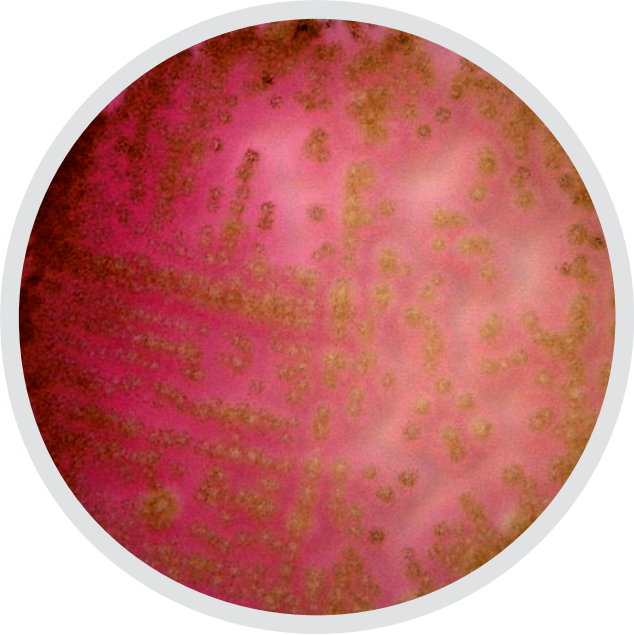
ROSE BENGAL AGAR + CHLORAMPHENICOL + DICHLORAN (DRBC) is a selective medium recommended by ISO 21527-1 for the enumeration of yeasts and molds, by means of the colony count technique, in foods products for human consumption and animals feeding stuffs which have a water activity greater than 0.95, such as meat, eggs, dairy products (except milk powder), fruits, fresh pastes, vegetables, etc. This formula is a modification of Rose Bengal Agar.

Peptone provides the nitrogen, vitamins, minerals and amino acids source. Dextrose is the fermentable carbohydrate as a carbon and energy source. Potassium phosphate is the buffer. Magnesium sulfate provides sulfur and other trace elements. Rose bengal is a selective agent that inhibits the growth of bacteria and limits the size and height of faster-growing molds, allowing the development and detection of other slower-growing yeasts - molds that appear pink colored. Chloramphenicol serves as a selective agent, inhibiting bacterial growth. It is a recommended antibiotic for neutral media due to its heat stability and wide bacterial spectrum. The addition of Dichloran prevents the fast spreading of mucoraceous fungi and also restricts the size of the colonies of other genera, improving the colony count. Bacteriological agar is the solidifying agent.

The inoculation can be carried out from a diluted source, either by the extension of 0.1 ml of each dilution into the prepared plates, or by the pouring method, depositing 1 ml of each dilution into the empty plate, pouring the medium immediately after (once it has been cooled to 45°C). Incubate at 25 ± 1ºC during 3, 4 and 7 days. Select the dishes containing less than 150 colonies and count these colonies report as number of colonies per gram of food.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 25 ± 1°C and observed after 3, 4 and 7 days.



Aspergilus brasiliensis
ATCC 16404

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Saccharomyces cerevisae ATCC 9763	Good
Rhodotorula mucilaginosa DMS 70403	Good (orange colony)
Mucor racemosus ATCC 42647	Moderate
Bacillus subtilis ATCC 6633	Inhibited
Escherichia coli ATCC 25922	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

King; D.A. and Pitt, J.I(1 979) Dichloran-rose Bengal medium for enumeration and isolation of moulds from foods. Appl. Environm. Microbiol. 37 959-964

ISO 21527 - Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0,95

ROTHE BROTH (GLUCOSE BROTH WITH AZIDE)

CAT. 1238

For the quantitative determination of fecal enterococci

FORMULA IN g/l

Peptone Mixture	**15.00**	Beef Extract	**4.50**
Glucose	**7.50**	Sodium Azide	**0.20**
Sodium Chloride	**7.50**		
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 34.7 grams of the medium in one liter of distilled water (69.4 grams if double concentration is desired). Mix well and dissolve by heating with frequent agitation until boiling point. DO NOT OVERHEAT. Dispense into appropriate containers and sterilize in autoclave at 118°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is yellowish brown.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

ROTHE BROTH (Glucose Broth with Azide) is a selective medium recommended by Malmann and Seligmann for the quantification of enterococci in water, food and other materials suspect of being contaminated by wastewaters. Enterococci are the best indicators of fecal contamination in water as *Escherichia coli* is very resistant to chloride.

The presence of enterococci is an indicator for fecal contamination, especially when it occurred a long time ago and the less resistant coliform bacteria, including *Escherichia coli*, may already be dead when the analysis is carried out.

Peptone mixture and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The use of Sodium azide to selectively inhibit Gram-negative bacteria first appeared in the studies of EDWARDS (1938) on the isolation of *Streptococcus agalactiae*, it was later showed that Sodium azide can also be used for the isolation of enterococci from water.

Rothe Broth is ideal for the enumeration of enterococci by the serial dilution method. Inoculate 10 ml of the sample in 10 ml tubes of double-strength Rothe Broth (or 1 ml of the sample in 10 ml of a single - strength medium). Use 5 tubes for each dilution (according to Mallmann and Seligmann).

Incubate all tubes at 35 ± 2°C for 24 - 48 hours. Confirmation of fecal enterococci is obtained by the subsequent inoculation of positive tubes into EVA Broth (**Cat. 1230**).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aures ATCC 25923	Inhibited
Enterococcus faecalis ATCC 19433	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Mallmann W.L. Seligmann E.B. AJPH, 1950. 40 286-289 Standard Methods for the Examination of Water and Wastewater. Eleventh Edition APHA Inc. New-York 1960

Edwards S.J. (1933) J. Comp. Path Therap., 46.211.

SABOURAUD DEXTROSE AGAR EUROPEAN PHARMACOPOEIA

CAT. 1024

For the cultivation of yeasts and molds

FORMULA IN g/l

Dextrose	**40.00**	Bacteriological Agar	**15.00**
Mixture of Peptic Digest of Animal Tissue and Pancreatic Digest of Casein (1:1)	**10.00**		
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 65 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 118 - 121ºC for 15 minutes. AVOID OVEARHEATING as it facilitates the hydrolysis of the components and the medium remains soft. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Candida albicans
ATCC 10231

USES

SABOURAUD DEXTROSE AGAR can be used for cultivating yeasts, molds and aciduric microorganisms. It is used for cultivating pathogenic fungi, particularly those associated with skin infections. This medium is also used for determining the microbial and fungal content of cosmetics and for the mycological evaluation of food.

The formula is based on the European Pharmacopoeia. Dextrose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. The high dextrose concentration and acidic pH make this medium selective for fungi.

Georg *et al* demonstrated that the basic agar fortified by three antibiotics considerably improves the isolation of pathogenic fungi from heavily contaminated sources. To prepare a selective culture medium aseptically add the following antibiotics: Cycloheximide, Penicillin and Streptomycin.

The incubation of the plates should be at 30ºC for 3 - 7 days.

The European Pharmacopoeia recommends this medium in the Paragraph 2.6.12 Microbiological examination of non- sterile products: Microbial enumeration test. For promoting the growth of *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 to inoculate ≤ 100 CFU at 20 - 25ºC for ≤ 5 days.

The European Pharmacopoeia recommends in the Paragraph 2.6.13 "Microbiological examination of non-sterile products"

Test for specified microorganisms

Use Dextrose Sabouraud Agar for growth promotion. After incubation at 30 - 35ºC for 3 - 5 days in Dextrose Sabouraud Broth subculture on the plate of Dextrose Sabouraud Agar and incubate at 30 - 35ºC for 24 - 48 hours.

Interpretation

Growth of white colonies may indicate the presence of *Candida albicans*. This is confirmed by identification tests.

The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30ºC and observed after 3 - 7 days.

Microorganisms	Inoculum (cfu/ml)	Growth	Recovery Rate (%)
* *Aspergillus brasiliensis* ATCC 16404	≤100	Good	≥ 70
**Candida albicans* ATCC 10231	≤100	Good	≥ 70
Escherichia coli ATCC 25922	≤100	Moderate-Good	≥ 70
Escherichia coli ATCC 8739	≤100	Moderate-Good	≥ 70
Lactobacillus casei ATCC 9595	≤100	Good	≥ 70
Saccharomyces cerevisiae ATCC 9763	≤100	Good	≥ 70

According European Pharmacopoeia 7.0

* Incubate at 30 - 35ºC for 24 - 48 hours. Total recount ≤100 cfu/ml to incubate at 20 - 25ºC for ≤ 5 days

** Total recount ≤100 cfu/ml to incubate at 20 - 25ºC for ≤ 5 days

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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European Pharmacopoeia. 7.0

SABOURAUD DEXTROSE AGAR 2%

CAT. 1166

For the cultivation of dermatophytes

FORMULA IN g/l

Dextrose	**20.00**	Bacteriological Agar	**17.00**
Peptone	**10.00**		
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 47 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 121ºC for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD DEXTROSE AGAR 2% is recommended for the cultivation of dermatophytes, particularly those associated with skin infections. The fungi colonies can be observed macro and microscopically.

It is demonstrated that the basic agar, fortified by three antibiotics, considerably improves the isolation of pathogenic fungi from heavily contaminated sources. To prepare a selective culture medium, aseptically add Cycloheximide, Penicillin and Streptomycin.

Dextrose is the fermentable carbohydrate providing carbon and energy. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent.

The high dextrose concentration makes this medium selective for fungi. The low pH of 5.6 approximately is appropriate for the growth of dermatophytes, as well as being slightly inhibitory to contaminating bacteria in clinical specimens.

Inoculate and incubate at 28 ± 2ºC and observe after 7 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 28 ± 2ºC and observed after 7 days.

Microorganisms	Growth
Trichophyton mentagrophytes ATCC 9533	Good
Trichophyton rubrum ATCC 2794	Moderate
Microsporum canis ATCC 10214	Good
Candida albicans ATCC 10231	Good

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

GEORG, L.K,AJELLO, L., a PAPAGEORGE; C: Use of cycloheximide in the selective isolation of fungi pathogenic to man.

SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL

CAT. 1090

For the selective cultivation and isolation of yeasts and molds

FORMULA IN g/l

Dextrose	**40.00**	Chloramphenicol	**0.50**
Peptone Mixture	**10.00**	Bacteriological Agar	**15.00**
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 65.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 118 - 121ºC for 15 minutes. AVOID OVEARHEATING as it facilitates the hydrolysis of the components and the medium remains soft. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL is a selective medium that can be used for the cultivation of yeast, molds and aciduric microorganisms. It is used for cultivating pathogenic fungi, particularly those associated with skin infections. This medium is also used for determining the microbial and fungal content of cosmetics and for the mycological evaluation of food.

Dextrose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. The high dextrose concentration and acidic pH make this medium selective for fungi.

This medium is a modification of the Dextrose Agar described by Sabouraud, with the addition of Chloramphenicol, which inhibits the great majority of bacterial contaminants.

Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum.

The incubation of the plates should be at 30°C for 3 - 7 days.

This medium has a higher concentration of Chloramphenicol than the medium of the same name (**Cat. 1134**), and, as such, is more inhibiting to contaminant bacteria.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 3 - 7 days.

Microorganisms	Growth
Candida albicans ATCC 2091	Good
Candida tropicalis ATCC 750	Good
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Jarett, L., and A.C. Sonnenwirth (ed) 1980. Gradwohl's clinical laboratory methods and diagnosis, 8th ed. CV Mosby.

Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (ed) 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.

SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL EUROPEAN PHARMACOPEIA

CAT. 1134

For the selective cultivation and isolation of yeasts and molds

FORMULA IN g/l

Dextrose	**40.00**	Chloramphenicol	**0.05**
Peptone (Meat & Casein)	**10.00**	Bacteriological Agar	**15.00**
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 65 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 118 - 121ºC for 15 minutes. AVOID OVEARHEATING as it facilitates the hydrolysis of components and the medium remains soft. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL is a selective medium that can be used for the cultivation of yeasts, molds and aciduric microorganisms. It is used for cultivating pathogenic fungi, particularly those associated with skin infections. This medium is also used for determining the microbial and fungal content of cosmetics and for the mycological evaluation of food.

The formula is based on the European Pharmacopoeia. Dextrose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. The high dextrose concentration and acidic pH make this medium selective for fungi.

This medium is a modification of the Dextrose Agar described by Sabouraud, with the addition of Chloramphenicol.

Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum.

This medium has a lower concentration of Chloramphenicol than the medium of the same name (**Cat. 1090**), making it less inhibitory to contaminant bacteria.

The European Pharmacopoeia recommends subculturing positive fungal strains separately to the medium at 20 - 25°C for 48 hours for *Candida albicans*, and at 20 - 25°C for 7 days for *Aspergillus brasiliensis*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 3 - 7 days.

Microorganisms	Growth	Inoculum (cfu/ml)	Recovery Rate (%)
* *Candida albicans* ATCC 10231	Good	10^2–10^3	≥ 70
* * *Aspergillus brasiliensis* ATCC 16404	Good	10^2–10^3	≥ 70
Escherichia coli ATCC 25922	Inhibited	10^2–10^3	≤ 0.01
Staphylococcus aureus ATCC 25923	Inhibited	10^2–10^3	≤ 0.01

* According to European Pharmacopeia incubate at 20 - 25ºC for 48 hours

* * According to European Pharmacopeia incubate at 20 - 25ºC for 7 days

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Association, Washington, D.C:

European Pharmacopoeia. 6th Edition. 2007

SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL + CYCLOHEXIMIDE

CAT. 1089

For the selective cultivation and isolation of pathogenic fungi

FORMULA IN g/l

Dextrose	**40.00**	Cycloheximide	**0.40**
Peptone Mixture	**10.00**	Bacteriological Agar	**15.00**
Chloramphenicol	**0.50**		
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 65.9 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and

sterilize in autoclave at 118 - 121ºC for 15 minutes. AVOID OVEARHEATING as it facilitates the hydrolysis of the components and the medium remains soft. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL + CYCLOHEXIMIDE can be used for cultivating yeasts, molds and aciduric microorganisms. It is used for cultivating pathogenic fungi, particularly those associated with skin infections. This medium is also used for determining the microbial and fungal content of cosmetics and for the mycological evaluation of food.

Dextrose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. The high dextrose concentration and acidic pH make this medium selective for fungi.

This medium is a modification of the Dextrose Agar described by Sabouraud, with the addition of Chloramphenicol and Cycloheximide. Chloramphenicol is an antibiotic which aids isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum. Cycloheximide is an antibiotic which inhibits saprophytic fungi but allows for the growth of pathogenic fungi: *Cryptococcus neoformans, Aspergillus fumigatus* and some species of *Candida* (*albicans, krusei*).

Inoculate sample and incubate at 30°C during 3 days and for up to 7 days if necessary.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 3 - 7 days.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Candida tropicalis ATCC 750	Partially Inhibited
Escherichia coli ATCC 25922	Inhibited
Trichophyton mentagrophytes ATCC 9533	Good
Penicillium spp.	Partially Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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SABOURAUD DEXTROSE AGAR + CYCLOHEXIMIDE

CAT. 1088

For the selective cultivation of yeasts and molds

FORMULA IN g/l

Dextrose	**40.00**	Cycloheximide	**0.40**
Peptone Mixture	**10.00**	Bacteriological Agar	**15.00**
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 65.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 118 - 121ºC for 15 minutes. AVOID OVEARHEATING as it facilitates the hydrolysis of the components and the medium remains soft. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

SABOURAUD DEXTROSE AGAR + CYCLOHEXIMIDE is a selective medium that can be used for cultivating yeasts, molds and aciduric microorganisms. It is also used for cultivating pathogenic fungi, particularly those associated with skin infections. This medium is at the same time used for determining the microbial and fungal content of cosmetics and for the mycological evaluation of food.

Dextrose is the carbohydrate energy source. Peptone mixture is the nitrogen, vitamins and amino acids source and the Bacteriological agar is the solidifying agent. The high dextrose

concentration and acidic pH make this medium selective for fungi.

This medium is a modification of the Dextrose Agar described by Sabouraud, with the addition of Chloramphenicol and Cycloheximide. Cycloheximide is an antibiotic which inhibits saprophytic fungi but allows for the growth of pathogenic fungi: *Cryptococcus neoformans*, *Aspergillus fumigatus* and some species of *Candida* (*albicans, krusei*).

Inoculate sample and incubate at 30°C during 3 days and for up to 7 days if necessary.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 3 - 7 days.

Microorganisms	Growth
Candida albicans ATCC 2091	Good
Escherichia coli ATCC 25922	Good/Moderate
Aspergillus brasiliensis ATCC 16404	Inhibited/Light
Penicillium spp.	Inhibited/Light
Trychophyton mentagrophytes ATCC 9533	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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SABOURAUD DEXTROSE BROTH EUROPEAN PHARMACOPOEIA

CAT. 1205

For the cultivation of yeasts and molds

FORMULA IN g/l

Dextrose	**20.00**	Mixture of Peptic of Animal Tissue and Pancreatic Digest of Casein (1:1)	**10.00**

Final pH 5.6 ± 0.2 at 25ºC

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 118 -121°C for 15 minutes. DO NOT OVEARHEAT. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD DEXTROSE BROTH is a liquid medium used for culturing yeasts, molds and aciduric microorganisms. The high dextrose concentration and acidic pH make this medium selective for fungi from clinical samples and other materials.

This medium is a modification of the Dextrose Agar described by Sabouraud, with half the Dextrose and without the agar. It is used for cultivating molds, yeasts and pathogenic fungi, particularly those associated with skin infections. It is also used in tests for sterility.

The formula is based on the European Pharmacopoeia. Dextrose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. The high dextrose concentration and acidic pH make this medium selective for fungi.

Inoculate and incubate at 30ºC for 18 - 48 hours.

The European Pharmacopoeia recommends in the Paragraph 2.6.13 " Microbiological examination of non-sterile products: test for specified microorganisms for *Candida albicans* " to prepare the product to be examined using 10 ml of the quantity corresponding to not less than 1 g o 1 ml to inoculate 100 ml of Sabouraud Dextrose Broth and to mix it carefully. Incubate at 30 - 35ºC for 3 - 5 days. Subculture on a plate of Sabouraud Dextrose Agar (**Cat. 1024**) and incubate at 30 - 35ºC for 24 - 48 hours.

This medium is also recommended by European Pharmacopoeia in the Paragraph 2.6.12 " Microbiological examination of non-sterile products: Microbial enumeration test" for the preparation of test strains of *Candida albicans*. Inoculate and incubate at 20 - 25ºC for 2 - 3 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 ± 2ºC and observed after 18 - 48 hours.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Candida albicans ATCC 26790	Good
* *Candida albicans* ATCC 10231	Good
Escherichia coli ATCC 25922	Partially inhibited
Escherichia coli ATCC 8739	Partially inhibited
Lactobacillus casei ATCC 9595	Good
Saccharomyces cerevisiae ATCC 9763	Good

* According to European Pharmacopeia. For preparation of test strains, incubate at 20 - 25ºC for 2 - 3 days and for specified microorganisms at 30 - 35ºC for 3 - 5 days.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD

European Pharmacopoeia. 7.0.

SABOURAUD FLUID MEDIUM USP

CAT. 1506

For the cultivation of yeasts and molds

FORMULA IN g/l

Dextrose	**20.00**	Meat Peptone	**5.00**
Casein Peptone	**5.00**		
Final pH 5.7 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. DO NOT OVERHEAT, since the medium contains high levels of carbohydrates that can caramelize (darken) and lose effectiveness. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD FLUID MEDIUM is employed in sterility test procedures for determining the presence of molds, yeasts and aciduric microorganisms. Sabouraud Fluid Medium is also used in the sterility tests of pharmaceutical products, in special parenterals, such as antisera, antibiotic preparations, venipuncture equipment and saline and glucose solutions. It is recommended in the USP for the determination of the fungistatic activity of pharmaceutical and cosmetic products to prevent false sterility tests.

Dextrose is the fermentable carbohydrate providing carbon and energy. Casein and Meat peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. The acidic pH of the final medium is inhibitory to a large number of bacteria and makes the medium particularly well suited for cultivating fungi and acidophilic microorganisms.

Inoculate sample and incubate at 30 ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Candida albicans ATCC 26790	Good
Escherichia coli ATCC 25922	Partially Inhibited
Lactobacillus casei ATCC 9595	Good
Saccharomyces cerevisiae ATCC 9763	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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SABOURAUD MALTOSE AGAR

CAT. 1054

For the cultivation of yeasts and molds

FORMULA IN g/l

Maltose	**40.00**	Bacteriological Agar	**15.00**
Peptone Mixture	**10.00**		
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 65 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118 - 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. AVOID OVERHEATING as it facilitates the hydrolysis of the components and the medium remains soft. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD MALTOSE AGAR is a modification of Sabouraud Dextrose Agar with maltose substituting dextrose. It is a selective medium due to its acidic pH. Davidson, Dawding and Buller reported that Sabouraud Maltose Agar was a satisfactory medium for isolating *Trichophyton gypseum* from a case of *Tinea barbae* and in their studies of the infections caused by *Microsporum audouinii* and *Trichophyton gypseum*.

Maltose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. The low pH suits fungi growth, particularly dermatophytes, and is slightly inhibitory to contaminating bacteria in clinical specimens. Bacteriological agar is the solidifying agent.

Georg *et al*. demonstrated that basic agar fortified by three antibiotics considerably improves the isolation of pathogenic fungi from heavily contaminated sources.

To prepare a selective culture medium aseptically add the following for every liter of the medium before use: Penicillin + Streptomycin or Chloramphenicol or Neomycin. Inoculate sample and incubate at 30°C for up to 7 days.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30°C and observed after 7 days.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Candida albicans ATCC 26790	Good
Trichophyton mentagrophytes ATCC 9533	Good
Escherichia coli ATCC 25922	Partially Inhibited
Lactobacillus casei ATCC 9595	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Chapman G. H. (1 952). Trans. New York Acad. Sci., Series II 1 4(6).254.

SABOURAUD MALTOSE BROTH

CAT. 1213

For the cultivation of yeasts, molds and acidophilic bacteria, as well as for sterility tests

FORMULA IN g/l

Maltose	**40.00**	Peptone Mixture	**10.00**
Final pH 5.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SABOURAUD MALTOSE BROTH is a modification of Sabouraud Dextrose Broth in which maltose has been substituted for dextrose. It is a selective broth because of its acidic pH.

Maltose is the fermentable carbohydrate providing carbon and energy. Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. The low pH suits fungi growth, particularly dermatophytes, and is slightly inhibitory to contaminating bacteria in clinical specimens.

Inoculate sample and incubate at 30°C for 40 - 72 hours.

The growth of molds appears as cotton balls in the medium. Initially they form a membrane at the top of the liquid/air surface.

The growth of yeasts and bacteria are manifested by a homogeneous turbidity, which can be then stained and viewed microscopically.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30°C and observed after 40 - 72 hours.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Candida albicans ATCC 26790	Good
Trichophyton mentagrophytes ATCC 9533	Good
Escherichia coli ATCC 25922	Partially Inhibited
Lactobacillus casei ATCC 9595	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

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Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersdburg, MD.

SALINE PEPTONE WATER ISO 6887

CAT. 1405

Recommended as a diluent and for the homogenization of microbiological samples

FORMULA IN g/l

Sodium Chloride	**8.50**	Casein Peptone	**1.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 9.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The medium is colorless.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SALINE PEPTONE WATER (Maximum Recovery Diluent) is an isotonic diluent used for maximum recovery of microorganisms, and for the growth of bacterial cultures, principally marine bacteria.

ISO 6887 recommends this medium as a diluent for the preparation of initial suspension for microbiological samples. The low concentration of peptone does not cause a multiplication of the organisms within 1 - 2 hours of dilution of the sample. It is also used for carbohydrate fermentation tests in many food and environment studies, amongst others. To determine carbohydrate fermentation patterns, add 1.8 ml of 1% Phenol red to reconstitute the dry medium. After dispensing into test

tubes with Durham gas collecting vials for gas detection, sterilize at 121°C for 15 minutes. Aseptically add sterile carbohydrate solution (Dextrose) to yield 1% final concentration. Distribute carbohydrate in the tube by rotating gently.

Peptone is the nutrient source of nitrogen, vitamins, amino acids and minerals. Sodium chloride maintains the osmotic balance.

Inoculate tubes with a sample and incubate at 35 ± 2°C for 18 - 24 hours. The fermentation of carbohydrate produces acid, causing a drop in the pH and a change of color to yellow; gas production is indicated by gas bubbles.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Salmonella typhimurium ATCC 14028	Good
Staphylococcus aureus ATCC 25923	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Coccolin L, Manzano M. Cantur C., Comi G. App. Environ Microbiolog 2001. nov. 67 (11) 5113-21. Destoumieux – garzon D. Saulnier, D. Garnier 3. et al.

J. Biol Chem. Vol. 276. Issue 50 -47070-47077 (14 December-2001).

ISO 6887 Microbiology – general guidance for the preparation of dilutions for microbiology examinations.

SALMONELLA CHROMOGENIC AGAR

CAT. 1122

For the isolation of *Salmonella spp.* in clinical samples and foods

FORMULA IN g/l

Sodium Citrate	**8.50**	Beef Extract	**5.00**
Chromogenic Mixture	**5.81**	Bacteriological Agar	**12.80**
Casein Peptone	**5.00**		
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 37.1 grams of the medium in one liter of distilled water at 80ºC. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent. It is recommended to prepare the plates on the same day of their use.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

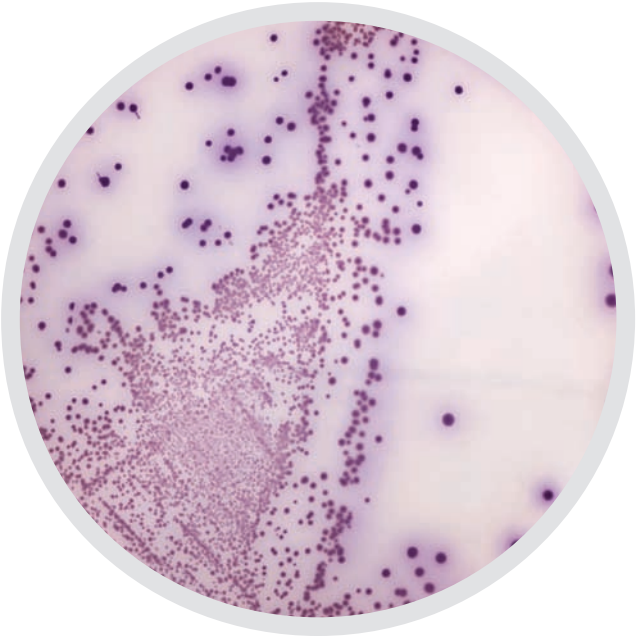
USES

SALMONELLA CHROMOGENIC AGAR is a selective chromogenic medium, used for the detection and presumptive identification of *Salmonella* species from clinical samples, foods and waters. The media traditionally used to differentiate species of *Salmonella* from the rest of the Enterobacteriaceae family, based on their capacity to produce hydrogen sulfide and their inability to ferment lactose, are not really adequate as there are more than 2000 species of *Salmonella* which do not have these characteristics.

Casein peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Chromogenic mixture, in conjunction with Sodium citrate, aids in inhibiting Gram-positive organisms, *Proteus* and coliforms. Bacteriological agar is the solidifying agent.

To identify *Salmonella* species, this chromogenic agent is based on the combination of two chromogenic substrates that ease quick identification. These two chromogenes are X-gal and Magenta-caprylate. X-gal is a substrate incorporated to visualize the enzyme ß-D-galactosidase - producing organisms as blue-green colonies. Magenta colonies are a result of the hydrolysis of Magenta-caprylate by the *Salmonella* species due to the inability to utilise another chromogenic substrate. Thus, non-*Salmonella* organisms appear blue-green or are not stained by any of the chromogenes of the medium.

Inoculate with the sample and incubate at 35 ± 2°C for 18 - 24 hours.



Salmonella enteritidis
ATCC 13076

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth	Colony color
Escherichia coli ATCC 25922	Partially inhibited	Blue-green
Salmonella enteritidis ATCC 13076	Good	Magenta
Salmonella typhi ATCC 19430	Good	Magenta
Salmonella typhimurium ATCC 14028	Good	Magenta
Proteus vulgaris ATCC 13315	Inhibited	Colorless
Salmonella lactose (+)	Good	Magenta

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Journal Clinical Microbiology, Vol. 41 nº 7 p. 3229-3232. July 2003 Robert Cassar and Paul Cuschieri.

J.D. Perry, Michael Furs, Jeffrey Taylor, Et. Al. Journal Clinical Microbiology, March 1999, pag. 766-768 Vol. 37. nº 3

Gallioto di camillo, p. Et. Al. (J. Clinil Microbiol. March 1999.

SALMONELLA SHIGELLA AGAR (SS AGAR)

CAT. 1064

Selective medium for the isolation of *Salmonella* and *Shigella*

FORMULA IN g/l

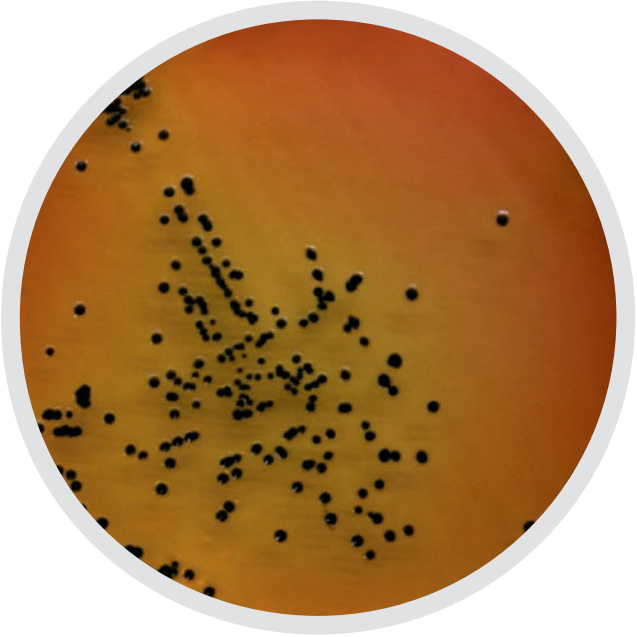
Lactose	**10.00**	Peptone Mixture	**5.00**
Bile Salts Mixture	**8.50**	Ferric Citrate	**1.00**
Sodium Citrate	**8.50**	Neutral Red	**0.025**
Sodium Thiosulfate	**8.50**	Brilliant Green	**0.0003**
Beef Extract	**5.00**	Bacteriological Agar	**13.50**

Final pH 7.0 ± 0.2 at 25ºC

PREPARATION

Suspend 60 grams of the medium in one liter of distilled water. Mix well until a homogeneous suspension is obtained. Heat with frequent agitation and boil for one minute. DO NOT AUTOCLAVE. Cool to 45 - 50°C and distribute in Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.



Salmonella typhimurium
ATCC 14028

USES

SALMONELLA SHIGELLA AGAR (SS AGAR) is a selective and differential medium widely used in sanitary bacteriology to isolate *Salmonella* and *Shigella* from feces, urine, and fresh and canned foods.

Due to its strong inhibitory power, SS Agar can be streaked with a heavy inoculum, but other less inhibitory media, such as Desoxycholate Agar (**Cat. 1020**), MacConkey Agar (**Cat. 1052**), Eosin Methylene Blue (EMB) Agar (**Cat. 1039**), XLD Agar (**Cat. 1080**) and Hektoen Enteric Agar (**Cat. 1030**), should be streaked in parallel. Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

Beef extract and Peptone mixture provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Bile Salts Mixture, Sodium citrate and Brilliant green inhibit Gram-positive bacteria, most coliform bacteria and swarming *Proteus spp.*, while allowing *Salmonella spp.* to grow. Neutral red is the pH indicator. Sodium thiosulfate and Ferric citrate allow the detection of the H_2S producing bacteria, such as *Proteus* and some strains of *Salmonella*, since they produce colonies with black centers and a clear halo.

Non-lactose fermenting bacteria (supposed pathogens) produce clear colonies, transparent or colorless, while coliforms are sufficiently inhibited, and form small colonies that vary from pink to red in color. The plates of the medium can be kept for at least a week in refrigeration.

This formulation, highly selective, is not recommended for the primary isolation of *Shigella*. Some *Shigella spp.* may be inhibited.

CHARACTERISTICS OF THE COLONIES

BACTERIA	COLONIES
Shigella and the majority of *salmonellae*	Clear, colorless, transparent
Escherichia coli	Small, pink to red
Enterobacter, Klebsiella	Larger than *E. coli*, mucoid, pale, opaque cream to pink
Proteus and some *Salmonella*	Colorless, transparent, with a black center if H_2S is produced

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after anaerobic incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Inhibited	—
Enterobacter aerogenes ATCC 13048	Partially inhibited	Cream-pink
Salmonella enteritidis ATCC 13076	Good	Colorless with black center
Salmonella typhi ATCC 6539	Good	Colorless with black center
Salmonella typhimurium ATCC 14028	Good	Colorless with black center
Shigella flexneri ATCC 12022	Good	Colorless
Enterococcus faecalis ATCC 19433	Inhibited	—

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Pub. Health Reports. 65:1075. 1950. Paper Read at Microbiological Congress, 1950. Proc. 22nd Ann. Meet. Northeastern Conf. Lab. Workers in Pullorum Disease Control Burlington, Vermont, June 20-21. 1950.

SALMONELLA SHIGELLA AGAR, MODIFIED

CAT. 1186

Selective medium for the isolation of *Salmonella* and *Shigella*

FORMULA IN g/l

Lactose	**10.00**	Beef Extract	**3.00**
Sucrose	**10.00**	Sodium Tiosulfate	**2.00**
Bile Salts N° 3	**5.00**	Ferric Ammonium Citrate	**1.00**
Sodium Citrate	**5.00**	Neutral Red	**0.02**
Animal Tissue Peptic Digest	**4.00**	Bromocresol Purple	**0.01**
Pancreatic Digest of Casein	**4.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25°C			

PREPARATION

Suspend 59 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50°C, mix well and dispense into plates. AVOID FREEZING. The prepared medium should be stored at 8 - 15°C. The color is purple.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

USES

SALMONELLA SHIGELLA AGAR, MODIFIED is a selective and differential medium widely used in sanitary bacteriology to isolate *Salmonella* and *Shigella* from feces, urine and fresh and canned foods.

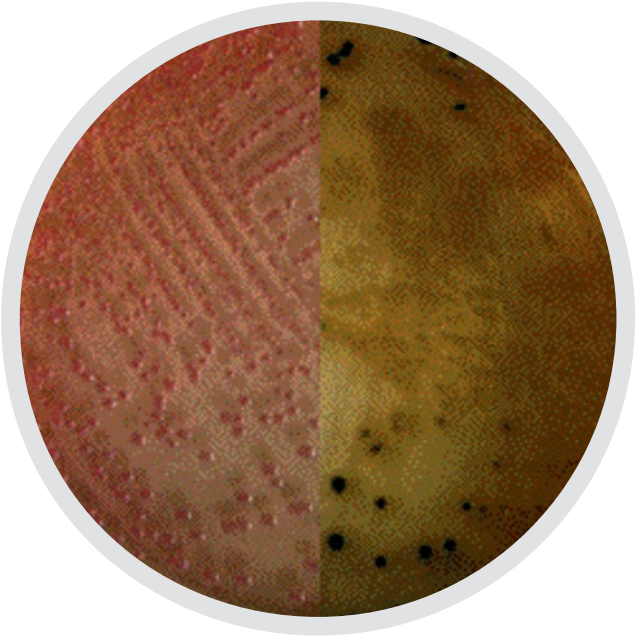
Modified Salmonella Shigella Agar is an improved formula of Salmonella Shigella Agar (SS Agar) (**Cat.1064**). Modified Salmonella Shigella Agar yields more Salmonellas and Shigellas and inhibits more *Escherichia coli*. *Shigella sonnei* colonies are pink, making the differentiation of Salmonella easier.

Due to its strong inhibitory power, SS Agar Modified can be streaked with a heavy inoculum but other less inhibitory media such as Desoxycholate Agar (**Cat. 1020**), MacConkey Agar (**Cat. 1052**), Eosin Methylene Blue (EMB) Agar (**Cat. 1039**), XLD Agar (**Cat. 1080**), and Hektoen Enteric Agar (**Cat. 1030**) should be streaked in parallel.

Animal tissue peptic digest, Beef extract and Pancreatic digest of casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium thiosulfate and Ferric ammonium citrate allow the detection of the H_2S producing bacteria such as some strains of *Salmonella*, as they produce colonies with black centers and a clear halo. Lactose and sucrose are the fermentable carbohydrates providing carbon and energy. Non-lactose fermenting bacteria (supposed pathogens) produce clear colonies, transparent or colorless, while coliforms are sufficiently inhibited, and form small colonies that vary from pink to red in color. Inhibition of Gram-positive microorganisms is obtained by the bile salts mixture. Bacteriological agar is the solidifying agent.

The plates of the medium can be kept for at least a week in refrigeration at 8 -15ºC.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.



Shigella sonnei ATCC 25931 | *Salmonella typhimurium* ATCC 14028

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after anaerobic incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Salmonella enteritidis ATCC 13076	Good	Colorless
Salmonella typhi ATCC 6539	Good	Colorless
Salmonella typhimurium ATCC14028	Good	Colorless
Shigella sonnei ATCC 25931	Good	Pink
Escherichia coli ATCC 25922	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Pub. Health Reports. 65:1075. 1950. Paper Read at Microbiological Congress, 1950.

Proc. 22nd Ann. Meet. Northeastern Conf. Lab. Workers in Pullorum Disease Control Burlington, Vermont, June 20-21. 1950. ISO 6579:2002.

SAN FRANCISCO MEDIUM, MODIFIED

CAT. 1413

Recommended for the growth of *Lactobacillus*

FORMULA IN g/l

Tryptone	**10.00**	Beef Extract	**2.00**
Yeast Extract	**7.00**	Sodium Gluconate	**2.00**
Glucose	**7.00**	Tween 80	**1.00**
Fructose	**7.00**	L-Cysteine HCl	**0.50**
Maltose	**7.00**	Magnesium Sulfate	**0.20**
Sodium Acetate	**5.00**	Manganase Sulfate	**0.05**
Diammonium Citrate	**5.00**	Ferric Sulfate	**0.01**
Dipotassium Acid Phosphate	**2.50**	Bacteriological Agar	**15.00**

Final pH 5.4 ± 0.2 at 25ºC

PREPARATION

Suspend 71.26 grams of the medium in 850 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, and aseptically add 150 ml of a fresh solution of Yeast Extract. The prepared medium should be stored at 8 - 15°C. The color is pale amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and pale beige in color. If there are any physical changes, discard the medium.

USES

SAN FRANCISCO MEDIUM, MODIFIED is a medium described by Vogel *et al*. for the identification of lactobacilli from Sourdough in 1994. Picozzi *et al*. modified this medium by adding 150 ml of a fresh Yeast extract solution instead of rye or wheat flour and baker's yeast components since these made the agar plates turbid and spongy.

Tryptone and Beef Extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Glucose, Fructose and Maltose are the fermentable carbohydrates providing carbon and energy. Yeast extract is a source of vitamins, particularly of the B-group. Sodium gluconate has been added as a stabilizing agent. Sodium acetate is added as a carbon source. Ammonium citrate at a low pH inhibits most microorganisms, including streptococci and molds, and limits swarming, but allows the growth of lactobacilli. Dipotassium acid phosphate is a buffer. Sulphate salts are ions required in a big variation of enzymatic reactions. L-Cysteine hydrochloride is the reducing agent. Bacteriological agar is the solidifying agent.

Incubate at 35 ± 2°C and observe alter 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C, and observed after 18 - 24 hours.

Microorganisms	Growth
Lactobacillus acidophilus ATCC 4356	Good
Lactobacillus casei ATCC 393	Good
Escherichia coli ATCC 25922	Moderate-Good
Pseudomonas aeruginosa ATCC 27853	Null-Light

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Vogel et al. (1994). Identification of Lactobacilli from Sourdough and Description of Lactobacillus pontis sp. nov. International Journal of Systematiac Bacteriology. April. 1994, p. 223-229

Picozzi et al (2005) Comparison of cultural media for the enumeration of sourdough lactic acid bacteria. Annals of Microbiology, 55 (4) 317-320

SCHAEDLER AGAR

CAT. 1066

For the cultivation of anaerobic microorganisms from contaminated specimens

FORMULA IN g/l

Trypticasein Soy Broth	**10.00**	Tris (Hydroxymethyl Aminomethane)	**3.00**
Peptone Mixture	**5.00**	Hemin	**0.01**
Dextrose	**5.00**	L-Cystine	**0.40**
Yeast Extract	**5.00**	Bacteriological Agar	**13.50**
Final pH 7.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 41.9 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and, if desired, add 5% sterile defibrinated blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and pale beige in color. If there are any physical changes, discard the medium.

USES

SCHAEDLER AGAR is prepared according to the formulation described by Schaedler, Dubos, and Costello, and modified by Mata *et al*. It can easily support the growth of anaerobes from the intestinal and digestive tracts and other organs without the interference of the accompanying aerobic flora because of its superior nutritive properties and its low oxidation-reduction potential. In normal conditions, the multiplication of anaerobes is diminished by the rapid increase of enterococci, *Escherichia coli*, *Enterobacter* and other intestinal facultative bacteria.

Although thioglycollate is widely used to lower the oxidation-reduction potential favoring the development of anaerobes, it has been proved that it is an inhibitor of other organisms. In this case the medium includes Cystine, which together with Dextrose, acts as a reducing agent. Trypticasein soy broth, Peptone and Yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is a carbon source. Tris (Hydroxymethyl Aminomethane) acts as a buffer system. Hemin stimulates organism growth. L-Cystine is a reducing agent. Bacteriological Agar is the solidifying agent.

It is recommended to consult methods for the cultivation of anaerobic organisms in food analysis.

Suspend a determined amount of the sample in a known volume of physiological saline. Take a small aliquot and make serial dilutions. With a calibrated loop inoculate duplicate plates, previously dried, and incubate for the appropriate time and temperature. For enumeration, select those plates that contain 30 to 100 colonies.

For the enumeration of *Enterococcus faecalis*, the aerobe and facultative anaerobe, which is an indicator of fecal contamination, Schaedler Agar can be used in the following manner:

Inoculate the food sample (frozen, pre-cooked) in suspension by streaking. Incubate aerobically at 25°C and at 35°C for 24 to 48 hours, and count *E. faecalis*.

If testing pre-cooked meat, also inoculate the base medium (with added neomycin) to investigate the presence and number of *Clostridium welchii*. Incubate anaerobically.

Schaedler can be used adding to it selective substances for the isolation and recovery of lactobacilli, streptococci, clostridia, *Bacteroides*, and *Flavobacterium* from feces and contents of the intestinal tract.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures after anaerobic incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Bacteroides fragilis ATCC 25285	Good
Clostridium butyrium ATCC 9690	Good
Clostridium perfringens ATCC 13124	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Schaedler, R.W. Dubos, R. and Costello, R., 1965. The Development of the Bacterial Flora in the Gastrointestinal Tract of Mice. J. Exp. Med. 1965. 122. 59-66. Mata L.J. Carrillo and Villatoro E., 1966.

Fecal Microflora in a Preindustrial Region. Appl. Microbiol, 17. 396:602.

SCHAEDLER BROTH

CAT. 1218

For the cultivation of anaerobes present in clinical samples and food

FORMULA IN g/l

Trypticasein Soy Broth	**10.00**	Casein Peptone	**2.50**
Dextrose	**5.00**	Meat Peptone	**2.50**
Yeast Extract	**5.00**	L- Cystine	**0.40**
Tris (Hydroxymethyl Aminomethane)	**3.00**	Hemin	**0.01**

Final pH 7.6 ± 0.2 at 25ºC

PREPARATION

Suspend 28.4 grams of the medium in one liter of distilled water .Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber and may present slight precipitate.

The dehydrated medium should be homogeneous, free-flowing and clear toasted in color. If there are any physical changes, discard the medium.

USES

SCHAEDLER BROTH is a liquid medium rich in nutrients, like Schaedler Agar (**Cat. 1066**) but lacking the agar. A large number of pathogenic anaerobic organisms involved in diverse human and animal diseases grow abundantly in this medium.

Schaedler Broth is excellent for the primary isolation of anaerobes, for blood cultures and other clinical materials. It is useful for the determination of the minimum inhibitory concentration (MIC) of antimicrobials used in sensitivity tests. The solid medium is not used to perform sensitivity tests because there is no effective agreement between the concentration of the drug and the diameters of the zones of inhibition that are observed when the solid medium is used.

TSB Broth, Casein peptone and Meat peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Tris (hydroxymethyl Aminomethane) is used to buffer the medium. Hemin stimulates organism growth. L-Cystine is a reducing agent.

To determine the MIC in this medium, Fass and collaborators described a simple method that does not require an anaerobic atmosphere. They recommend placing a glass bead of 6 mm in diameter at the botton of the test tube before sterilizing. The bacterial growth is observed after 18 - 24 hours of incubation at 35 ± 2°C.

To cultivate anaerobic cocci, the authors recommend adding 1 ml of inactivated horse serum for every 100 ml of broth.

In order to know if the Schaedler Broth that has been stored has deteriorated or oxidized, add 0.01 grams of resazurin for each 100 ml of the medium.

The addition of sodium polyanethol-sulphonate (SPS) and carbon dioxide to Schaedler Broth enables it to be used as a blood culture medium and for the cultivation of especially fastidious *Bacteroides* species.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after anaerobic incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Bacteroides fragilis ATCC 25285	Good
Clostridium butyrium ATCC 9690	Good
Clostridium perfringens ATCC 13124	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Fass R.J. Prior R.B. and Rotille C. A. 1975

Antimicrobial Agents Chemother. 8, 444-452.

Rotille C.A. and Col. 1075 Antimicrob. Agents Chemother. 7. 311-315.

Isenberg HD. (ed) 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, DC. Atlas RM. 1993 Handbook of microbiological media, p. 794-795 CRC Press, Boca Raton, FL.

SELENITE CYSTINE BROTH

CAT. 1220

For the selective enrichment of *Salmonella spp.* and some strains of *Shigella* in feces, urine (from clinical samples) and other materials of sanitary importance

FORMULA IN g/l

Sodium Phosphate	**10.00**	Sodium Selenite	**4.00**
Peptone Mixture	**5.00**	L-Cystine	**0.01**
Lactose	**4.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 23 grams of the medium in one liter of distilled water. Mix well and heat gently until dissolved. Dispense and sterilize by exposing the medium to flowing steam for 5 minutes. Excessive heating is detrimental. DO NOT AUTOCLAVE. If the broth is to be used immediately, sterilization is unnecessary. Broth that has been tubed and steamed may be kept for months under refrigeration. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is clear amber to slightly dark amber. After a long storage period of the dehydrated medium, the color of the prepared broth might change to reddish/red. The microbiological performance however is not affected.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

SELENITE CYSTINE BROTH is used for the selective enrichment of *Salmonella spp.*, and is a modified enriched medium by the addition of the amino acid cystine. This amino acid establishes a redox potential that seems to be very good for the enrichment and recovery of *Salmonella* and some strains of *Shigella*, present in limited numbers in feces, diverse foods and other products of sanitary concern.

Selenite Cystine Broth is used particularly to limit the loss of sensitivity that affects other enrichment media especially in food products with a high content of organic material, for example, foods containing egg or egg powder.

Selenite Cystine Broth is recommended for the detection of *Salmonella* in the non-acute stages of illness when the organisms occur in low numbers in the feces, and for epidemiological studies to encourage the detection of low numbers of organisms from asymptomatic or convalescent patients.

Selenite Cystine Broth inhibits the early multiplication of bacteria such as coliforms, but allows the Salmonellae to grow with ease. Peptone mixture is a source of nitrogen, vitamins and amino acids essential for growth. Lactose is the carbohydrate energy source. Sodium selenite inhibits Gram-positive bacteria and most enteric Gram-negative bacteria, except *Salmonella*. L-Cystine lowers the toxicity of Sodium selenite and adds an additional organic sulphur.

Suspend 1 - 2 gr of specimen in the broth (approximately 10 - 15% by volume) for feces, food samples or other solid materials.

Inoculate and incubate at 35 ± 2°C for 18 - 24 hours.

Note that after 18 hours of incubation, the commensal microorganisms rapidly increase and begin to impede the isolation of Salmonellae, so it is necessary to subculture before the elapse of this critical time. These inoculations to the differential solid media, such as SS Agar (**Cat. 1064**), MacConkey Agar (**Cat. 1052**), XLD Agar (**Cat. 1080**) and Chromogenic

Salmonella Agar (**Cat. 1122**) should be performed after 6 - 8 hours of incubation and again after 12 - 24 hours.

Follow the usual methods used in the microbiological analysis of food.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Partial to complete inhibition
Salmonella pullorum ATCC 9120	Good
Salmonella choleraesuis ATCC 12011	Good
Salmonella typhi ATCC 6539	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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American Public Health Association (1976) Compendium of Methods for the Microbiological Examination of Foods. Fricker CR. (1987) J. Appl. Bact. 63: 99-1 16.

SELLERS AGAR

CAT. 1065

Differential medium for studies of Gram-negative, non-fermenting bacteria

FORMULA IN g/l

Gelatin Peptone	**20.00**	Sodium Nitrate	**1.00**
D-Mannitol	**2.00**	Yeast Extract	**1.00**
Sodium Chloride	**2.00**	Sodium Nitrite	**0.35**
Magnesium Sulfate	**1.50**	Bromothymol Blue	**0.04**
Dipotassium Phosphate	**1.00**	Phenol red	**0.008**
L-Arginine	**1.00**	Bacteriological Agar	**13.50**
Final pH 6.7 ± 0.2 at 25°C			

PREPARATION

Suspend 43.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121°C for 10 minutes. Allow to cool in a slanted position in order to obtain butts of 3.5 cm depth and a slant length of 7 - 7.5 cm. The prepared medium should be stored at 8 - 15°C. The color is green.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Important: Immediately before inoculation, add 0.15 ml or 2 drops of 50% dextrose aqueous solution, allowing it to run down the side of the tube opposite to the slant.

USES

SELLERS AGAR is a very useful medium to identify and differentiate Gram-negative, non-fermenting bacilli, such as *Pseudomonas aeruginosa* and *Alcaligenes faecalis*. The differentiation is based on the detection of fluorescence, glucose oxidation, production of nitrogen gas and pH changes, from clinical samples and other materials.

Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Magnesium sulfate is a cofactor for various metabolic reactions. Sodium nitrite provides nitrogen to some organisms. L-Arginine provides amino acids for growth. Sodium chloride maintains the osmotic balance. Dipotassium phosphate acts as a buffer system. D-Mannitol fermentation is detected by Bromothymol blue as a yellow halo around the colonies. Phenol red is a pH indicator. Bacteriological agar is the solidifying agent.

Inoculate medium by stabbing the base of the tube with a needle and streaking the slant. Incubate at 35 ± 2°C for 18 - 24 hours. *Acinetobacter calcoaceticus* morphologically resemble *Neisseria* and are frequently erroneously reported as causes of gonococcal urethritis and meningococcal meningitis (resistant to penicillin). To aid in the identification of the non-fermenters, other media such as OF Basal Medium (**Cat. 1500**), Indole Nitrate Medium (**Cat. 1504**), etc., should be used.

Under UV light only the *Pseudomonas* exhibit fluorescence, which is stimulated by magnesium and mannitol in the medium. At times, it is necessary to hold the tubes for 2 days for *Pseudomonas* to produce a typical alkaline (blue color) reaction in the medium. After incubation, check for glucose oxidation by the appearance of a yellow band, which can disappear after 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	*Acinetobacter calcoaceticus* ATCC 19606	*Acinetobacter lwoffii* ATCC 9957	*Alcaligenes faecalis* ATCC 8750	*Pseudomonas aeruginosa* ATCC 27853
Growth	Good	Good	Good	Good
Slide	Blue	Blue	Blue-green	Blue-green
Butt	Green	Blue	Blue-green	Blue-green
Strip	Yellow			Blue
Fluorescence	-	-	-	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Sellers J. Bact. 87: 46. 1964 Lennette E.H., Spaulding H.E. and Truant P.J. Manual of Clinical Microbiology, 2nd Ed. 1974.

SIM MEDIUM

CAT. 1514

For the identification and differentiation of Enterobacteriaceae

FORMULA IN g/l

Caseine Peptone	**20.00**	Sodium Thiosulfate	**0.20**
Meat Peptone	**6.10**	Bacteriological Agar	**3.50**
Ferric Ammonium Sulfate	**0.20**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SIM MEDIUM is a semisolid medium used for the differentiation of enteric bacteria through sulfide production, indole formation and motility. The Sulfur reduction test is useful in differentiating enteric organisms, especially *Salmonella* and *Shigella*. The Indole test is used for differentiating the Enterobacteriaceae. The Motility test is useful for testing a wide variety of organisms. The medium is also useful in the differentiation of *Klebsiella* from *Enterobacter* and *Serratia* species.

Casein and Meat peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Casein peptone is rich in tryptophan which is reduced and produces indole. Sodium thiosulfate provides Sulphur and Ferric ammonium citrate is the indicator for H_2S production under alkaline conditions. Bacteriological agar is the solidifying agent in a low-concentration to enable the motility to be seen.

Inoculate the pure culture by stabbing to a depth of 3/4 of the tube. Incubate at 35 ± 2°C for 18 - 24 hours and read the results. Darkening indicates the production of H_2S. Motility is indicated by a diffuse turbidity away from the line of inoculation. Growth only along the inoculation line indicates non-motility. The presence of indole is tested by adding Kovac's Reagent (**Cat. 5205**) giving a purple-red coloration to the reagent.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	H_2S	Indole	Motility
Escherichia coli ATCC 25922	Good	-	+	+
Salmonella typhimurium ATCC 14028	Good	+	-	+
Shigella flexneri ATCC 12022	Good	-	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

S.A.B. Manual of Microbiological Mc. Graw-Hill, Book Co. New York, 1957.

Greene, Bilum de Cora, Fairchail, Kaplan, Landau and Sharp. J. Bact. 63:347. 1951.

Harrigan WF. And MacCarice ME (1966) Laboratory Methods in Microbiology Academic Press., 53.

SIMMONS CITRATE AGAR ISO 10273

CAT. 1014

For differentiation of Enterobacteriaceae on the basis of citrate utilization

FORMULA IN g/l

Sodium Chloride	**5.00**	Magnesium Sulfate	**0.20**
Sodium Citrate	**2.00**	Bromothymol Blue	**0.08**
Ammonium Dihydrogen Phosphate	**1.00**	Bacteriological Agar	**15.00**
Dipotassium Phosphate	**1.00**		
Final pH 6.9 ± 0.2 at 25ºC			

PREPARATION

Suspend 24.3 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121ºC for 15 minutes. Allow to cool in a slanted position in order to obtain short butts of 1 - 1.5 cm. depth. Alternatively, the medium can be poured into Petri dishes. The prepared medium in tubes should be stored at 2 - 8°C. In plates, it should be stored at 8 - 15ºC. The color is bluish-green.

The dehydrated medium should be homogeneous, free-flowing and green in color. If there are any physical changes, discard the medium.

USES

SIMMONS CITRATE AGAR is used to differentiate Gram-negative enteric bacilli on the basis of Sodium citrate as a source of carbon and inorganic Ammonium salt as a source of nitrogen. It is recommended for the differentiation of coliforms isolated from water and clinical samples.

It is used in the same manner as Koser Citrate Broth (**Cat. 1200**) for the utilization of citrate as one of the IMVIC reactions. Magnesium sulfate is a cofactor for various metabolic reactions. Sodium chloride maintains the osmotic balance. Dipotassium phosphate acts as a buffer system. Bromothymol blue is a pH indicator. Ammonium dihydrogen phosphate is the sole source of nitrogen. Sodium citrate is the sole source of carbon. Bacteriological agar is the solidifying agent.

It can be poured into plates or dispensed into tubes with long slants. The surface of the slant is inoculated and the base stabbed. The tubes are incubated at 35 ± 2°C for 4 days.

Only those organisms capable of utilizing citrate as a source of carbon grow on the slant and produce a color change from green to blue (alkaline), whilst when no citrate utilization takes place (negative test), the color of the medium remains the same.

Escherichia coli, alongside *Shigella, Yersinia* and *Edwardsiella* species, do not grow on the medium. *Serratia* and most *Enterobacter, Citrobacter, Klebsiella, Proteus* and *Providencia* species, except for *Morganella morganii* and *Klebsiella rhinoscleromatis*, utilize citrate and produce the typical blue coloration.

Simmons Citrate Agar is also used to differentiate citrate-positive *Salmonella enteritidis* and members of *Salmonella* subgenus II, III and IV from the citrate-negative *Salmonella typhi, Salmonella paratyphi A*, *Salmonella pullorum* and *Salmonella gallinarum*.

ISO 10273 recommends this medium for the confirmation of *Yersinia enterocolitica*. Inoculate and incubate at 30ºC during 24 hours. The medium remains green since *Yersinia enterocolitica* does not use citrate as the sole source of carbon.

If good results are not obtained, as in the case of some *Providencia* strains, incubate for 7 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Medium Color
Enterobacter aerogenes ATCC 13048	Good	Blue
Escherichia coli ATCC 25922	Inhibited	Green
Salmonella enteritidis ATCC 13076	Good	Blue
Shigella dysenteriae ATCC 13313	Inhibited	Green
Salmonella typhimurium ATCC 14028	Good	Blue
Salmonella typhi ATCC 19430	Good	Green
**Yersinia enterocolitica* ATCC 27729	Inhibited	Green

* Inoculate and incubate at 30ºC for 24 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD ISO

Simmons. J. Inf. Dis. 39:209, 1926. Standard Methods for the Examination of Water and Wastewater. Eleventh Edition. APHA Inc. New York, 1960. Edwards & Ewing. Enterobacteriaceae. USPHS. Publications 743. Washington, 1972.

Torregrosa and Ortiz, Pediatrics 59:35. 1961.

ISO 10273. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica

SLANETZ-BARTLEY MEDIUM ISO 7899-2

CAT. 1109

For the detection and enumeration of enterococci in water by the membrane filtration technique

FORMULA IN g/l

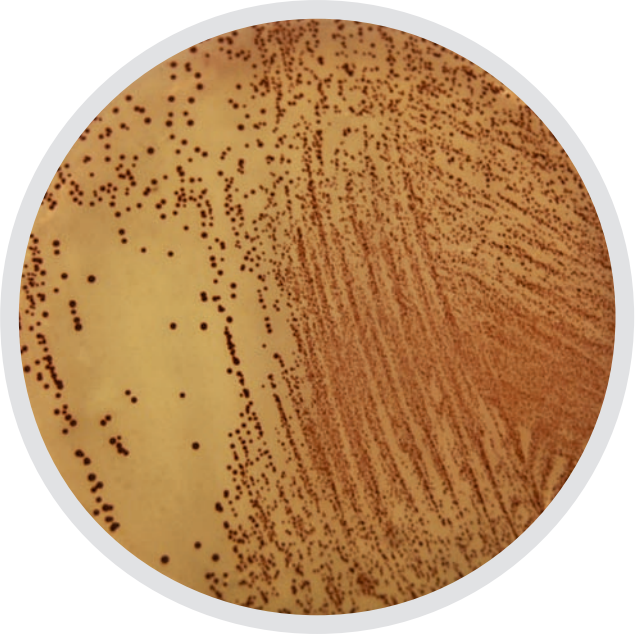
Tryptose	**20.00**	Sodium Azide	**0.40**
Yeast Extract	**5.00**	Triphenyltetrazolium Chloride (TTC)	**0.10**
Dipotassium Phosphate	**4.00**	Bacteriological Agar	**10.00**
Glucose	**2.00**		
Final pH 7.2 ± 0.1 at 25ºC			

PREPARATION

Suspend 41.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent with a pink tint.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.



Enterococcus faecalis
ATCC 11700

USES

SLANETZ-BARTLEY MEDIUM is very selective for enterococci. Burkwall and Hartman demonstrated that the addition of 0.5 ml of Tween 80 and 20 ml of a 10% Sodium carbonate or bicarbonate solution to each liter of the medium was valuable when investigating enterococci in frozen foods.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate providing carbon and energy. Dipotassium phosphate is a buffer. Sodium azide inhibits Gram-positive bacteria. Triphenyltetrazolium chloride is reduced to formazan by the enterococci. Bacteriological agar is the solidifying agent.

The ISO standard 7899-2 recommends this medium for the enumeration of enterococci in water systems. Water is filtered through a membrane, which is then placed on the surface of a plate of Slanetz-Bartley Medium. The plate is incubated at 36 ± 2°C for 44 ± 4 hours. The membrane is examined with a magnifying lens under good light and all red or brown colonies are counted as presumptive enterococci.

With a positive presumptive result, the membrane with the typical colonies is transferred to a dish with Bile Esculin Azide Agar (**Cat. 1005**), pre-warmed to 44°C. The plates are incubated at 44 ± 0.5°C for 2 hours. After incubation typical colonies (brown-black surrounding medium) are counted as intestinal enterococci.

This medium also complies with the recommendations of the British Ministry of Health – Report 71, and the German DIN Regulations 10181 and 10160 for the examination of milk, meat and meat sub-products.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 36 ± 2°C and observed after 44 ± 4 hours.

Microorganisms	Growth	Red Colonies
Streptococcus pyogenes ATCC 12344	Moderate	-
Streptococcus agalactiae ATCC 13813	Null/light	-
Enterococcus faecalis ATCC 11700	Good	+
Enterococcus faecalis ATCC 19433	Good	+
Staphylococcus aureus ATCC 25923	Null	-
Escherichia coli ATCC 25922	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Slanetz L.W. and Bartley C.H. 1957. J. Bact. 74; 591 -595.

ISO 7899-2. Water quality-Detection and enumeration of intestinal enterococci-Part2: Membrane filtration method. Nordic Committee of Food analysis 1968 Leaflet 68.

Department of Health and Social Security report 711982.

The Bacteriological examination of drinking water supplies, HMBO, London

SLANETZ-BARTLEY MEDIUM WITHOUT TTC ISO 7899-2

CAT. 1435

For the detection and enumeration of enterococci in water by the membrane filtration technique

FORMULA IN g/l

Tryptose	**20.00**	Glucose	**2.00**
Yeast Extract	**5.00**	Sodium Azide	**0.40**
Dipotassium Phosphate	**4.00**	Bacteriological Agar	**10.00**
Final pH 7.1 ± 0.1 at 25ºC			

PREPARATION

Suspend 41.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50 - 60ºC and aseptically add 10 ml of 1% TTC solution, previously sterilized by filtration with a 0.22 µm membrane. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent with a pink tint.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

SLANETZ-BARTLEY MEDIUM I with TTC added is a very selective medium recommended for the isolation and enumeration of enterococci in water and foodstuffs by the membrane filtration technique. It is also suitable for the industrial preparation of poured plates.

Burkwall and Hartman demonstrated that the addition of 0.5 ml of Tween 80 and 20 ml of a 10% Sodium carbonate or bicarbonate solution to each liter of the medium was valuable when investigating enterococci in frozen foods.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate providing carbon and energy. Dipotassium phosphate is a buffer. Sodium azide inhibits Gram-positive bacteria. Triphenyltetrazolium chloride is reduced to formazan by the enterococci. Bacteriological agar is the solidifying agent.

The ISO standard 7899-2 recommends this medium for the enumeration of enterococci in water systems. Water is filtered through a membrane, which is then placed on the surface of a plate of Slanetz-Bartley Medium. The plate is incubated at 36 ± 2°C for 44 ± 4 hours. The membrane is examined with a magnifying lens under good light and all red or brown colonies are counted as presumptive enterococci.

With a positive presumptive result, the membrane with the typical colonies is transferred to a dish with Bile Esculin Azide Agar (**Cat. 1005**), pre-warmed to 44°C. The plates are incubated at 44 ± 0.5°C for 2 hours. After incubation, typical colonies (brown-black surrounding the medium) are counted as intestinal enterococci.

This medium also complies with the recommendations of the British Ministry of Health – Report 71, and the German DIN Regulations 10181 and 10160 for the examination of milk, meat and meat products.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 36 ± 2°C and observed after 44 ± 4 hours.

Microorganisms	Growth	Red Colonies
Streptococcus pyogenes ATCC 12344	Moderate	-
Streptococcus agalactiae ATCC 13813	Null/light	-
Enterococcus faecalis ATCC 11700	Good	+
Enterococcus faecalis ATCC 19433	Good	+
Staphylococcus aureus ATCC 25923	Null	-
Escherichia coli ATCC 25922	Null	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Slanetz L.W. and Bartley C.H. 1957. J. Bact. 74; 591 -595.

ISO 7899-2. Water quality-Detection and enumeration of intestinal enterococci-Part2: Membrane filtration method. Nordic Committee of Food analysis 1968 Leaflet 68.

Department of Health and Social Security report 711982.

The Bacteriological examination of drinking water supplies, HMBO, London

SODIUM SELENITE BROTH

CAT. 1222

For the selective enrichment of *Salmonella spp.* in foods, feces, urine (from clinical samples) and other materials of sanitary importance

FORMULA IN g/l

Sodium Phosphate	**10.00**	Lactose	**4.00**
Peptone Mixture	**5.00**	Sodium Selenite	**4.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 23 grams of the medium in one liter of distilled water. Mix well and heat gently until dissolved. Dispense and expose the medium to flowing steam for 5 minutes. Excessive heating is detrimental. DO NOT STERILIZE IN AUTOCLAVE. If the broth is to be used immediately, the flowing stream is unnecessary. Broth which has been tubed and steamed may be kept for months under refrigeration. The prepared medium should be stored at 2 - 8°C. The color is clear amber to slightly dark amber. After a long storage period of the dehydrated medium, the color of the prepared broth might change to reddish/red. The microbiological performance however is not affected.

The dehydrated medium should be homogeneous, free-flowing and whitish in color. If there are any physical changes, discard the medium.

CAUTION: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

SODIUM SELENITE BROTH is a selective medium for the enrichment of *Salmonella* that may be present in small numbers and competing with intestinal flora. The broth medium can be made more selective for the isolation of *Salmonella* in meat products when it is incubated for 16 to 18 hours at 43°C instead of 37°C.

The enrichment medium is frequently used for the detection of pathogens in fecal specimens as these pathogens usually represent a small proportion of the intestinal flora.

Peptone mixture is a source of nitrogen, vitamins and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Sodium selenite inhibits Gram-positive bacteria and most enteric Gram-negative bacteria, except *Salmonella*. Sodium phosphate is a buffer.

Inoculate medium and incubate at 35 ± 2°C for 18 - 24 hours. After incubation, subculture to MacConkey Agar (**Cat. 1052**), SS Agar (**Cat. 1064**), XLD Agar (**Cat. 1080**) or Chromogenic Salmonella Agar (**Cat. 1122**), and incubate again for confirmation.

The broth is also recommended for the transport of strains of *Vibrio cholerae* because these organisms can survive 2 to 5 days in Sodium Selenite Broth. If the pH is adjusted to 7.8 by means of the addition of Sodium carbonate, the *Vibrio* survive 8 to 10 days at temperatures between 22 and 25°C.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Partially inhibited
Salmonella choleraesuis ATCC 12011	Good
Salmonella typhi ATCC 6539	Good
Salmonella typhimurium ATCC 14028	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Georgala and Boothroyd J. App. Bact. 28:210. 1965.

Harvey and Thompson. Mon. Bull. Ministry Health Lab. Serv. 12:149, 1953.

Harvey and Phillips J. Hyg. 59:93. 1961.

Felsenfeld, Waters, and Ishihara. Illinois Branch Meeting. Soc. Exper. Biol. and Med., 1950.

SPS AGAR (SULFITE POLYMYXIN SULFADIAZINE)

CAT. 1082

For the isolation and enumeration of *Clotridium perfringens* from food and all types of foodstuffs

FORMULA IN g/l

Caseine Peptone	**15.50**	Sulfadiazine	**0.12**
Yeast Extract	**10.00**	Polymyxin B Sulfate	**0.01**
Ferric Citrate	**0.50**	Bacteriological Agar	**13.00**
Sodium Sulfite	**0.50**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 39.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense

into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SPS AGAR (Sulfite Polymyxin Sulfadiazine Agar) is a moderately selective medium to recover *Clostridium perfringens* from fresh or preserved foods and food ingredients.

The medium was modificated by Angelotti, incorporating Sulfadiazine and Polymyxin B sulphate to the more recent Mossel formula for the recovery of *Clostridium perfringens*.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Ferric citrate and Sodium sulfite are H_2S indicators. *C. perfringens* reduces the sulfite to sulfide which in turn reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies.

Material samples are prepared in a homogenizer, or other equipment, and serial dilutions are plated in SPS Agar, previously cooled to 45 - 50°C. Incubate anaerobically. (The authors used a mixture of 90% nitrogen and 10% CO_2).

Serial dilutions in tubed media can also be made and incubated aerobically at 35 ± 2°C for 24 - 48 hours.

A few microorganisms other than *C. perfringens* also grow on SPS Agar so it is best to perform a Gram stain and look for spores. Many common microorganisms are totally or partially inhibited, but if they develop, they generally do not form black colonies nor spores, nor do they reduce nitrate and are non-motile Gram-positive vegetative bacilli.

The lack of motility and the capacity to reduce nitrate can be determined on Indole Nitrate Medium (**Cat. 1504**) with 2 g/l of added agar.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Clostridium perfringens ATCC 12919	Good	Black
Clostridium sporogenes ATCC 11437	Moderate	Black
Escherichia coli ATCC 25922	Inhibited	
Staphylococcus aureus ATCC 6538	Moderate inhibited	White

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Angelotti, Nall, Foter y Lewis. Applied Microbiol. 10: 193. 1962. Mossel. J.SCI. Agr. 10: 662. 1959. Mossel de Bruin Van Diepen, Vendrig y Zoutwelle J. Applied Bact, 19: 142. 1956.

STANDARD METHODS AGAR (P.C.A) Acc. to APHA and ISO 4833

CAT. 1056

For total microbial plate count in milk and other materials of sanitary significant (APHA* formula) and ISO 4833

FORMULA IN g/l

Enzymatic Digest of Casein	**5.00**	Glucose (anhydrous)	**1.00**
Yeast Extract	**2.50**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 23.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored from 8 - 15°C. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light toasted in color. If there are any physical changes, discard the medium.

USES

STANDARD METHODS AGAR (P.C.A.) (PLATE COUNT AGAR) is recommended by APHA when enumerating bacteria of sanitary interest, which are indicators of contamination or microbial load in foods.

Enzymatic Digest of Casein provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological agar is the solidifying agent.

In general, 1 ml of the appropriate test dilution is added to the sterile medium at a temperature of 44 - 45°C, mixed gently and poured into sterile Petri dishes. Alternatively, dispense a portion of each test dilution (e.g., 0.1, 0.01 ml) into separate sterile Petri dishes. Add 10 - 12 ml of tempered (45°C) Standard Methods Agar to Petri dishes containing test dilutions. Swirl the dishes to thoroughly mix the medium and test dilution. Allow plates to cool and solidify.

Incubate the Petri dishes at 32 ± 2°C for 18 - 48 hours and count the developed colonies. Consult the specific texts of APHA for the particular sample applications.

This medium is recommended by the ISO normative 4833 for the colony count technique of microorganisms at 30ºC. Inoculate 1ml of the sample, (if necessary 2 continuous decimal dilutions to be able to count between 15 - 300 colonies per plate), put 12 - 15 ml per plate of agar cooled to 44 - 47ºC in each Petri dish. The time of preparation should not exceed 45 minutes. Invert the plates and incubate at 30 ± 1ºC for 72 ± 3 hours. Post incubation count the colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 32 ± 2ºC and observed after 18 - 48 hours.

Microorganisms	Growth
**Escherichia coli* ATCC 25922	Good
Escherichia coli ATCC 8739	Good
Staphylococcus aureus ATCC 25923	Good
**Staphylococcus aureus* ATCC 6538	Good
Staphylococcus epidermidis ATCC 12228	Good
**Bacillus subtilis* ATCC 6633	Good

* According ISO 4833 Incubate at 30ºC for 72 ± 3 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

International Standard ISO 4833 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 ºC

Standard Methods for the Examination of Dairy Products, 13th Ed. APHA, 1972. American Public Health Association.

Recommended Methods for the Microbiological Examination of Foods, APHA Inc. New York, 1958. Standard Methods for the

Examination of Water and Wastewater, APHA Inc. New York, 1960.

**APHA: American Public Health Association Inc.*

STANDARD NUTRIENT AGAR I

CAT. 1177

For the cultivation and enumeration of fastidious bacteria

FORMULA IN g/l

Peptones	**15.00**	Dextrose	**1.00**
Yeast Extract	**3.00**	Bacteriological Agar	**12.00**
Sodium Chloride	**6.00**		
Final pH 7.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 37 grams of the medium in one liter of distilled water .Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

STANDARD NUTRIENT AGAR I is a medium suitable for the cultivation and enumeration of fastidious bacteria. The addition of blood, ascites fluidt or serum makes it also suitable to cultivate streptococci, pneumococci and other microorganisms. It is employed for the enumeration and isolation of bacteria, and also as high-grade base for preparing special culture media.

The Peptones present in the formula provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Incubate the Petri dishes aerobically at 35± 2ºC during 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 25923	Good
Streptococcus pyogenes ATCC 12344	Good
Streptococcus pneumoniae ATCC 6301	Good
Listeria monocytogenes ATCC 19118	Good
Escherichia coli ATCC 25922	Good

Microorganisms	Growth
Shigella flexneri ATCC 12022	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

American Public Health Association 1917. Standard methods of water analysis, 3rd Ed. American Public Health Association, Washing D.C

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Eaton, A.D., L. S Clesceri, and A. E. GreenBurg (Ed). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, D. C

Vanderzant C., and D. F. Splittsotoeesser (Ed). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association. D. C.

STANDARD NUTRIENT BROTH I

CAT. 1286

For the enrichment and cultivation of fastidious bacteria

FORMULA IN g/l

Peptones	**15.00**	Dextrose	**1.00**
Sodium Chloride	**6.00**	Yeast Extract	**3.00**
Final pH 7.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

STANDARD NUTRIENT BROTH I is a medium suitable for the cultivation of fastidious bacteria. The addition of blood, ascitic fluid or serum makes it also suitable to cultivate streptococci, pneumococci and other microorganisms.

It is used for the enumeration, isolation and enrichment of bacteria, and also as high-grade bases for the preparation of special culture media.

Peptone presents in the formula provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Yeast extract is a source of vitamins, particularly of the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Inoculate and Incubate at 35 ± 2ºC during 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18- 24 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 25923	Good
Streptococcus pyogenes ATCC 12344	Good
Streptococcus pneumoniae ATCC 6301	Good
Listeria monocytogenes ATCC 19118	Good
Escherichia coli ATCC 25922	Good
Shigella flexneri ATCC 12022	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Eaton, A.D., L. S Clesceri, and A. E. GreenBurg (Ed). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, D. C

Vanderzant C., and D. F. Splittsotoeesser (Ed). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association. D. C.

STAPHYLOCOCCUS AGAR Nº 110

CAT. 1032

Selective medium for the isolation of pathogenic staphylococci

FORMULA IN g/l

Sodium Chloride	**75.00**	Dipotassium Phosphate	**5.00**
Gelatin	**30.00**	Yeast Extract	**2.50**
Casein Peptone	**10.00**	Lactose	**2.00**
D-Mannitol	**10.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 149.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and clear in color. If there are any physical changes, discard the medium.

USES

STAPHYLOCOCCUS AGAR Nº110 is a selective medium used to isolate pathogenic staphylococci from clinical and non-clinical samples based on mannitol fermentation, pigment formation and gelatinase activity.

Staphylococci are responsible for many cases of pneumonia, meningitis, furunculosis, urethritis, vaginitis, etc. This medium is also used for isolating staphylococci which contaminate a wide variety of foods and produce food poisoning.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly the B-group. Lactose and D-Mannitol are the fermentable carbohydrates as energy sources. Dipotassium phosphate is the buffer. Sodium chloride supplies essential electrolytes for transport and osmotic balance and, in high concentra-tion, inhibits most bacteria except staphylococci. Gelatin is included to test liquefaction. Bacteriological agar is the solidifying agent.

Inoculate the plates and incubate at 35 ± 2°C for 18 - 48 hours.

Pathogenic staphylococci (coagulase-positive staphylococci) resist the high NaCl concentration and form golden yellow colonial pigments.

Mannitol fermentation, that produces acid, is detected by adding a few drops of Bromothymol blue to a plate and looking for a yellow halo around the colonies.

Staphylococci liquefy gelatin, producing clearing zones around the colonies. One plate can be filled with 5 ml of a saturated solution of ammonium sulfate, or with a drop of 20% sulfosalicylic acid and incubated for 12 minutes to observe the hydrolysis of the gelatin: a clearing around the colony constitutes a positive hydrolysis (Stone's Reaction).

MICROBIOLOGICAL TEST

TThe following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Pigment Production
Bacillus subtilis ATCC 6633	Good	-
Escherichia coli ATCC 25922	Inhibited	-
Staphylococcus aureus ATCC 25923	Good	+
Staphylococcus aureus ATCC 6538	Good	+
Staphylococcus epidermidis ATCC 12228	Good	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Chapman J. Bact. 51:409, 1946. Chapman J. Bact. 63:147. 1952.

Mac Faddin, J.F. 1985 Media for isolation cultivation identification maintenance of medical bacteria, vol. 1 p. 722-726. Willians & Wilkins, Baltimore, MD.

Association of Off icial Analytical Chemists 1995. Bacteriological analytical manual, 8th ed. AOAC Internationel, Cait hersburg, MD.

STUART TRANSPORT MEDIUM

CAT. 1518

For transport and maintenance of all kind of samples

FORMULA IN g/l

Sodium Glycerophosphate	**10.00**	Methylene Blue	**0.002**
Sodium Thioglycollate	**1.00**	Agar nº2	**3.00**
Calcium Chloride	**0.10**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 14.1 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in screw-

capped tubes and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is blue in surface.

The dehydrated medium should be homogeneous, free-flowing and cream in color. If there are any physical changes, discard the medium.

USES

STUART TRANSPORT MEDIUM is a semisolid medium used in the transport and preservation of specimens for the cultivation of diverse organisms such as gonococci, streptococci, Enterobacteriaceae, etc.

It is essentially non-nutritive and contains Sodium thioglycollate to retard oxidation. Calcium chloride along with sodium glycerophosphate act as good buffering agent and also maintains osmotic equilibrium in the medium. Methylene blue acts as the redox indicator, the blue color indicates the presence of oxygen.

The original formula was developed by Stuart for the preservation and transport of *Neisseria gonorrhoeae* and *Trichomonas vaginalis*. Later, Stuart *et al*. demonstrate that the medium could be used in the handling and cultivation of *Haemophilus influenzae*, alpha and beta hemolytic streptococci, pneumococci, and Enterobacteriaceae which can survive at an ambient temperature for 6 to 8 weeks.

It is, however, recommended to send the sample to the laboratory as soon as possible. For the transport of delicate microorganisms it is advisable to use cotton swabs impregnated with charcoal which are commercially available.

The survival of bacteria in a transport medium depends on various factors such as bacteria type and concentration in the specimen, transport medium formulation, and transport temperature and duration. Optimal growth and typical morphology can only be expected if direct inoculation and appropriate cultivation are followed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures kept at different temperatures (4°C and room temperature: 22°C) up to 72 hours.

Microorganisms	Recovery at 4°C	Recovery at 25°C
Bordetella pertussis ATCC 9340	≥50%	≥50%
Haemophilus influenzae ATCC 19418	≥50%	≥50%
Neisseria gonorrhoeae ATCC 19424	≥50%	≥50%
Neisseria meningitidis ATCC 13090	≥50%	≥50%
Shigella flexneri ATCC 12022	≥50%	≥50%
Streptococcus pneumoniae ATCC 6301	≥50%	≥50%

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Beakley, J. W. 1975. The toxicity of wooden applicator sticks for Neisseria gonorrhoeae. Pub. Hith, Lab. 15 (1), 11:16.

Stuart, R.D. Toshach, Sh. R., and Patsula, M. T.: 1954. The problem of transport of specimen for cultura of gonococci. Canad. J. Publ. Hlth. 45(2), 13:83.

Stuart, R. D. 1954. Transport medium for specimens in Public Health Bacteriology. Pub. Hlth. Rep. Wash. 74(5), 431:438.

SULFITE TRYPTOSE BROTH

CAT. 1378

For the detection of *Clostridium perfringens*

FORMULA IN g/l

Tryptose	**15.00**	Sodium Metabisulfite	**1.00**
Soy Peptone	**5.00**	Ferric ammonium Citrate	**1.00**
Yeast Extract	**5.00**		
Final pH 7.6 ± 0.1 at 25ºC			

PREPARATION

Suspend 27 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute in tubes in amounts of 18 ml. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add 0.4 grams of D-cylcoserine and homogenize gently. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SULFITE TRYPTOSE BROTH is a liquid nutrient medium for *Clostridium perfringens*.

Tryptose and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Cycloserine inhibits the accompanying bacterial flora and causes the colonies, which develop, to remain smaller. Microorganisms producing hydrogen sulfide are characterized by a blackening of the tube due to the reaction of Sodium metabisulfite and the Ferric ammonium citrate salt. The containers showing a blackening indicate the presence of *C. perfringens*.

Inoculate and incubate at 37 ± 1°C for 20 ± 4 hours and after 44 ± 4 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the respective supplements added, after incubation at a temperature of 37 ± 1°C and observed after 20 ± 4 hours and after 44 ± 4 hours.

Microorganisms	Growth	Blackering
Clostridium perfringens ATCC 12919	Good	+

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Sahidi S.A. and Ferguson A.R. (1971) Appl. Microbiol.,21 500-506. Harmon S.M., Kauttar D.A. and Peeler J.T.(1971) Appl. Microbiol. 21. 922-927. Hauschild A.H.W and Hilsheimar R. (1973) Appl. Microbiol.27. 78-82.

TBX CHROMOGENIC AGAR (TRYPTONE BILE X-GLUCURONIDE) ISO 16649-2,3

CAT. 1151

Selective medium for the detection and enumeration of *Escherichia coli* in foods

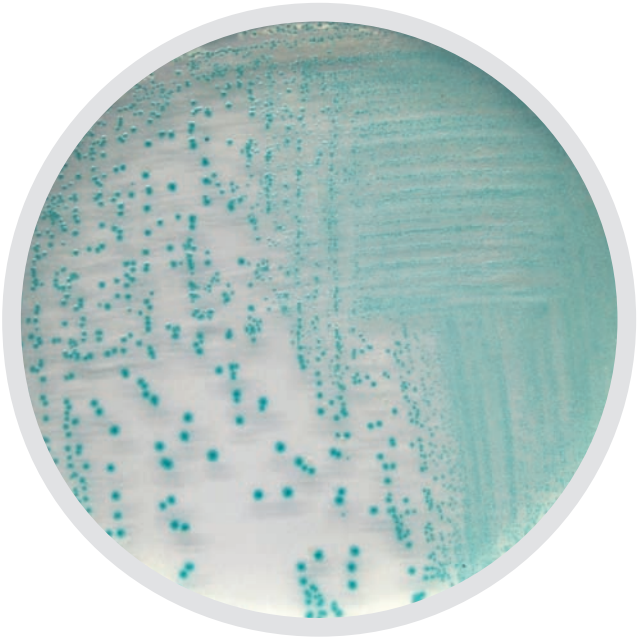
FORMULA IN g/l

Casein Peptone	**20.00**	X-β-Glucuronide	**0.075**
Bile Salts	**1.50**	Bacteriological Agar	**15.00**
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 36.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.



Escherichia coli
ATCC 25922

USES

TBX CHROMOGENIC AGAR is based on Tryptone Bile Salts Agar medium, used to detect and enumerate *E. coli* in foods, with the addition of a chromogenic agent, x-ß-Glucuronide, to detect the presence of the enzyme glucuronidase, which is highly specific for *E. coli*.

The released chromophore in TBX Agar is colored and target colonies are easily identified. *E. coli* absorbs the chromogenic agent x-ß-glucuronide, and the intracellular glucuronidase enzyme activity breaks the bond between the chromophore and the glucuronide. The released chromophore is colored and builds up within the cells, causing the *E. coli* colonies to be blue-green colored.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bile Salts are inhibitors to other Gram-positive organisms and suppress coliform bacteria. Bacteriological agar is the solidifying agent.

ISO 16649 specifies a horizontal method for the enumeration of ß-glucuronidase-positive *E. coli* in products intended for human consumption or for the feeding of animals. In part 2, it uses a colony count technique at 44°C on a solid medium containing a chromogenic ingredient for the enzyme ß-glucuronidase. Inoculate and incubate at a temperature of 44ºC for 18 - 24 hours.

ISO 16649-3 uses the most probable number technique. The most probable number of β-glucuronidase-positive *E. coli* are determined according to the number of tubes of Minerals Modified Glutamate Broth (**Cat. 1365**) whose subcultures have produced blue or blue-green colonies on tryptone bile glucuronide agar. Inoculate and incubate at a temperature of 44°C for 20 - 24 hours.

Note: The negative ß-glucuronidase-negative *E. coli* colonies are colorless, e.g. *E. coli* O157:H7. The high temperatures (44°C) inhibit the growth of E. coli O157:H7.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 44°C and observed after 18 - 24 hours.

Microorganisms	Growth		Colony Color
	44°C	37°C	
Escherichia coli ATCC 25922	Good	Good	Blue-Green
Salmonella typhimurium ATCC 14028	Inhibited	Inhibited	Colorless
Enterococcus faecalis ATCC 19433	Inhibited	Inhibited	-
Klebsiella pneumoniae ATCC 13883	Inhibited	Inhibited	Colorless

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

International standard ISO 16649-2 Microbiology of food animal feeding stuffs. Horizontal method for the enumeration of presumptive ß-glucuronidase –positive. Part 2:Colony-count technique at 44°C using 5-bromo-4-chloro-3-indoly ß-D-glucuronide. Part 3: Most probable number technique using 5-bromo-4-chloro-3-indoly ß-D-glucuronide.

TCBS AGAR

CAT. 1074

For the selective isolation of *Vibrio* from a variety of clinical samples and other materials

FORMULA IN g/l

Sucrose	**20.00**	Ox Bile	**5.00**
Sodium Chloride	**10.00**	Sodium Cholate	**3.00**
Sodium Thiosulfate	**10.00**	Ferric Ammonium Citrate	**1.00**
Sodium Citrate	**10.00**	Thymol Blue	**0.04**
Meat Peptone	**5.00**	Bromothymol Blue	**0.04**
Casein Peptone	**5.00**	Bacteriological Agar	**14.00**
Yeast Extract	**5.00**		

Final pH 8.6 ± 0.2 at 25ºC

PREPARATION

Suspend 88 grams of the medium in one liter of distilled water. Mix for 10 to 15 minutes. Dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. DO NOT OVEARHEAT. DO NOT AUTOCLAVE. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is green.

The dehydrated medium should be homogeneous, free-flowing and light toasted with a green tint in color. If there are any physical changes, discard the medium.

USES

TCBS AGAR is a selective medium widely used to isolate and cultivate practically all bacteria of the genus *Vibrio*, including *V. cholerae* and *V. alginolyticus*, pathogenic to humans causing cholera, choleral diarrhea or food poisoning from contaminated foods and from stool specimens. The last 2 conditions especially can be caused by ingesting raw or partially processed fish or seafood containing *Vibrio parahemolyticus*. The only *Vibrio* that does not grow in TCBS is *V. hollisae*.

The Meat and Casein peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium citrate, Sodium thiosulfate and Ox bile are the selective agents, inhibiting the Gram-positive bacteria. Sodium thiosulfate provides sulphur, and Ferric citrate is the indicator for H_2S production. Sucrose is the carbohydrate energy source. Bromothymol blue and Thymol blue are pH indicators. Sodium chloride promotes growth (*Vibrio* grows well in salty media). Bacteriological agar is the solidifying agent. The alkaline pH of the medium enhances the recovery of *V. cholerae*.

The suspect material (feces, vomit, rectal swabs, fish, and other food) is heavily inoculated on the surface of the plate, incubated at 35 ± 2°C for 18 - 24 hours. Sucrose-positive vibrios, such as *Vibrio cholerae* and *Vibrio alginolyticus*, are yellow on TCBS. Sucrose-negative ones, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, produce blue-green colonies.

Almost all *Vibrio* ferment sucrose and yield yellow colonies from the production of acid. Some types of *Proteus* (fermenters of sucrose) can form yellow colonies similar to those of *Vibrio*.

MICROBIOLOGICAL TEST

The following results in the were obtained performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Vibrio cholerae Inaba	Good	Yellow
Vibrio cholerae Ogawa	Good	Yellow
Vibrio alginolyticus ATCC 19108	Moderate	Yellow
Vibrio parahemolyticus ATCC 17802	Good	Blue
Enterobacter cloacae ATCC 13047	Inhibited	Yellow
Proteus mirabilis ATCC 14273	Moderate	Light-blue

Microorganisms	Growth	Colony Color
Escherichia coli ATCC 25922	Null	
Pseudomonas aeruginosa ATCC 27853	Moderate	Blue

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Cholera Information (WHO, 1965). WHO Expert Committee on Cholera (2 and Rep. Techn., Rep. Series No. 352. 1967. Felsemfeld, Bull World Otg. 34:161. 1966. Kobayashi. T. Enomoto S. Sakasaki, R. Y.

Kwajaras, S., Jap. J. Bact. 18 387 291. 1963.

TETRATHIONATE BROTH BASE USP

CAT. 1114

For the selective enrichment of *Salmonella* species from foods, water, feces, urine and other materials of sanitary interest

FORMULA IN g/l

Sodium Thiosulfate	**30.00**	Peptone Mixture	**5.00**
Calcium Carbonate	**10.00**	Bile Salts	**1.00**
Final pH 8.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 46 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50ºC and aseptically add 20 ml per liter of an iodine solution to the medium on the same day of its use. Homogenize gently and dispense into sterile containers. Prepare the solution iodine-iodide by dissolving 6 g of Iodine crystals and 5 g of potassium iodide in 20 ml of distilled water. Dispense 10 ml into tubes, continually swirling the flask to maintain homogeneity. The prepared medium should be stored at 2 - 8°C and must be used immediately. The color is milky white with a white dense precipitate at the bottom.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

TETRATHIONATE BROTH BASE with added iodine-iodide solution is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in low numbers or have been injured during food processing, and compete with other microorganisms and intestinal flora. Even though cells which have been injured might not form colonies on selective media, they can cause disease if ingested. This formulation conforms to the United States Pharmacopoeia USP.

Tetrathionate is formed by the iodine reaction with Sodium thiosulfate. This combination (Sodium thiosulfate and Tetrathionate) suppresses commensal intestinal organisms. The organisms which have the enzyme tetrathionate reductase, such as *Salmonella*, proliferate in this medium. However, *Proteus* also contains this enzyme, but its growth can be minimized by adding 4 mg/l of novobiocin before adding the iodine solution. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bile salts are inhibitors of other Gram-positive organisms. Calcium carbonate neutralizes and absorbs toxic metabolites.

Inoculate each 10 ml tube with 1 - 2 g of the sample (feces, wastewater, etc.) and incubate at 35 ± 2°C for 18 - 24 hours. Growth is indicated by turbidity in the medium.

After incubation, subculture onto selective plated media for *Salmonella*, such as MacConkey Agar (**Cat. 1052**), Bismuth Sulfite Agar (**Cat. 1011**), Desoxycholate Agar (**Cat. 1020**), Brilliant Green Agar (**Cat. 1078**), XLD Agar (**Cat. 1274**) or Hektoen Enteric Agar (**Cat. 1030**), and incubate at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the additive added, after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Salmonella choleraesuis ATCC 12011	Good
Salmonella typhi ATCC 6539	Good
Salmonella typhimurium ATCC 14028	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

American Public Health Association Recommended Methods for the Microbiological Examination of Foods, APHA, Inc. New York, 1958. American Public Health Association Standard Methods for the Examination of Dairy products. Eleventh Edition, APHA, Inc. New York, 1960.

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United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmaceopeial Convention, Inc., Rockville, Md.

THIOGLYCOLLATE BROTH NIH & USP

CAT. 1241

For sterility assays of biological and pharmaceutical products

FORMULA IN g/l

Caseine Peptone	**15.00**	Sodium chloride	**2.50**
Yeast Extract	**5.00**	Sodium Thioglycollate	**0.50**
Dextrose	**5.00**	L-Cystine	**0.50**
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 28.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The medium does not contain agar or Resazurin and is preferable that it is freshly prepared, eliminating any dissolved oxygen before use by heating in boiling water or in a water bath. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

THIOGLYCOLLATE BROTH (NIH & USP) is prepared according to the formula of the National Institute of Health (NIH) and the United States Pharmacopoeia (USP). Is used in detecting microorganisms in normally sterile materials, and is an alternative to certain products that are turbid or cannot readily culture because of the viscosity.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium thioglycollate and L-Cystine lower the oxidation-reduction potential by removing oxygen to maintain a low Eh. Dextrose is the carbohydrate energy source and allows for a rapid and vigorous growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Inoculate and incubate at 35 ± 2°C for 18 - 48 hours. Anaerobic conditions can also be used with this medium.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Bacillus subtilis ATCC 6633	Good
Candida albicans ATCC 10231	Good
Clostridium sporogenes ATCC 19404	Good
Streptococcus pyogenes ATCC 19615	Good
Bacteroides fragilis ATCC 25285	Good
Escherichia coli ATCC 25922	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

U.S. Pharmacopoeia XVI, 1960.

THIOGLYCOLLATE FLUID MEDIUM EUROPEAN PHARMACOPOEIA

CAT. 1508

For the cultivation of aerobic and anaerobic microorganisms in sterility tests

FORMULA IN g/l

Pancreatic Digest of Casein	**15.00**	L-Cystine	**0.50**
Glucose Monohydrate	**5.00**	Sodium Thioglycollate	**0.50**
Yeast Extract	**5.00**	Resazurin	**0.001**
Sodium Chloride	**2.50**	Bacteriological Agar	**0.75**
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 29.0 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into 15 x 2 cm test tubes (15 ml in each tube) and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is clear amber with a pink upper layer. Cool before using and store in the dark.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

Once prepared it can be used some time after preparation until it is 30% oxidized, which is indicated by a pink color on the Resazurin surface. If the oxidation is greater, reheat the medium only once, with steam or boiling water, cool it and use.

USES

THIOGLYCOLLATE FLUID MEDIUM is used for detecting microorganisms in sterility tests, according to the formula specified in the US Pharmacopoeia and conforms to formulations detailed in the British and European Pharmacopoeia in the Paragraph 2.6.1 Sterility.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium thioglycollate neutralizes the bacteriostatic effect of the compounds used as preservatives in pharmaceutical preparations, especially injectables. Sodium thioglycollate and L-Cystine lower the oxidation-reduction potential by removing oxygen to maintain a low pH. Dextrose is the carbohydrate energy source and allows for a rapid and vigorous growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Resazurin is an oxidation indicator, turning from pink (aerobic) to colorless (anaerobic conditions). Bacteriological agar delays the dispersion of CO_2 and diffusion of O_2.

With this medium it is not necessary to use a cap of sterile paraffin oil or incubate in special containers for anaerobes. The anaerobic organisms develop at the bottom of the tube, the microaerophiles in the middle of the medium and the aerobes in the top oxidized layer.

Inoculate and incubate at a temperature of 35 ± 2°C. no more than 3 days.

The European Pharmacopoeia recommends in the Paragraph 2.6.1 Sterility the following:

For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycolate medium incubate at 20 - 25°C may be used instead of soya-bean casein digest medium.

For growth promotion test of aerobes, anaerobes and fungi, inoculate portions of Fluid thioglycollate medium with a small number, not more than 100 CFU, of *Clostridium sporogenes* ATCC 11437, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538. For sterility, incubate portions of the media for 14 days. No growth of microorganisms occurs.

When the material in study contains other preservatives, use a sufficient amount of thioglycollate to dilute the inoculum beyond its bacteriostatic strength level.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Inoculum (cfu/ml)	Recovery Rate (%)
Bacillus subtilis ATCC 6633	Good	10 -10^{-2}	≥ 50
Candida albicans ATCC 10231	Good	≤ 100	≥ 50
Neisseria meningitidis ATCC 13092	Good	10 -10^{-2}	≥ 50
* *Staphylococcus aureus* ATCC 6538	Good	≤ 100	≥ 70
Clostridium sporogenes ATCC 11437	Good	≤ 100	≥ 50
Pseudomonas aeruginosa ATCC 9027	Good	≤ 100	≥ 50
Bacteroides fragilis ATCC 25285	Good	10 -10^{-2}	≥ 50
Staphylococcus aureus ATCC 25923	Good	10 -10^{-2}	≥ 70
Streptococcus pyogenes ATCC 19615	Good	10 -10^{-2}	≥ 50
Aspergillus brasiliensis ATCC 16404	Good	≤ 100	≥ 50

*According to European Pharmacopeia, Inoculum no more than100 CFU. Incubate at 30 - 35ºC not more than 3 days

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

Brewer. JAMA, 115. 1940. Vera. J. Bact. 47:59, 1944. Pittman. J. Bact. 51:19, 1946.

Kurtin A. J. Clin. Path. 30:229, 1958.

European Pharmocopoeia 7.0. 2.6.1 Sterility

THIOGLYCOLLATE MEDIUM USP ISO 7937

CAT. 1533

For the sensitivity test according to American Pharmacopoeia, and for the cultivation of *Clostridium perfringens* according ISO 7937

FORMULA IN g/l

Enzymatic Digest of Casein	**15.00**	Sodium Thioglycollate	**0.50**
Dextrose	**5.50**	L-Cystine	**0.50**
Yeast Extract	**5.00**	Resazurin	**0.001**
Sodium Chloride	**2.50**	Bacteriological Agar	**0.75**

Final pH 7.1 ± 0.2 at 25ºC

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Cool to room temperature (25ºC). If the stored medium exhibits more than 20% pink color (due to oxidation), the tubes

should be reheated in a water bath to expel the oxygen. Do not reheat more than once. The prepared medium should be stored at 2 - 8°C. The color is clear amber with a pink upper layer.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

THIOGLYCOLLATE MEDIUM (U.S.P) (ISO 7937) is prepared according to the American Pharmacopoeia to perform sterility testing of pharmaceutical products and other devices. This medium is excellent for the cultivation of aerobic and anaerobic microorganisms without the need for an anaerobic jar or paraffin, or a special seal.

It is well buffered so that acid or alkaline inocula will hardly alter the reaction of the medium. Thioglycollate Medium is also recommended for the cultivation of *Clostridium* and *Desulfotomaculum nigrificans*.

Sodium thioglycollate in the medium neutralizes the bacteriostatic effect produced by mercurial compounds used as preservatives in pharmaceutical solutions, making Thioglycollate Media useful in testing material which contain heavy metals. It is necessary to establish the bacteriostatic activity of the product by the method described in the USP (1970) in order to avoid false negative results.

The small quantity of agar assists in the detection of contaminants during sterility testing as it delays the dispersion of CO_2, diffusion of O_2 and reducing substances. The Nitrogen source is provided by the Enzymatic Digest of Casein and the vitamins by the Yeast extract. Sodium thioglycollate and L-Cystine lower the oxidation-reduction potential of the medium by removing the O_2 to maintain a low Eh, therefore preventing the accumulation of peroxides which can be toxic to some organisms. Resazurin is an indicator of oxidation by turning pink. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The medium is used in liquid form in test tubes or as a slanted solid with added agar (1.5%). The medium or slant agar tube can be inoculated directly and incubated at 35 ± 2°C for 18 - 24 hours.

The medium is recommended by the ISO normative 7937 for the enumeration of *Clostridium perfringens* using the colony count technique. Before using the tubes they should be left to air. Incubate under anaerobic conditions at 37°C for 18 - 24 hours. The turbidity should be 1-2 F.T.U.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Bacillus subtilis ATCC 6633	Good

* According to ISO 7937, incubate under anaerobic conditions at 37°C for 18 - 24 hours.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Clostridium sporogenes ATCC 11437	Good
**Clostridium perfringens* ATCC 13124	Good
Streptococcus pyogenes ATCC 19615	Good
Bacillus fragilis ATCC 25285	Good
Escherichia coli ATCC 25922	Good

* According to ISO 7937, incubate under anaerobic conditions at 37°C for 18 - 24 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO USP

Brewer, J. Bact. 39:10. 1940. Hansen, Price, and Clements. J. Bact. 64:772. 1952.

Vera. J. Bact. 47:59, 1944. King. Annals. N.Y. Acad. Sci. 98:615. 1962. Alvarez, A.J.: Med. Tech. 21:249, 1955. Vera and Petran.

Bull. Natl. Assn. Clin. Lab. 5:90. 1964. Tarshis J. Lab. and Clin. Med., 54:630. 1959.

The United States Pharmacopoeial Convention, 1995. 23th ed.

International Standard ISO 7937: Microbiology of food and animal feeding stuffs – Horizontal Method for the enumeration of Clostridium perfringens – Colony-count technique

THIOGLYCOLLATE MEDIUM WITHOUT INDICATOR USP

CAT. 1516

For the cultivation and isolation of obligate and facultative aerobic, anaerobic and microaerophilic bacteria

FORMULA IN g/l

Caseine Peptone	**17.00**	Sodium Thioglycollate	**0.50**
Dextrose	**6.00**	L-Cystine	**0.25**
Soy Peptone	**3.00**	Sodium Sulfite	**0.10**
Sodium Chloride	**2.50**	Bacteriological Agar	**0.75**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15

minutes. The prepared medium should be stored at 2 - 8°C. For optimal performance the tubes should be boiled and cooled to ambient temperature before use. Boiling restores the uniformly hazy appearance of the medium. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

THIOGLYCOLLATE MEDIUM WITHOUT INDICATOR is an enriched general-purpose medium for the recovery of a wide variety of microorganisms

The medium is characterized by its ability to support the growth, from a minimal inoculum, of a great variety of aerobes, anaerobes and microaerophilic microorganisms. The lack of an indicator avoids possible toxicity to organisms, making this a choice medium for diagnostics, particularly useful for sterile materials which contain mercurial preservatives.

Casein and Soy peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium thioglycollate and L-Cystine lower the oxidation-reduction potential by removing oxygen to maintain a low Eh. Dextrose is the carbohydrate energy source and allows a rapid and vigorous growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar delays the dispersion of CO_2 and diffusion of O_2.

This medium supports a minimal inoculation with early visible signs of growth. Strict aerobes develop in the upper part, whereas anaerobes develop at the bottom of the medium tube. This medium supports the growth of aerobic microorganisms such as members of the genus *Brucella*, of strict anaerobes such as *Clostridium acetobutyricum, Clostridium novyi, Actinomyces bovis, Bacteroides, Lactobacillus*, and other bacteria. Pathogenic fungi frequently grow well in this medium. The medium can be used with the addition of 10% serum for the cultivation of *Trichomonas vaginalis* and other microorganisms that utilize serum for added growth.

Inoculate and incubate at 35 ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Bacillus subtilis ATCC 6633	Good
Candida albicans ATCC 10231	Good
Streptococcus pyogenes ATCC 19615	Good
Bacteroides vulgatus ATCC 8482	Moderate
Neisseria meningitidis ATCC 13090	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

USP

Brewer. JAMA, 115. 1940. Vera. J. Bact. 47:59, 1944. Pittman. J. Bact. 51:19, 1946.

Kurtin A. J. Clin. Path. 30:229, 1958.

Baron, E.J. C.R. Peterson, S.M. Finegold 1994. Bailey and Scott's diasnostic Microbiology, 9th ed. MosBy-Year Book, Ing; St. Louis, M.O. The United States Pharmacopoeial Convention, 1995. 23th ed. P. 1686-1690.

TODD HEWITT BROTH

CAT. 1236

For the cultivation of β-hemolytic streptococci for serologic typing from clinical samples

FORMULA IN g/l

Bacteriological Peptone	**20.00**	Dextrose	**2.00**
Heart Infusion	**3.10**	Sodium Chloride	**2.00**
Sodium Carbonate	**2.50**	Disodium Phosphate	**0.40**
Final pH 7.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and clear beige in color. If there are any physical changes, discard the medium.

USES

TODD HEWITT BROTH is recommended for the cultivation of streptococci and other fastidious microorganisms. It was originally developed for the production of streptococcal hemolysin. The broth was modified by Updyke and Nickle and is used preferentially to cultivate beta-hemolytic strains, especially for serological typing, from clinical specimens and for epidemiological studies.

The medium is also recommended as an enrichment medium for the growth of streptococcal cells in the identification of Groups A and B. This medium was used as an enrichment broth for Group A streptococci in a comparison study of a rapid antigen test.

Bacteriological Peptone and Beef Heart infusion provide nitrogen, vitamins, minerals and amino acids essential for growth. Disodium phosphate and Sodium carbonate act as a buffer to prevent the destruction of the hemolysin by the acid produced through fermentation of the carbohydrate Dextrose, source of carbon and energy. Sodium chloride maintains the osmotic balance of medium.

Inoculate and incubate tubes at 35 ± 2°C for 18 - 48 hours.

To prepare Todd Hewitt Agar, add 13 - 1 5 g/l of Bacteriological Agar (**Cat. 1800/1802**) to the broth and sterilize as above.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 48 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good
Streptococcus mitis ATCC 9895	Good
Streptococcus pneumoniae ATCC 6303	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Todd and Hewitt J. Path I Bact. 35:973. 1932 Updyke and Nickle. Applied. Microbiol 2: 117. 1954

Diagnostic Procedures and Reagents. 4th Ed. APHA Inc. New York 1963.

Isenberg H.D. (ed) 1992. Clinical Microbiology procedures handbook, American Society for Microbiology, Washington, D.C. Murray, P.R., E. J. Baron, M.A. Pfaller, F,C, Tencver and R.H. Yolken (ed) 1995 Manual of clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C

TRIPLE SUGAR IRON AGAR (TSI) EUROPEAN PHARMACOPOEIA

CAT. 1046

For the identification and differentiation of Enterobacteria

FORMULA IN g/l

Peptones (Beef & Casein)	**20.00**	Glucose Monohydrate	**1.00**
Lactose Monohydrate	**10.00**	Ferric Ammonium Citrate	**0.30**
Sucrose	**10.00**	Sodium Thiosulfate	**0.30**
Sodium Chloride	**5.00**	Phenol red	**0.025**
Beef Extract	**3.00**	Bacteriological Agar	**12.00**
Yeast Extract	**3.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 64.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121ºC for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5 - 2.0 cm. depth. The prepared medium should be stored at 2 - 8°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

TRIPLE SUGAR IRON AGAR (TSI) is a differential medium used to differentiate enteric Gram-negative Enterobacteria on the basis of carbohydrate fermentation and H_2S production. It is used as an aid in the identification of pathogenic and saprophytic Enterobacteria isolated from routine bacteriological analysis of material samples such as feces. This medium is used to initiate the identification of Enterobacteria in some FDA schemas.

Peptone mixture and the Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. TSI contains three carbohydrates (Dextrose, Sucrose and Lactose) as sources of carbon and energy. When these are fermented the acid production is indicated by the Phenol red indicator, being the color changes yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to Hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Ferric ammonium citrate is a H_2S indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The glucose concentration in the medium is one-tenth the concentration of lactose or sucrose in order to facilitate the detection of organisms that only ferment glucose. The fermentation of glucose produces a small amount of acid in the inclination of the tube, which is rapidly oxidized and the medium remains red or revert to an alkaline pH. On the other hand, the

same acid reaction in the butt of the tube keep the acid pH (yellow) due to the lower oxygen tension. When all glucose is used, organisms able to ferment lactose or glucose will begin to utilize them. In order to enhance the free exchange of air in the slant of the tube, the tube cap must be closed loosely.

The mode of action is similar to Kligler Iron Agar (**Cat. 1042**) which contains two sugars. The addition of 1% Sucrose in TSI Agar allows to differentiate between *Proteus* and *Salmonella*. The fermentation of sucrose by *Proteus* turns the color of the Phenol red indicator in the slant from red to yellow. Dextrose-positive and lactose-negative members of the genus *Salmonella* all cause a reddening of the slant and acidify the depths of the agar tubes.

The European Pharmacopoeia recommends Triple Sugar Iron (TSI) Agar as one of the media used for the confirmation of *Salmonella*. The Eur. Pharmacopoeia recommends transferring separately a few of the suspect colonies to TSI Agar using surface and deep inoculation. The presence of salmonellae is provisionally confirmed if in the deep inoculation, but not in the surface culture, there is a change of color from red to yellow and, usually, a formation of gas, with or without production of Hydrogen sulphide in the agar. Precise confirmation may be carried out by the appropriate biochemical and serological tests. The product passes the test if colonies of the type described do not appear, or if the confirmatory biochemical and serological tests are negative.

Inoculate with sample and incubate at 35 ± 2 for 18 - 72 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 72 hours.

Microorganisms	Growth	Slant	Depth	H_2S	Gas
Escherichia coli ATCC 25922	Good	Yellow	Yellow	-	+
Proteus vulgaris ATCC 13315	Good	Yellow	Yellow	+	+
Salmonella typhimurium ATCC 14028	Good	Red	Yellow	+	+
Shigella flexneri ATCC 12022	Good	Red	Yellow	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

Standard Methods for the Examination of Dairy Products. APHA, 1972.

Food and Drug Administration. Bacteriological Analytical Manual, 1976.

Vanderzant, C. and D.F. Splitt stresser (ed) 1992. Conpendium of methods for the microbiological examination of foods, 3rd ed.

American Public Health Association, Washington D.C.

European Pharmacopoeia. 6th Edition. 2007.

TRIPLE SUGAR IRON AGAR (TSI) ISO 6579

CAT. 1172

For the biochemical confirmation of *Salmonella*

FORMULA IN g/l

Peptone	**20.00**	D-Glucose	**1.00**
Lactose	**10.00**	Ferric Ammonium Citrate	**0.30**
Sucrose	**10.00**	Sodium Thiosulfate	**0.30**
Sodium Chloride	**5.00**	Phenol red	**0.024**
Beef Extract	**3.00**	Bacteriological Agar	**12.00**
Yeast Extract	**3.00**		

Final pH 7.4 ± 0.2 at 25ºC

PREPARATION

Suspend 64.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121ºC for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5 - 2.0 cm. depth. The prepared medium should be stored at 2 - 8°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

TRIPLE SUGAR IRON AGAR (TSI) is recommended by ISO 6579 for the biochemical confirmation of *Salmonella*.

Peptone and the Beef Extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. The three carbohydrates (Glucose, Sucrose and Lactose) are the fermentable carbohydrates providing carbon and energy. When these are fermented the acid production is indicated by the Phenol red indicator, being the color changes yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. The Ferric ammonium citrate is a H_2S indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The glucose concentration in the medium is one-tenth the concentration of lactose or sucrose in order to facilitate the detection of organisms that only ferment glucose. The fermentation of glucose produces a small amount of acid in the

inclination of the tube, which is rapidly oxidized and the medium remains red or revert to an alkaline pH. On the other hand, the same acid reaction in the butt of the tube keep the acid pH (yellow) due to the lower oxygen tension. When all glucose is used, organisms able to ferment lactose or glucose will begin to utilize them. In order to enhance the free exchange of air in the slant of the tube, the tube cap must be closed loosely.

The addition of 1% Sucrose in the TSI Agar allows differentiating between *Proteus* and *Salmonella*. The fermentation of the sucrose by *Proteus* turns the color of the Phenol red indicator in the slant from red to yellow. Dextrose positive, lactose negative members of the genus *Salmonella* all cause a reddening of the slant and acidify the depths of the agar tubes. Inoculate with sample and incubate at 37 ± 1°C for 24 ± 3 hours, according to ISO 6579.

Escherichia coli
ATCC 25922

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1°C and observed after 24 ± 3 hours.

Microorganisms	Growth	Slant	Depth	H_2S	Gas
Escherichia coli ATCC 25922	Good	Yellow	Yellow	-	+
Proteus vulgaris ATCC 13315	Good	Yellow	Yellow	+	+
Salmonella enteritidis ATCC 13076	Good	Red	Yellow	+	+
Shigella flexneri ATCC 12022	Good	Red	Yellow	-	-
Pseudomonas aeruginosa ATCC 9027	Good	Red	Red	-	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 6579 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp.

Standard Methods for the Examination of Dairy Products. APHA, 1972.

Food and Drug Administration. Bacteriological Analytical Manual, 1976.

Vanderzant, C. and D.F. Splitt stresser (ed) 1992. Conpendium of methods for the microbiological examination of foods, 3rd American Public Health Association, Washington D.C.

European Pharmacopoeia. 4th Edition. 2002.

TRYPTICASEIN DEXTROSE MEDIUM

CAT. 1003

For the differentiation of aerobic and anaerobic microorganisms, based on dextrose fermentation and motility

FORMULA IN g/l

Casein Peptone	**20.00**	Bromothymol Blue	**0.01**
Dextrose	**5.00**	Bacteriological Agar	**3.50**
Final pH 7.3 ± 0.2 at 25°C			

PREPARATION

Suspend 28.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes, filling to half capacity. Sterilize in autoclave at 118 - 121°C for 15 minutes. Cool to 45 - 50°C, mix well and dispense into plates. Cool and tighten caps to prevent dehydration. The prepared medium should be stored at 2 - 8°C. Stored at room temperature, the medium can be used several weeks after preparation. The color is green-beige.

The dehydrated medium should be homogeneous, free-flowing and beige to clear green in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN DEXTROSE MEDIUM is a semi-solid medium used to differentiate organisms based on dextrose fermentation and motility.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. When the dextrose is fermented, the acid production is demonstrated by a reaction of the Bromothymol blue pH indicator changing color from purple to yellow (acid). The presence of gas is observed by the

formation of bubbles in the agar or foam on the surface of the tube. Motility is seen by the diffusion away from the line of inoculation (positive) and the medium becomes cloudy. Non-motile organisms only grow along the inoculation line.

Inoculate by stabbing the medium. Reactions are generally complete after incubation at 35 ± 2°C for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Motility	Colony color
Escherichia coli ATCC 25922	Good	+	Yellow
Staphylococcus aureus ATCC 25923	Good	-	Yellow

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Recommended Methods for the Microbiological Examination of Foods APHA Inc., New York. Compendium of Methods for the Microbiological examination of food. 3rd edition APHA 1992.

Standard Methods for the Examination of Dairy Products. 1 1th Edition. APHA., Inc. New York, 1960.

Greenberg and Cooper Can. Med. Assn. J. 83:143. 1960. Wetmore and Gochenour J. Bact. 72:79, 1956.

TRYPTICASEIN GLUCOSE EXTRACT AGAR

CAT. 1041

For the plate count of bacteria in potable water, wastewater, air, milk and dairy products

FORMULA IN g/l

Casein Peptone	**5.00**	D- Glucose	**1.00**
Beef Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 24 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN GLUCOSE EXTRACT AGAR is used for the enumeration of bacteria from potable water, wastewater, air, milk and dairy products by the plate count method. This medium is recommended by APHA for the heterotrophic plate count procedure in testing bottled water.

Casein peptone and Beef extract provide provide nitrogen, vitamins, minerals and amino acids essential for growth. D-Glucose is a source of fermentable carbohydrate as the carbon and energy source. Bacteriological agar is the solidifying agent.

Inoculate plates by appropriate dilution samples. Follow the procedures of dilutions, plating and incubation in the current Standard Methods. Incubate at 35 ± 2°C for 18 - 24 hours. The recovery of microorganisms present in the sample is excellent.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 25923	Good
Enterococcus faecalis ATCC 11700	Good
Escherichia coli ATCC 25922	Good
Salmonella typhimurium ATCC 14028	Good
Pseudomonas aeruginosa ATCC 27853	Good (pigment production)
Bacillus cereus ATCC 11778	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Standard Methods for the Examination of Water and Wastewater. 1 1th Edition APHA Inc. New York, 1960.

Standard Methods for the examination of dairy products, 1 6th ed. American Public Health Association; Washington D.C. Marshall, R.T. (1993).

Standard Methods for the examination of water and wastewater 1 8th ed. American Public Health Association, Washington D.C. 1992.

TRYPTICASEIN SOY AGAR (TSA) EUROPEAN PHARMACOPOEIA

CAT. 1068

For general bacteriology methods and the determination of hemolytic reactions

FORMULA IN g/l

Pancreatic Digest of Casein	**15.00**	Sodium Chloride	**5.00**
Papaic Digest of Soya Bean	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Large quantities may require a longer sterilization time, but the temperature should not be increased. To prepare blood plates for hemolysis studies, add 5 - 10% of defibrinated sterile blood to the sterile medium, previously cooled to 45°C. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15ºC. The color with the blood added is cherry red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN SOY AGAR (TSA) is a general purpose medium very rich in nutrients for general use in microbiological laboratories and for the cultivation and isolation of fastidious or non fastidious microorganisms, or for the maintenance of stock culture. It supports the abundant growth of fastidious organisms such as pneumococci, streptococci, *Neisseria*, etc. from clinical samples.

Containing two peptones as rich nitrogen sources, obtained by the enzymatic hydrolysis of Casein and Soy proteins, this medium supports the growth of a great variety of microorganisms, including fastidious aerobes and anaerobes. Soy peptone also contains natural sugars which promote bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Some of the microorganisms that grow on this medium are the following: *Streptococcus, Neisseria, Brucella, Corynebacteria, Listeria, Pasteurella, Vibrio, Haemophilus vaginalis, Candida*, etc.

Since it lacks carbohydrates it is very useful in the study of hemolytic reactions and also in the preparation of chocolate agar. If desired, antibiotics can easily be incorporated as well as other supplements or inhibitory agents. Inoculate and incubate at 30 - 35ºC and observe after 18 - 24 hours.

The European Pharmacopoeia recommends this medium in the Paragraph 2.6.12 "Microbiological examination of non-sterile products: Microbial enumeration test" for the preparation of test strain.

Inoculate and incubate at 30 - 35ºC for 18 - 24 hours for *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633. For total aerobic microbial count (growth promotion) of *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 6633, inoculate ≤100 cfu/ml at 30 - 35ºC for ≤ 3 days, of and for *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 inoculate ≤100 cfu/ml at 30 - 35 ºC ≤ 5 days. For total aerobic microbial count (Suitability of counting method in the presence of the product) inoculate ≤100 cfu/ml of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* at 30 - 35ºC for ≤ 3 days, and for *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 inoculate ≤100 cfu/ml at 30 - 35 ºC ≤ 5 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with the blood added, from type cultures after incubation at a temperature of 30 - 35ºC and observed after 18 - 24 hours.

Microorganisms	Growth	Growth with 5% sheep's Blood	Hemolysis	Inoculum Cfu/ml	Recovery Rate %
Staphylococcus aureus ATCC 25923	Good	Good	Beta	10^2-10^3	≥70
**Staphylococcus aureus* ATCC 6538	Good			≤100	
Staphylococcus epidermidis ATCC 12228	Good	Good		10^2-10^3	≥70
Streptococcus pneumoniae ATCC 6303	Good	Good	Alpha	10^2-10^3	≥70
Streptococcus pyogenes ATCC 19615	Good	Good	Beta	10^2-10^3	≥70
**Pseudomonas aeruginosa* ATCC 9027	Good			≤100	
**Bacillus subtilis* ATCC 6633	Good			≤100	
***Candida albicans* ATCC 10231	Good			≤100	
***Aspergillus brasiliensis* ATCC 16404	Good			≤100	

*According to European Pharmacopeia. Total recount of aerobics ≤100 cfu/ml to incubate at 30 - 35ºC for ≤ 3 days and preparation of test strains for 30 - 35ºC during 18 - 24 hours.

** Total recount of aerobics ≤100 cfu/ml to incubate at 30 - 35ºC for ≤ 5 days and preparation of test strains 30 - 35ºC during 18 - 24 hours

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Curry, A.S., G. Joyce and G.N. Mcerven, Jr. 1993 CTFA Microbiology guideline. The Cosmetic To iletry and Fragance Association, Inc. Washington D.C.

European Pharmacopoeia. 7.0

TRYPTICASEIN SOY AGAR (T.S.A) Nº2

CAT. 1561

For the isolation, cultivation and detection of hemolytic activity of fastidious microorganisms

FORMULA IN g/l

Tryptone H	**15.00**	Soy Peptone	**5.00**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Allow to stand for 5 minutes and mix well until a uniform suspension is obtained. Heat with gentle agitation and boil for one minute. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45 - 50°C, and add 5 - 10% sterile defibrinated blood, homogenize and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 2 - 8°C. The color without blood is amber, slightly opalescent. The color with blood is cherry red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN SOY AGAR BASE (T.S.A) Nº2 is an improved formulation of the original TSA Agar, to be used with animal blood supplements with 5 or 10% sheep blood. It is used for the isolation, cultivation and detection of hemolytic reactions of fastidious microorganisms. These hemolytic reactions are important to differentiate bacteria from clinical samples, especially species of *Streptococcus*.

Trypticasein Soy Agar Nº2, supplemented with 5% sheep blood, offers clear and visible hemolytic reactions with group A streptococci (*Streptococcus pyogenes*).

Tryptone H enhances hemolysin production. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. Blood is an additional source that provides growth factors for the microorganisms and is the basis for determining haemolytic reactions

Inoculate and incubate the plates at a temperature of 35 ± 2°C in atmosphere with 5 - 10% CO_2 and observed after 18 - 24 hours. Hemolytic streptococci can be seen as translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2 - 4 mm) colonies, surrounded by a hemolysis zone. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with 5% defibrinated sheep blood added, after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2, and observed after 18 - 24 hours.

Microorganisms	Growth	Hemolysis
Neisseria meningitidis ATCC 13090	Good	–
Staphylococcus aureus ATCC 25923	Good	Beta
Staphylococcus epidermidis ATCC 12228	Good	
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

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TRYPTICASEIN SOY BLOOD AGAR BASE

CAT. 1189

For the cultivation of fastidious microorganisms and detection of hemolytic activity of fastidious microorganisms

FORMULA IN g/l

Pancreatic Digest of Casein	**14.50**	Growth Factors	**1.50**
Soy Peptone	**5.00**	Bacteriological Agar	**14.00**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45 - 50°C and add 50 ml of sterile defibrinated blood. Homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood. The prepared medium should be stored at 8 - 15ºC. The color without blood is amber, slightly opalescent. The prepared medium with blood is red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN SOY BLOOD AGAR BASE is used for the isolation, cultivation and detection of hemolytic reaction of fastidious microorganisms.

It is suitable for isolating and cultivating a wide range of microorganisms with difficult growth characteristics. Upon adding blood, it can be utilized for determining hemolytic reactions. Pancreatic digest of casein and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The addition of blood provides extra growth factors for fastidious microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the type of blood or base medium used. For example, defibrinated sheep blood allows the recovery of *Thermophilus* species and gives best results for Group A streptococci.

Inoculate and incubate at 35 ± 2ºC for 18 - 24 hours.

Results:

1. Alpha-hemolysis: greenish discoloration of medium
2. Beta-hemolysis: clear zone surrounding colony
3. Gamma-hemolysis: no change

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with 5% of defibrinated sheep blood, after incubation at temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Hemolysis
Staphylococcus aureus ATCC 25923	Good	Beta
Streptococcus pneumoniae ATCC 6305	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Alpha
Escherichia coli ATCC 25922	Good	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Altwegg. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Snavely and BrahierA. J. Clin. Path. 33:511. 1 960.Hosty, Freeman and Irwin, Public, Health. Lab, 1953.

TRYPTICASEIN SOY BROTH (TSB) EUROPEAN PHARMACOPOEIA

CAT. 1224

For general laboratory use and to cultivate fastidious microorganisms

FORMULA IN g/l

Pancreatic Digest of Casein	**17.00**	Glucose Monohydrate	**2.50**
Sodium Chloride	**5.00**	Dipotassium Phosphate	**2.50**
Papaic Digest of Soy Bean	**3.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Larger quantities may require a longer sterilization time, but the temperature should not be increased. The prepared medium should be stored at 2 - 8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN SOY BROTH is a medium very rich in nutrients for general use in microbiological laboratories. It supports the abundant growth of fastidious organisms such as pneumococci, streptococci, Neisseriae, etc.

The medium is used frequently in many procedures of diagnostic research or microbiology. For example, it is used for the isolation and sensitivity testing of all types of pathogens, and for the production of antigens for agglutination and serological tests.

Containing two peptones as rich nitrogen sources, obtained by the enzymatic hydrolysis of Casein and Soy proteins, this medium supports the growth of a great variety of microorganisms, including fastidious aerobes and anaerobes. Soy peptone also contains natural sugars which promote bacterial growth. Glucose is a carbohydrate and carbon source. Sodium chloride supplies essential electrolytes for transport and osmotic balance, and Dipotassium phosphate is a buffering agent.

The European Pharmacopoeia recommends this medium in Paragraph 2.6.13 "Microbiological examination of non-Sterile products" for the test of specified microorganisms, as a pre-enrichment medium for sample preparations for testing of products. Inoculate and incubate at 30 - 35ºC and observe after 18 - 24 hours.

In Paragraph 2.6.12 "Microbiological examination of non-sterile products: Microbial enumeration test", it is indicated to inoculate and incubate at 30 - 35ºC ≤ 3 days for the total count of aerobics ≤100 cfu/ml.

For a growth promotion test of aerobes, anaerobes and fungi, inoculate the medium with a small number of microorganisms: no more than 100 cfu of *Aspergillus brasiliensis* ATCC 16404, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231. Incubate for no more than 3 days in the case of bacteria, and no more than 5 days in the case of fungi. If desired, antibiotics can easily be incorporated as well as other supplements or inhibitory agents. Sterility Test: Incubate portions of the media for 14 days. If no growth of microorganisms occurs, the product is sterile.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 35ºC and observed after 18 - 24 hours.

Microorganisms	Growth	Inoculum cfu	Recovery
Brucella abortus ATCC 4315	Good	10^2-10^3	≥50
**Staphylococcus aureus* ATCC 6538	Good	≤100	≥70
Escherichia coli ATCC 25922	Good	10^2-10^3	≥70
Escherichia coli ATCC 8739	Good	10^2-10^3	≥70
Enterobacter aerogenes ATCC 13048	Good	10^2-10^3	≥70
***Candida albicans* ATCC 10231	Good	≤100	≥70
Streptococcus pyogenes ATCC 19615	Good	10^2-10^3	≥70
Streptococcus pneumoniae ATCC 6303	Good	10^2-10^3	≥70
**Pseudomonas aeruginosa* ATCC 9027	Good	≤100	≥70
Salmonella typhimurium ATCC 14028	Good	10^2-10^3	≥70
* *Bacillus subtilis* ATCC 6633	Good	≤100	≥70
***Aspergillus brasiliensis* ATCC 16404	Good	≤100	≥70

*According to European Pharmacopeia. Total recount of aerobiosis ≤100 cfu/ml. Incubate at 30 - 35ºC for ≤ 3 days.

** According to European Pharmacopeia. Total recount of yeast and molds ≤100 cfu/ml. Incubate at 30 - 35ºC for ≤ 5 days.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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European Pharmacopoeia. 7.0

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Muey and Edward. Proc. Soc. Exper. Biol. and Med., 97:550. 1958. Steward and Kelly. J. Bacteriol., 77:101. 1959.

MacFaddin, J.D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 797. vol. 1. Williams & Wilkins, Baltimore, MD.

TRYPTONE BILE SALTS AGAR ISO 9308-1

CAT. 1013

For the detection and enumeration of *E. coli* and other coliforms in water, by the membrane filtration technique

FORMULA IN g/l

Tryptone	**20.00**	Bacteriological Agar	**15.00**
Bile Salts	**1.50**		

Final pH 7.2 ± 0.1 at 25ºC

PREPARATION

Suspend 36.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTONE BILE SALTS AGAR is used in the quick test detection and enumeration of *Escherichia coli* and others coliforms in foods and waters by the membrane filtration technique as per the Standard ISO 9308-1.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bile salts are inhibitors to Gram-positive organisms and suppress coliform bacteria. Bacteriological agar is the solidifying agent.

The fecal presence and level is an important factor in the evaluation of a water mass and the infection risk which it poses to human health. The analysis of water samples to detect *E. coli*, which usually is found in the intestinal tract of humans and other warm-blooded animals, provides an indication of this type of contamination.

This medium is recommended by the ISO 9308-1 for the quick test method which is based on the fact that 99% of *Escherichia* strains produce indole from tryptophan at 44ºC. The filtration membrane, after incubation at 36 ± 2ºC during 4 - 5 hours in Tryptone Soy Agar (**Cat. 1138**), is transferred to Tryptone Bile Salts Agar and incubated at 44 ± 0.5°C during 19 - 20 hours.

If desired, the two agar media can be combined in a petri dish in a double layer. In this case, it is convenient to place the membrane over a recently prepared double-layered plate of TSA (**Cat. 1138**) and TBA, incubate at 36 ± 2ºC during 4 - 5 hours, and incubate again at 44 ± 0.5ºC during 19 - 20 hours.

The indole test is carried out: all indole-positive strains which give red colonies when stained with indole reagent are considered *E. coli*.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures after incubation at a temperature of 44 ± 0.5°C and observed after 19 - 20 hours.

Microorganisms	Growth	Colony Color	Indole
Escherichia coli ATCC 25922	Good	Red	+
Klebsiella pneumoniae ATCC 13833	Null		

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

International Standard ISO 9308-1 Water quality. Detection and enumeration of Escherichia coli and coliform bacteria. Part 1 Membrane filtration method

Sahidi S.A. and Ferguson A.R. (1971) Appl. Microbiol.,21 500-506. Harmon S.M., Kauttar D.A. and Peeler J.T.(1 971) Appl. Microbiol. 21. 922-927. Hauschild A.H.W and Hilsheimar R. (1973) Appl. Microbiol.27. 78-82.

TRYPTONE SOY AGAR ISO 9308-1

CAT. 1138

For the detection and enumeration of *E. coli* in water

FORMULA IN g/l

Casein Triptic Digest	**15.00**	Sodium chloride	**5.00**
Soy Peptone	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.2 ± 0.1 at 25ºC			

PREPARATION

Suspend 40 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTONE SOY AGAR is used for the quick and standard test for the detection and count of *E. coli* and other coliforms by the membrane - filter technique as directed by the ISO 9308-1.

It is also a general purpose solid medium, particularly useful for the isolation, sensitivity testing and determination of hemolysis with fastidious microorganisms as it does not contain any sugars. Another use of the Tryptone Soy Agar is for the oxidase test according to the traditional method as described in ISO 9308-1.

Casein and Soy peptone Casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride maintains the osmotic balance and Bacteriological agar is the solidifying agent.

According to ISO 9308-1, the filtration membrane is placed in the Tryptone Soy Agar and incubated at 36 ± 2°C during 4 - 5 hours. The membrane is then transferred to Tryptone Bile Salts Agar (**Cat. 1013**) and incubated at 44 ± 0.5°C during 19 - 20 hours.

If desired, both media can be combinated into one double-layer Petri dish. In this case, it is advisable to place the membrane on a plate freshly prepared with a double layer consisting of Tryptone Soy Agar and TBA and incubated at 36 ± 2 º C during 4 - 5 hours and then incubated at 44 ± 0.5 º C for 19 - 20 hours.

All indole positive strains which give red colonies when stained with indole reagent are considered as *E. coli*.

For the preparation of blood-plates for hemolysis studies, add 5 - 10% sterile defibrinated blood to sterilized and cooled to 45°C medium.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 36 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Klebsiella pneumoniae ATCC 13833	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 9308-1 Water quality. Detection and enumeration of Escherichia coli and coliform bacteria. Part 1 Membrane filtration method

Regulation water quality- Detection and count of Escherichia coli and coliform bacteria. Anon. 1987 J. Food Microbiol., 5: 291 -296.

TRYPTOPHAN CULTURE BROTH ISO 9308-1

CAT. 1237

For the detection of *Escherichia coli* and other coliforms by indole production

FORMULA IN g/l

Tryptone	**10.00**	L-Tryptophan	**1.00**
Sodium Chloride	**5.00**		
Final pH 7.5 ± 0.1 at 25ºC			

PREPARATION

Suspend 16 grams of the medium in one liter of distilled water .Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute in test tubes, 3 ml in each. Close the tubes with cotton or with a plastic or metallic cap and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTOPHAN CULTURE BROTH is used for the quick and standard test for the detection of *E. coli* and other coliforms by indole production. Indole formation depends exclusively on the development of bacteria producing enzymes called tryptophanases, which oxidize the L-tryptophan essential amino acid producing indole, skatole (methyl indole) and indole acetate. Several bacteria genera have species that are indole positive, particularly *Proteus, Escherichia, Edwardsiella, Flavobacterium, Aeromonas, Plesiomonas, Bacillus*, etc.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. No fermentable carbohydrates in the medium allow for the good synthesis of trypthophanase and therefore indole production.

The norm ISO 9308-1 recommends to inoculate the charachteristics colonies isolated from TTC Chapman Agar (**Cat. 1076**) incubated at 36 ± 2ºC for 21 ± 3 hours and incubate at 44 ± 0.5°C for 21 ± 3 hours. Incubation at 44°C inhibits growth of many indole-positive genera except *E. coli*.

Indole production is determined by adding a few drops of Kovac's Reagent. (**Cat. 5205**) A positive test is indicated by the development of a red color in the reagent layer.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures after incubation at a temperature of 44 ± 0.5°C and observed after 21 ± 3 hours, with the addition of Kovac's Reagent (**Cat. 5205**) for Indole production.

Microorganisms	Indole
Escherichia coli ATCC 25922	+
Klebsiella pneumoniae ATCC 13833	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 9308-1. Water quality-Detection and enumeration of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method

TRYPTOSE AGAR

CAT. 1047

For the cultivation of a wide variety of fastidious microorganisms, particularly *Brucella*

FORMULA IN g/l

Tryptose	**20.00**	Thiamine Chlorhydrate	**0.005**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Dextrose	**1.00**		
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 41 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTOSE AGAR with thiamine is a general purpose non selective medium recommended for the cultivation of *Brucella spp.*

Sanders and Huddleson demonstrated that the addition of Dextrose and Thiamine chlorohydrate to the medium stimulates the growth of some species of *Brucella*. The Tryptose Agar with Thiamine is also recommended for the cultivation of pathogen microorganisms without enrichment, for streptococci, pneumococci, meningococci and other fastidious bacteria.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose is the fermentable carbohydrate providing carbon and energy. Thiamine is a growth factor. Bacteriological agar is the solidifying agent.

The high productivity of the Tryptose Agar used for the cultivation and isolation of *Brucella* confirms its value for the primary cultivation of *Brucella*, as other fastidious organisms.

Inoculate and incubate at 35 ± 2ºC under 5 -10% of CO_2 for 40 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC under 5 - 10% of CO_2, and observed after 40 - 48 hours.

Microorganisms	Growth
Brucella abortus ATCC 4315	Good
Brucella melitensis ATCC 4309	Good
Streptococcus pyogenes ATCC 19615	Good
Streptococcus pneumoniae ATCC 6306	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Jones, L.M., Wundt, W.; International Committee on Nomenclature of Bacteria, Sub-committee on the Taxonomy of Brucella. – Int. J. Syst. Bact., 21; 126-128 (1971)

Picket, M.J., Nelson, E.L., a. Liberman, J.D.; Specification within ghe Genus Brucella. II. Evaluation of Differential Dye, Biochemical, and Serological Tests. – J. Bact, 66; 210-219 (1953)

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Silverman, S.J., a. Elberg, S.S.; The antigenic relationships of native antigens of species of Brucella. – J. Immunol., 65; 163-174 (1950)

TRYPTOSE BROTH

CAT. 1322

For the cultivation of a wide range of fastidious microorganisms, particularly *Brucella* from clinical samples

FORMULA IN g/l

Tryptose	**20.00**	Dextrose	**1.00**
Sodium Chloride	**5.00**	Thiamine Chlorhydrate	**0.005**
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 26 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTOSE BROTH is used for the cultivation of *Brucella* and other fastidious microorganisms from clinical samples.

The media with Tryptose, and in this case with thiamine, are recommended for the isolation, cultivation and differentiation of *Brucella*. Sanders and Huddleson demonstrated that the addition of Dextrose and Thiamine Chlorhydrate to the media stimulates the growth of some species of *Brucella*.

Tryptose is a source of nitrogen, vitamins and amino acids. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance, and Thiamine Chlorhydrate is a growth factor.

Tryptose Broth with Thiamine is also recommended for the cultivation of pathogen microorganisms for *Streptococcus, Pneumococcus, Meningococcus* and other fastidious bacteria.

Inoculate and incubate at 35 ± 2°C, under 5 - 10% CO_2, during 40 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2, and observed after 40 - 48 hours.

Microorganisms	Growth
Brucella abortus ATCC 4315	Good
Brucella melitensis ATCC 4309	Good
Streptococcus pyogenes ATCC 19615	Good
Streptococcus pneumoniae ATCC 6306	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Jones, L.M., a, Wundt, W.; International Committee on Nomenclature of Bacteria, Subcommittee on the Taxonomy of Brucella. – Int. J. Syst. Bact., 21; 126-128 (1971)

Picket, M.J., Nelson, E.L., a. Liberman, J.D.; Specification within ghe Genus Brucella. II. Evaluation of Differential Dye, Biochemical, and Serological Tests. – J. Bact, 66; 210-219 (1953)

Schindler, R.: Untersuchungen A1/4ber die Differenzierung von Brucellatypen. – Zbl. Bakt., I. Orig., 164; 93-95 (1955)

Silverman, S.J., a. Elberg, S.S.; The antigenic relationships of native antigens of species of Brucella. – J. Immunol., 65; 163-174 (1950)

TRYPTOSE PHOSPHATE BROTH

CAT. 1243

For the cultivation of fastidious microorganisms

FORMULA IN g/l

Casein Peptone	**13.00**	Disodium Phosphate	**2.50**
Proteose Peptone N°3	**7.00**	Dextrose	**2.00**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 29.5 grams of the medium in one liter of distilled water .Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TRYPTOSE PHOSPHATE BROTH is recommended for the cultivation of fastidious and pathogenic microorganisms.

Peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The buffering capacity is provided by the Disodium phosphate.

The addition of 0.1 - 0.2 % agar to Tryptose Phosphate Broth delays the dispersion of CO_2 and diffusion of O_2 and facilitates the anaerobic growth. The low concentration of agar provides conditions for both aerobic growth in the upper zone and microaerophilic and anaerobic growth in the lower zone.

Inoculate and incubate at 35 ± 2°C for 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed alter 18 - 48 hours.

Microorganisms	Growth
Nelsselra meningitidis ATCC 13090	Good
Staphylococcus epidermidis ATCC 12228	Good
Streptococcus pneumoniae A1CC 6305	Good
Streptococcus pyogenes A1CC 19615	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Gray, M.L., Stafseht, H.J., a. Thorp, F.JR.: The use of potassium tellurite, sodium azide and acetic acid in a selective medium for the isolation of Listeria monocytogenes. - J. Bact., 59: 443-444 (1950)

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T.S.A. AGAR Nº 2 MODIFIED

CAT. 1198

For the isolation, cultivation and detection of hemolytic activity of fastidious microorganisms

FORMULA IN g/l

Tryptone	**15.00**	Growth Factors	**4.50**
Soy Peptone	**5.00**	Bacteriological agar	**15.00**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 44.5 grams of the medium in one liter of distilled water. Mix well until a uniform suspension is obtained. Heat with gentle agitation and boil for one minute. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45 - 50°C, and aseptically add 7% sterile defibrinated sheep blood. Homogenize and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium without blood is amber, slightly opalescent. The color of the prepared medium with blood is red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

T.S.A. AGAR Nº2 MODIFIED has been especially formulated for the production of blood plates for the isolation and cultivation of fastidious microorganisms from clinical samples, and produces enhanced hemolysis due to the special growth factors included in the formula.

It is a medium which is very rich in nutrients and is recommended for general use in microbiological laboratories. It supports the abundant growth of fastidious organisms such as pneumococci, streptococci, *Neisseria*, etc.

Containing two peptones as rich nitrogen sources, this medium supports the growth of a great variety of microorganisms, including fastidious aerobes and anaerobes. Sodium chloride maintains the osmotic balance and the Bacteriological agar is the solidifying agent.

A list of microorganisms that grow on this medium are the following: *Streptococcus, Neisseria, Brucella, Corynebacteria, Listeria, Pasteurella, Vibrio, Haemophilus vaginalis, Candida*, etc.

Since it lacks carbohydrates, it is very useful in the study of hemolytic reactions and also in the preparation of chocolate agar. If desired, antibiotics can easily be incorporated as well as other supplements or inhibitory agents.

Incubate at 35 ± 2ºC and observe after 18 - 24 hours. Pneumococci frequently appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis. Hemolytic streptococci can be translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2 - 4 mm) colonies, surrounded by a hemolysis zone. Staphylococci are seen as opaque, white to gold-yellow colonies with or without zones of beta hemolysis. *Listeria* produces small zones of beta hemolysis. They can be distinguished by their rod shape in stains, and by motility at room temperature.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with the blood added, from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth with 7% sheep blood	Hemolysis
Neisseria meningitidis ATCC 13090	Good	—
Staphylococcus aureus ATCC 25923	Good	Beta
Staphylococcus epidermidis ATCC 12228	Good	—
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.

Facklam and Washington. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.),

T.S.C. AGAR BASE (TRYPTOSE SULFITE CYCLOSERINE) ISO 7937

CAT. 1029

For the detection and enumeration of *Clostridium perfringens*

FORMULA IN g/l

Caseine Peptone	**15.00**	Disodium Disulfite (Anhydrous)	**1.00**
Soy Peptone	**5.00**	Ferric Ammonium Citrate	**1.00**
Yeast Extract	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.6 ± 0.2 at 25ºC			

PREPARATION

Suspend 42 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 44 - 47ºC and aseptically add two vials of the selective supplement Clostridium perfringens (**Cat. 6020**) reconstituted in 5 ml of sterile distilled water. If desired, 25 ml of Egg Yolk Emulsion (**Cat. 5152**) can be added (Not indicated in ISO 7937). Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Clostridium perfringens supplement (Cat. 6020)

(1 vial for 500 ml of the medium)

D-Cycloserine ..... 200 mg

USES

T.S.C. AGAR BASE is a recommended medium in ISO 7937 for presumptive identification and enumeration of *Clostridium perfringens* by count technique. It is a nutrient medium for the cultivation and detection of *Clostridium perfringens* based on lecithinase detection, if the Egg Yolk Emulsion (5152) is added, and in hydrogen sulfide gas production. It is also useful for the recovery of stressed cultures.

The superior nutrient base provides optimal conditions for the development of Clostridia. Tryptose and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group essential for bacterial growth. Ferric Ammonium Citrate and Disodium disulfite are H_2S indicators. Bacteriological agar is the solidifying agent. Egg yolk Emulsion is added for the lecithin, utilizing the reduction capacity of certain *Clostridium perfringens* strains to produce an opaque area in the colony surroundings. Note that this is not recognized as a universal character for all *C. perfringens*. Cycloserine inhibits the accompanying bacterial flora and causes the colonies, which develop, to remain smaller. It also reduces, thus, disturbs the blackening around the *C. perfringens* colonies. Inoculate with sample and incubate anaerobically at 37°C for 20 ± 2 hours.

Colonies producing hydrogen sulfide are characterized by a blackening due to the reaction with the Ferric salt. The degradation of the egg yolk lecithin produces insoluble products which accumulate around the colonies, forming a white precipitate. After 24 hours incubation, all black colonies, lecithinase positive as well as the lecithinase negative ones, have to be considered as positive presumptive *C. perfringens* and the corresponding confirmation tests have to be made.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the respective supplements added, after incubation at a temperature of 37°C and observed after 20 ± 2 hours under anaerobic conditions.

Microorganisms	Growth	Colony Color
Clostridium perfringens ATCC 13124	Good	Black with opaque halo

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Sahidi S.A. and Ferguson A.R. (1971) Appl. Microbiol, 21 500-506. Harmon S.M., Kauttar D.A. and Peeler J.T. (1971) Appl. Microbiol. 21 922-927. Hauschild A.H.W. and Hilsheimar R. (1973) Appl. Microbiol. 27. 78-82.

International standard ISO 7937 Microbiology of food and animal feeding stuffs-Horizontal method for enumeration of Clostridium perfringens –colony count technique

TSN AGAR

CAT. 1075

For the selective isolation of *Clostridium perfringens* from foods and other material

FORMULA IN g/l

Casein Peptone	**15.00**	Neomycin Sulfate	**0.05**
Yeast Extract	**10.00**	Polymyxin B Sulfate	**0.02**
Sodium Sulfite	**1.00**	Bacteriological Agar	**13.50**
Ferric Citrate	**0.50**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TSN AGAR (TRYPTONE SULFITE NEOMYCIN) is a selective medium that can be used in tubes or plates for the identification and enumeration of *Clostridium perfringens* in foods and other materials, especially from mixed contaminating flora.

The Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Ferric citrate and Sodium sulfite are H_2S indicators. *C. perfringens* reduces the sulfite to sulfide which reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies. Bacteriological agar is the solidifying agent. Polymyxin and Neomycin sulfates inhibit the growth of the majority of Enterobacteria and *Clostridium bifermentans*. The incubation at a temperature of 46°C allows specific and quantitative results.

Inoculate medium with sample and incubate at 46 ± 1°C for 18 - 24 hours. Use an anaerobic jar for incubation in a H_2/CO_2 atmosphere if possible. For aerobic incubation in tubes, cover the tubes with a layer of sterile medium. Read within half an hour after taking the plates out of the jars and observe for black colonies which can lose their color by oxidation in air after this time period.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after anaerobic incubation at a temperature of 46 ± 1°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Clostridium perfringens ATCC 10543	Good	Black
Clostridium sporogenes ATCC 13124	Good	Black
Escherichia coli ATCC 25922	Inhibited	—
Pseudomonas aeruginosa ATCC 27853	Inhibited	—

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Angelotti, Nall, Foter y Lewis. Applied Microbiol. 10: 193. 1962. Mossel. J.SCI. Agr. 10: 662. 1959. Mossel de Bruin Van Diepen, Vendrig y Zoutwelle J. Applied Bact, 19: 142. 1956.

TSYEA AGAR (TRYPTONE SOY YEAST EXTRACT AGAR) ISO 11290-1

CAT. 1398

For the confirmation of *Listeria spp.*

FORMULA IN g/l

Tryptone	**17.00**	Dipotassium Phosphate	**2.50**
Yeast Extract	**6.00**	Glucose Monohydrate	**2.50**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Soy Peptone	**3.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 51 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TSYE AGAR (Tryptone Soy Yeast Extract) ISO 11290-1 is a general purpose medium which supports the growth of a wide variety of microorganisms.

The formula conforms to ISO 11290-1 and is used for the confirmation of *Listeria monocytogenes* colonies and to subculture suspected *Listeria* colonies.

Tryptone, Yeast extract and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Glucose is the fermentable carbohydrate providing carbon and energy. Dipotassium phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

This medium is used to select colonies for the confirmation of *Listeria spp.* After incubation in Listeria Oxford Agar (**Cat. 1133**) and Listeria Palcam Agar (**Cat. 1141**) , take 5 suspected *Listeria spp.* colonies, and inoculate them in TSYEA Agar.

Incubate at 35 - 37ºC during 18 - 24 hours or until growth is satisfactory. The rest of confirmatory tests should be carried out from pure cultures in TSYEA Agar.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 - 37º C and observed after 18 - 24 hours.

Microorganisms	Growth
Listeria monocytogenes ATCC 19111	Good
Listeria innocua ATCC 33090	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 11290-1.Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method

TSYEB BROTH (TRYPTONE SOY YEAST EXTRACT BROTH) ISO 11290-1

CAT. 1339

For the confirmation of *Listeria monocytogenes*

FORMULA IN g/l

Casein Peptone	**17.00**	Soy Peptone	**3.00**
Yeast Extract	**6.00**	Dipotassium Phosphate	**2.50**
Sodium Chloride	**5.00**	D-Glucose	**2.50**
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 36 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TSYEB BROTH ISO 11290-1 (Tryptone Soy Yeast Extract Broth) is a general purpose medium that supports the growth of a wide variety of microorganisms. This medium is formulated according to ISO 11290-1 and is used for the confirmation of *Listeria monocytogenes* colonies and for subcultivation of suspected *Listeria* colonies.

Casein peptone and Soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Glucose is the fermentable carbohydrate providing carbon and energy. Dipotassium phosphate acts as a buffer system. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

To test the use of carbohydrates inoculate all carbohydrate broths from one culture of TSYEB.

Inoculate and incubate at 35 ± 2°C during 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Staphylococcus aureus ATCC 6538	Good
Listeria monocytogenes ATCC 19117	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

UNE-EN-ISO 11290-1 Microbiology of food and animal feeding stuff. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part. 1: Detection method. Ammendment 1: Modification of the isolation media and the haemolysis test and inclusion of precision data.

TTC CHAPMAN AGAR (Lactose Agar TTC with Tergitol 7) ISO 9308-1

CAT. 1076

For the detection and enumeration of *E. coli* and coliforms in drinking water by membrane filtration technique

FORMULA IN g/l

Lactose	**20.00**	Sodium Heptadecyl Sulfate (Tergitol 7)	**0.10**
Meat Peptone	**10.00**	Bromothymol Blue	**0.050**
Yeast Extract	**6.00**	Bacteriological Agar	**15.00**
Beef Extract	**5.00**		
Final pH 7.2 ± 0.1 at 25ºC			

PREPARATION

Suspend 56.15 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add 2.5 ml of TTC 1% Supplement (**Cat. 6030**) previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. DO NOT OVERHEAT THE MEDIUM. The prepared medium should be stored at 8 - 15ºC. The color is green.

The dehydrated medium should be homogeneous, free-flowing and blue-green in color. If there are any physical changes, discard the medium.

TTC 1% SUPPLEMENT (CAT. 6030)
(1 Vial reconstructed in 5 ml of distilled water)

Triphenyltetrazolium Chloride .....50 mg

USES

TTC CHAPMAN AGAR (Lactose Agar TTC with Tergitol 7) is a selective and differential medium prepared according to ISO 9308-1, is used for the presumptive control of *E. coli* and coliforms in waters for human consumption by the membrane - filtration technique.

Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Lactose is a fermentable carbohydrate providing carbon and energy. Sodium heptadecylsulfate (Tergitol 7) and TTC inhibit most Gram-positive bacteria. Bromothymol blue is a pH indicator. Bacteriological agar is the solidifying agent.

The norm ISO 9308-1 recommends:

Two samples of water must be taken on two membranes and incubated in TTC Chapman Agar at 36 ± 2°C and 44 ± 4°C respectively. After 21 ± 3 hours of incubation:

- *E. coli* and *Citrobacter spp.* present yellow colonies with orange-colored center.

- *Enterobacter spp.* form red colored colonies and dark yellow with orange-colored center. The medium is yellow.

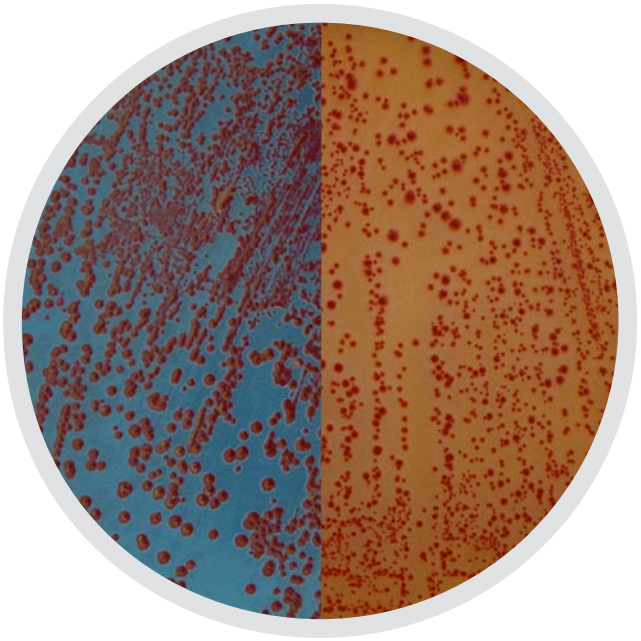
- *Klebsiella spp.* form red colored or yellow, but without center. The medium is yellow.

- Lactose non fermentative bacteria grow with purple colonies and change the medium to blue.

- *Klebsiella* and *Enterobacter* species can also produce yellow-green colonies.

The results will always refer to counts per 100 ml of sample (considering if it has been necessary to make dilutions). The colonies that grow at 36 ± 2°C will be considered as fecal coliforms and the colonies that grow at 44 ± 4°C are considered as *E. coli*.

Confirmation of the colonies in EMB Agar (**Cat. 1039**), Kligler Iron Agar (**Cat. 1042**), etc. is necessary for the verification of the biochemical characteristics. The Indole and Oxidase tests are carried out in the following media respectively: Tryptophan Culture Broth (**Cat. 1237**) and Tryptone Soy Agar (**Cat. 1138**).



Non fermenting species — *Escherichia coli* ATCC 25922

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the Supplement added, after incubation at a temperature of 36 ± 2°C and 44 ± 4°C and observed after 21 ± 3 hours.

Microorganisms	Growth 44ºC	Growth 36ºC	Colony Color
Escherichia coli ATCC 25922	Good	Good	Yellow with orange center
Citrobacter spp.	Null	Good	Yellow with orange center

Microorganisms	Growth 44°C	Growth 36°C	Colony Color
Klebsiella spp.	Null	Good	Red to yellow
Enterobacter aerogenes ATCC 13048	Null	Good	Red to dark yellow with orange center
Non fermenting species	Null	Good	Light violet

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Chapman G.H. 1946. A single culture medium for selective isolation of plasma coagulating staphylococci and for improved testing of chromogenesis (J. Bacteriol. 51: 409-410).

Tittsler R.P. and L.A. Sandholzer. 1936. The Use of Semi-Solid Agar for the detection of bacteria motility. (J. Bacteriol 31: 575-580)

ISO 9308-1.Water quality. Detection and enumeration of Escherichia coli and coli-form bacteria.PART.1. Membrane filtration method

UNIVERSAL BEER AGAR (UBA)

CAT. 1562

For the cultivation of important microorganisms in the beer industry

FORMULA IN g/l

Peptonized Milk	**15.00**	Sodium Chloride	**0.01**
Yeast Extract	**10.00**	Manganese Sulfate	**0.01**
Dextrose	**10.00**	Magnesium Sulfate	**0.01**
Tomato Juice	**7.00**	Ferrous Sulfate	**0.01**
Monopotassium Phosphate	**0.50**	Bacteriological Agar	**12.00**
Dipotassium Phosphate	**0.50**		

Final pH 6.3 ± 0.2 at 25ºC

PREPARATION

Suspend 55 grams of the medium in 750 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Add 250 ml of beer without gas when the medium is still hot. Mix with agitation. Dispense into appropriate containers and sterilize in autoclave at 121°C for 10 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15ºC. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

UNIVERSAL BEER AGAR is a non-selective agar rich in nutrients that supports the growth and recovery of microorganisms of significance in the brewing industry.

The medium isolates bacteria and yeasts, capable of growing under brewing conditions, which are of real significance for the brewer.

Yeast extract is a source of vitamins, particularly of the B-group. Peptonized milk contains lactose as an energy source. Tomato juice is a source of carbon, protein and nutrients. Dextrose is a fermentable carbohydrate providing carbon and energy. Potassium phosphates act as a buffer system. Magnesium sulfate, Ferrous sulfate and Manganese sulfate are sources of ions that stimulate metabolism. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. The incorporation of beer in the medium adds hop constituents and alcohol which eliminate many airborne contaminants not originating from pitching yeasts or beer, and therefore minimizing false positive results. Also, it stimulates the growth of beer spoilage organisms, such as lactobacilli, pediococci, *Acetobacter*, and *Zymomonas spp.*

For the detection of bacterial contaminants in pitching yeasts, cycloheximide (1 mg/l) may be added.

Incubate at 28 - 30ºC and observe after 3 days. Incubate aerobically to detect *Acetobacter* and anaerobically to detect *Lactobacillus microaerophilus*, pediococci and *Zymomonas*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 28 - 30ºC and observed after 3 days.

Microorganisms	Growth
Lactobacillus fermentarum ATCC 9339	Good
Pediococcus damnosus ATCC 29358	Scarce
Saccharomyces cerevisiae ATCC 9763	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Kozulis, J. A. AND Page, H.E.A. A. new universal beer agar medium for the enumeration of wort and beer microorganisms. Proc.Am.Brew. Chem 52-58, (1968)

UREA AGAR BASE (CHRISTENSEN) ISO 6579

CAT. 1110

For the differentiation of Enterobacteria on the basis of urease production

FORMULA IN g/l

Urea	**20.00**	Gelatin Peptone	**1.00**
Sodium Chloride	**5.00**	Dextrose	**1.00**
Monopotassium Phosphate	**2.00**	Phenol red	**0.012**
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Dissolve 29 grams of the medium in 100 ml of distilled water. Sterilize by filtration. Separately dissolve 15 grams of agar in 900 ml of distilled water by boiling. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 50°C and add to the 100 ml of the sterile Urea Agar Base. Mix well and dispense aseptically in sterile tubes. Leave the medium to set in a slanted position so as to obtain deep butts. Do not remelt the slanted agar. The prepared medium should be stored at 2 - 8°C. The color at a pH of 6.8 to 7.0 should have a light pinkish-yellow color.

The dehydrated medium should be homogeneous, free-flowing and orange-red in color. If there are any physical changes, discard the medium.

USES

UREA AGAR BASE (Christensen) may be used as an aid in the differentiation of microorganisms, particularly enteric Gram-negative Enterobacteria, on the basis of urea hydrolysis, from clinical samples and other materials. The formula is according to ISO 6579.

Urea Agar Base, with TSI Agar (**Cat. 1046**), may be used as a screening medium for the selection of *Salmonella* and *Shigella*. Urea Agar Base is used in spot tests for the rapid detection of urease activity and, when combined with results of other quick screening tests, it is the most common method to detect urease production by Enterobacteria. It is particularly recommended for the differentiation of members of the genus *Proteus* from those of *Salmonella* and *Shigella* in the diagnosis of enteric infections.

Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride maintains the osmotic balance. Monopotassium phosphate provides buffering capacity. Urea is a source of nitrogen for those organisms producing urease. Phenol red is the pH indicator.

To obtain good results, inoculate heavily over the slant as the speed of the reaction depends on the relation of organism amount and medium surface. Do not inoculate the butt of this medium as it is used as a negative color control. ISO 6579 recommends to incubate at 35 ± 2°C for 24 ± 3 hours. A positive test is denoted by a change in color, due to urease production, from yellow (pH 6.8) to a deep purple or bluish-red (pH 8.1) on the slant surface. Observations of the tubes should be made at 2 - 4 hours. Proteus and a few other organisms give a positive (purple) reaction.

Reincubate all negative cultures daily for up to 7 days for positives such as *Brucella*.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 ± 3 hours.

Microorganisms	Growth	Urease
Enterobacter aerogenes ATCC 13048	Good	- (no change of color in the medium)
Escherichia coli ATCC 25922	Good	- (no change of color in the medium)
Klebsiella pneumoniae ATCC 13883	Good	+ (red or purple medium)
Proteus vulgaris ATCC 13315	Good	+ (red or purple medium)
Salmonella typhimurium ATCC 14028	Good	- (no change of color in the medium)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Christensen J. Bact. 52:641. 1946. Thal and Chen J. Bact. 69:10. 1955. Ewing Enterobacteriaceae. USPHS, Publication 734.

Norma UNE-EN-ISO 6579. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Salmonella spp.

UREA BROTH

CAT. 1226

For the differentiation of Enterobacteria, particularly *Proteus* from *Salmonella* and *Shigella* from clinical samples

FORMULA IN g/l

Urea	**20.00**	Yeast Extract	**0.10**
Dipotassium Phosphate	**9.50**	Phenol red	**0.01**
Monopotassium Phosphate	**9.10**		
Final pH 6.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 3.87 grams of the medium in 100 ml of distilled water without heating. When the powder is dissolved, sterilize by filtration. Dispense quantities of 0.5 to 2 ml in small sterile tubes. Larger volumes can be used but the reactions will be slower. Do not sterilize in autoclave. Do not boil the medium. When there is no filter available, the medium can be sterilized at 100 - 110°C for 10 minutes. If the medium is prepared and inoculated immediately it provides good results without sterilizing. The prepared medium should be stored at 2 - 8°C. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

UREA BROTH can be used for the determination of the urea activity of Enterobacteriaceae, as well as microorganisms of the families of *Brucella, Bacillus, Micrococcus, Mycobacteria* and *Proteus.* It can be used for the identification of bacteria on the basis of urea utilization. It is especially recommended for the differentiation of members of the genus *Proteus* from those of *Salmonella* and *Shigella*.

Urea is a source of nitrogen for those organisms producing urease. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Potassium phosphates provide buffering capacity. Phenol red is the pH indicator.

Prepare a heavy suspension of the organism isolated from plated media and inoculate the Urea Broth tubes. Incubate at 35 ± 2°C for 18 - 24 hours.

When organisms utilize urea, ammonia is produced during incubation making the reaction of these media alkaline. Positive urease tubes turn the phenol indicator a deep violet-red color (alkalinization). Therefore, urease production may be detected by a change in the Phenol red indicator.

Developed by Rustigian and Stuart, this highly buffered medium usually reacts only to the high outputs of ammonia by *Proteus, Morganella* and *Providencia* in the first 24 hours of incubation.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Urease
Escherichia coli ATCC 25922	-
Klebsiella pneumoniae ATCC 13883	+
Proteus vulgaris ATCC 13315	+
Salmonella typhimurium ATCC 14028	-

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Rustigian and Stuart. Proc. Soc. Exp. Biol. and Med. 47:109, 1941.

McKay, Edwards and Leonar A. J. Clin. Path. 17:479, 1947.

Goldsmith and Latlief. Applied Microbiol., 3:195. 1955.

Gordon and Mihn. J. Gen. Microbiol., 21:736. 1959.

UREA INDOLE BROTH ISO 10273

CAT. 1227

For the differentiation of Enterobacteria on the basis of urease and indol production and the transdeamination of tryphophan (TDA) from clinical samples

FORMULA IN g/l

Urea	**20.00**	Dipotassium Phosphate	**1.00**
Sodium Chloride	**5.00**	Monopotassium Phosphate	**1.00**
L-Tryptophan	**3.00**	Phenol red	**0.025**
Final pH 6.8 ± 0.2 at 25°C			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water .Mix well. Add 10 ml of 95% ethanol. Dispense in 1 - 5 ml amounts into sterile tubes. AVOID OVERHEATING. DO NOT AUTOCLAVE. The prepared medium should be stored at 2 - 8°C. The color is orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

UREA INDOLE BROTH ISO 10273 can be used for the determination of urease and indole production by Enterobacteriaceae as well as microorganisms of the families of *Brucella, Bacillus, Micrococcus, Mycobacteria* and *Proteus*.

L-Tryptophan is an essential amino acid and is converted to skatole and indole. Sodium chloride maintains the osmotic balance. Potassium phosphates act as a buffer system. Urea is a source of nitrogen for those organisms producing urease. Phenol red is the pH indicator.

Prepare a heavy suspension of the organism isolated from plated media and inoculate the Urea Indole Broth tubes. Incubate at 35 ± 2°C for 18 - 24 hours. Observe at 3 - 4 hours for any positive urease in tubes that turn the indicator a deep violet red color

alinization), typical of *Proteus* or *Yersinia*. *Klebsiella* and some *Citrobacter* develop positive tubes after 18 hours.

Indole production is determined by adding a few drops of Kovacs Reagent (**Cat. 5205**). A positive test is indicated by the development of a red color in the reagent layer. Tryptophan deaminase (TDA) is demonstrated by adding to a 24 hours culture a few drops of a 30% solution, diluted 1:3. of iron perchloride. The appearance of a brown or red-brown color indicates a positive TDA.

ISO 10273 recommends this medium for the presumptive identification of *Yersinia enterocolitica*. Inoculate and incubate at 30ºC for 24 hours. If the medium is not inoculated with a sufficient quantity of inoculum, it is possible to find false negatives.

Escherichia coli ATCC 25922 — *Proteus vulgaris* ATCC 13315

CHARACTERISTICS OF THE COLONIES

Microorganisms	Urea	Indol	TDA
Escherichia coli	-	+	-
Shigella dysenteriae, boydii, flexneri	-	d	-
Shigella sonnei	-	-	-
Salmonella	-	-	-
Salmonella arizonae SG III	-	-	-
Citrobacter	-	-	-
Edwardsiella	-	+	-
Proteus vulgaris	+	+	+
Proteus rettgeri	+	+	+
Proteus morganii	+	+	+
Proteus mirabilis	+	-	+
Providencia	-	+	+
Yersinia enterocolitica	+	d	-
Y. pseudotuberculosis	+	-	-
Klebsiella pneumoniae	+(slow)	-	-
K. oxytoca	+(slow)	+	-
Enterobacter aerogenes	-	-	-
E. cloacae, E. hafniae	-	-	-
E. agglomerans	-	d	-
Serratia marcescens, liquefaciens	-	-	-

d = variable according to different biochemical types

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Urease	Indole
Escherichia coli ATCC 25922	-	+
Klebsiella pneumoniae ATCC 13883	+	-
Proteus vulgaris ATCC 13315	+	+
Salmonella typhimurium ATCC 14028	-	-
**Yersinia enterocolitica* ATCC 23715	+	±

*Incubate at 30ºC for 24 hours according to ISO 10273.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

Roland F. Bourbon D, Sztrum S. Ann. Inst. Pasteur, 73. 914-916.

ISO 10273 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica.

URINARY TRACT INFECTIONS CHROMOGENIC AGAR (UTIC)

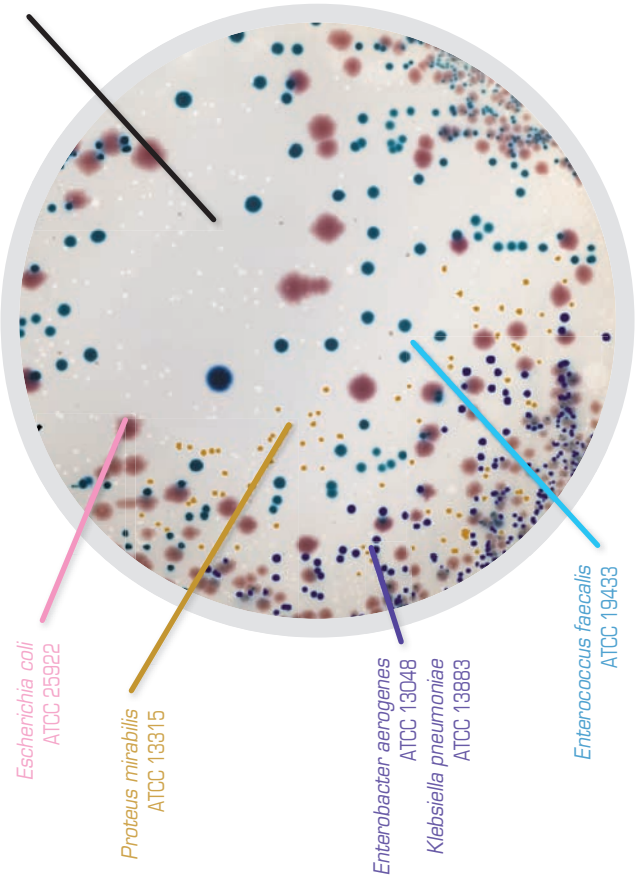
CAT. 1424

For the presumptive detection and differentiation of organisms causing urinary tract infections

FORMULA IN g/l

Peptone Mixture	**16.00**	Chromogenic Substrate	**0.50**
Growth factors	**13.00**	Bacteriological Agar	**16.00**
Tryptophan	**2.00**		
Final pH 7.2 ± 0.2 at 25ºC			

Staphylococcus aureus
ATCC 25923



PREPARATION

Suspend 47.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The medium should be stored at 8 - 15°C. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

URINARY TRACT INFECTION CHROMOGENIC AGAR (UTIC) is a chromogenic medium for the presumptive identification and confirmation of microorganisms causing urinary tract infections. Microorganisms which cause infections, in the urinary tract comes mainly from one species and are generally abundant: *E. coli* is the organism most frequently isolated.

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. The medium includes two chromogenic substrates which are cleaved by enzymes produced by *Enterococcus spp.*, *Escherichia coli* and coliforms. It also includes phenylalanine and tryptophan providing a presumptive indication of the tryptophan deaminase activity, which illustrates the presence of *Proteus spp.*, *Morganella spp.*, and *Providencia spp.* This is based on CLED Agar. Bacteriological agar is the solidifying agent.

One of the chromogenes is metabolised by β-glucosidase enzyme activity, allowing the specific detection of enterococci which form blue or turquoise colonies. The other chromogen is cleaved by β-galactosidase, an enzyme produced by *E. coli* which grows as pink colonies. (In case of unreliable colony results, carry out Indol test).

Cleavage of both chromogenes result in dark blue-purple colonies. Tryptophan in the medium acts as an indicator of the tryptophane deaminase producing colonies of *Proteus, Morganella*, and *Providencia spp.* of brown color.

It should be noted that, as with all chromogenic media, microorganisms with atypical enzyme patterns may give anomalous reactions. For example 45% of *Enterobacter cloacae* do not contain β-glucosidase, therefore resulting in pink colonies not distinguishable from *E. coli*. For confirmation, the Indol test must be performed.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed 18 - 24 hours.

Microorganisms	Growth	Colony color
Escherichia coli ATCC 25922	Good	Pink
Enterobacter aerogenes ATCC 13048	Good	Dark Blue
Klebsiella pneumonieae ATCC 13883	Good	Dark Blue
Proteus mirabilis ATCC 13315	Good	Light Brown
Staphylococcus aureus ATCC 25923	Good	(natural pigmentation) White Cream
Enterococcus faecalis ATCC 19433	Good	Light Blue

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

CE IVD

Samra Z, Heifetz M, Talmor J, Bain E and Bahar J. Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. J Clin Microbiol. 1998;36(4): 990-4.

UVM-I LISTERIA SELECTIVE ENRICHMENT BROTH, MODIFIED

CAT. 1279

For the selective enrichment of *Listeria spp.* in two stages from raw meat, food and clinical samples as per USDA-FSIS procedures

FORMULA IN g/l

Sodium Chloride	**20.00**	Yeast Extract	**5.00**
Disodium Phosphate	**12.00**	Potassium Phosphate	**1.35**
Tryptone	**5.00**	Esculin	**1.00**
Proteose Peptone	**5.00**	Nalidixic Acid	**0.020**
Beef Extract	**5.00**	Acryflavine	**0.012**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 54.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is yellow-green.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: this medium contains Acryflavine, a possible mutagen, do not inhale or contact with skin. Wear gloves and eye/face protection.

USES

LISTERIA SELECTIVE ENRICHMENT BROTH, MODIFIED (UVM FORMULA) is a modification of the formula described by Donnelly and Baigent; reducing the content of Nalidixic Acid in UVM-I and UVM-II from the previous formulation and increasing the concentration of acryflavine hydrochloride in the UVM-II Listeria Selective Enrichment Broth, Modified (**Cat. 1280**).

This modification and the two-stage selective enrichment method (USDA-FSIS) have meant the most isolation of *Listeria monocytogenes* in meat products and, additionally, have the advantage of doing so in only 3 - 4 days.

Tryptone, Proteose Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Potassium phosphates act as a buffer system. Esculin is hydrolyzed by all *Listeria* species. Nalidixic acid blocks the DNA replication of susceptible bacteria and acts against many Gram-negative bacteria. Acriflavine hydrochloride inhibits many Gram-positive bacteria.

USDA-FSIS method consists of inoculating 10 grams of the sample in 90 ml of UVM-I broth, incubating at 35 ± 2°C and observing after 18 - 48 hours. After this is complete, take 100 µl of the incubated UVM-I and inoculate in 9.9 ml of UVM-II broth (**Cat. 1280**). Incubate at 35 ± 2°C and observed after 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Listeria monocytogenes ATCC 19114	Good
Listeria monocytogenes NCTC 10527	Good
Staphylococcus aureus ATCC 6538	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

McClain, D., Lee W.H: Development of USDA-FSIS Method for isolation of Listeria monocytogenes from raw meat and poultry.- J. Assoc.Off.Anal. Chem., 71(3)

Donnelly C.W. y Baigent G.J.(1986) Appl.Environ.Microbial 52.689-695

UVM-II LISTERIA SELECTIVE ENRICHMENT BROTH, MODIFIED

CAT. 1280

For the selective enrichment of *Listeria spp.* in two stages from raw meat, food and clinical samples as per USDA-FSIS procedures

FORMULA IN g/l

Sodium Chloride	**20.00**	Yeast Extract	**5.00**
Disodium Phosphate	**12.00**	Monopotassium Phosphate	**1.35**
Tryptone	**5.00**	Esculin	**1.00**
Proteose Peptone	**5.00**	Nalidixic Acid	**0.020**
Beef Extract	**5.00**	Acryflavine	**0.025**
Final pH 7.2 ± 0.2 at 25°C			

PREPARATION

Suspend 54.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into

appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the medium is yellow-green.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

CAUTION: this medium contains Acryflavine, a possible mutagen, is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

LISTERIA SELECTIVE ENRICHMENT BROTH, MODIFIED (UVM FORMULA) is a modification of the formula described by Donnelly and Baigent;reducing the content of Nalidixic Acid in UVM-I and UVM-II from the previous formulation and increasing the concentration of acryflavine hydrochloride in the UVM-II Listeria Selective Enrichment Broth, Modified.

This modification and the two-stage selective enrichment method (USDA-FSIS) have meant the most isolation of *Listeria monocytogenes* in meat products and, additionally, have the advantage of doing so in only 3 - 4 days.

Tryptone, Proteose Peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Potassium phosphates act as a buffer system. Esculin is hydrolyzed by all *Listeria* species. Nalidixic acid blocks the DNA replication of susceptible bacteria and acts against many Gram-negative bacteria. Acriflavine hydrochloride inhibits many Gram-positive bacteria.

USDA-FSIS method consists of inoculating 10 grams of the sample in 90 ml of UVM-I Broth (**Cat. 1279**), incubating at 35 ± 2ºC and observing after 18 - 48 hours. After this is complete, take 100 µl of the incubated UVM-I and inoculate in 9.9 ml of UVM-II Broth. Incubate at 35 ± 2ºC and observe after 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 48 hours.

Microorganisms	Growth
Listeria monocytogenes ATCC 19114	Good
Listeria monocytogenes NCTC 10527	Good
Staphylococcus aureus ATCC 6538	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

McClain, D., Lee W.H: Development of USDA-FSIS Method for isolation of Listeria monocytogenes from raw meat and poultry.- J. Assoc.Off.Anal. chem.., 71(3)

Donnelly C.W. y Baigent G.J.(1986) Appl.Environ.Microbial 52.689-695.

VANCOMYCIN SCREEN AGAR

CAT. 1410

For the screening of enterococci resistant to vancomycin

FORMULA IN g/l

Peptic Digest of Casein	**16.00**	Disodium Phosphate	**2.50**
Brain Heart Infusion	**8.00**	Dextrose	**2.00**
Peptic Digest of Animal Tissue	**5.00**	FD & Yellow #5 Dye	**0.56**
Sodium Chloride	**5.00**	Bacteriological Agar	**13.50**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 52.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add 6.0 mg of Vancomycin. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15ºC. The color of the prepared medium is yellow.

The dehydrated medium should be homogeneous, free-flowing and pinkish beige in color. If there are any physical changes, discard the medium.

USES

VANCOMYCIN SCREEN AGAR AGAR is used for the screening of enterococci resistant to vancomycin.

Peptic digest of casein, Brain heart infusion and Peptic digest of animal tissue provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Disodium phosphate acts as a buffer system. FD & Yellow #5 Dye is inert and improves the visual identification in the medium.

Enterococci are known to be major causes of infections. Commonly, they infect the urinary tract, abdomen, and bile tract. *Enterococcus faecalis* cause 80 - 90% of the infections produced by enterococci and *Enterococcus faecium* the remaining 10 - 20% of the infections. Enterococci are the fourth cause of hospital infections and hence it is important to know the adequate treatment. With this medium it can be found out whether the enterococci are resistant or not to vancomycin.

Incubate at 35 ± 2ºC and observe for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Enterococcus faecalis ATCC 29212	Inhibited
Enterococcus faecalis ATCC 51299	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Jen, Huycke and Gilmore. 1994 Clin. Microbiol. Rev.7:462

Moellering 1992. Clin. Infect. Dis. 14:1173

Emori and Gaynes 1993. Clin. Microbiol. Rev 6-428

VEGETABLE PEPTONE BROTH (TSB VEGETABLE)

CAT. 1380

Universal culture medium without inhibitors and broad-spectrum indicators

FORMULA IN g/l

Vegetable Peptone	**20.00**	Dextrose	**2.50**
Sodium Chloride	**5.00**	Dipotassium Phosphate	**2.50**

Final pH 7.3 ± 0.2 at 25ºC

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

VEGETABLE PEPTONE BROTH is a non-animal Trypticasein Soy Broth medium very rich in nutrients for general use in microbiological laboratories.

It supports the abundant growth of fastidious organisms such as pneumococci, streptococci, Neisseriae, etc. The medium is used frequently in many procedures of diagnostic research or microbiology. It is used for the isolation and sensitivity testing of all types of pathogens, and for the production of antigens for agglutination and serological tests. The peptone used in this broth is from non-animal origin and conforms with the requirements of the pharmaceutical industry and biotechnology.

Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dipotassium phosphate is a buffering agent.

Inoculate and incubate at 35 ± 2 ºC for 18 - 24 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2 ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Staphylococcus aureus ATCC 6538	Good
Escherichia coli ATCC 25922	Good
Candida albicans ATCC 10231	Good
Streptococcus pneumoniae ATCC 6303	Good
Bacillus subtilis ATCC 6633	Good
Aspergillus brasiliensis ATCC 16404	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Deutsches Arzneibuch, 10. Auflage; European Pharmacopeia II, United States Pharmacopeia XXIII.

VIOLET RED BILE AGAR WITH GLUCOSE (VRBG) ISO 21528, EUROPEAN PHARMACOPEIA

CAT. 1092

For the cultivation and enumeration of Enterobacteria in water, foods and other materials

FORMULA IN g/l

Glucose Monohydrate	**10.00**	Bile Salts	**1.50**
Pancreatic Digest of Gelatin	**7.00**	Neutral Red	**0.03**
Sodium Chloride	**5.00**	Crystal Violet	**0.002**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 41.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Cool to 45ºC and dispense immediately. Alternatively, sterilize in autoclave at 118ºC for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

VIOLET RED BILE AGAR WITH GLUCOSE (VRBG) is a selective medium, containing Bile and Violet Red dye, for the isolation and enumeration of enterobacteria. It is based on MacConkey Medium (**Cat. 1052**) for the detection and enumeration of bile-tolerant Gram-negative Enterobacteriaceae in dairy products and foods. In this medium, the lactose is replaced by glucose as the carbohydrate. VRBG agar is becoming the preferred medium for use in investigations into raw materials, processed foods and plant hygiene.

The Enterobacteriaceae group includes lactose-fermenting coliforms bacteria and non-lactose fermenting species like *Salmonella* and *Shigella*.

Pancreatic digest of gelatin provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate providing carbon and energy. Glucose fermenters form red colonies in the presence of the pH indicator neutral red. Bile salts and crystal violet inhibit Gram-positive bacteria. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

When testing raw vegetables, for example, VRBL agar (**Cat. 1093**) is a more practical choice for a hygienic state because certain non-lactose fermenting but glucose utilizing organisms, e.g. *Pseudomonas* species, predominate amongst the naturally occurring flora and may easily overgrow the indicator on VRBG Agar.

The European Pharmacopoeia 7.0 recommends in Paragraph 2.6.13: "Microbiological examination of non-Sterile products: test for specified microorganisms" the testing of the product for bile-tolerant gram-negative bacteria. Use the volume of 1gr corresponding to product to inoculate with Mossel EE Broth (**Cat. 1202**) at 30 - 35ºC for 24 - 48 hours. Subculture on plates of Violet Red Bile Agar with Glucose. Incubate at 30 - 35ºC for 18 - 24 hours. The product complies with the test if there is no growth of colonies.

ISO 21528 also recommends this medium as a selective medium. The medium should be incubated at 37ºC when the enumeration of Enterobacteriaceae is for a hygienic indicator and alternatively, at a temperature of 30 °C when the enumeration of Enterobacteriaceae is conducted for technological purposes and includes psychrotrophic Enterobacteriaceae.

The pour plate method suppresses the growth of Gram-negative non-fermenting bacteria due to its anaerobic conditions. The fermentation of glucose is likewise stimulated and results in the formation of purple-red colonies, clearly visible, surrounded by a zone of the same color. Note that coliforms will ferment the glucose and produce acid with or without gas. *Klebsiella* and *Citrobacter* , which are more heat-resident than coliforms, also grow in this medium and can indicate a production process defect (insufficient heating).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 35ºC and observed after 18 - 24 hours. According to European Pharmacopoeia 7.0.

Microorganisms	Growth	Colony color
* *Escherichia coli* ATCC 11775	Good	Red
* *Escherichia coli* ATCC 8739	Good	Red
* *Salmonella gallinarum* NCTC 9240	Good	Red
Staphylococcus aureus ATCC 6538	Inhibited	–
* *Shigella flexneri* ATCC 29903	Good	Red
Pseudomonas aeruginosa ATCC 9027	Good	–
Enterococcus faecalis ATCC 29122	Inhibited	–

* According to ISO 21528. Incubation at 37ºC during 24 ± 2 hours.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO EP

D.A. Mossel, (1985) Media for Enterobacteriaceae (Inst. J. Food Microbiol 2:27).

ISO 21528. Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of Enterobacteriaceae.

ISO 7402 Microbiology -- General guidance for the enumeration of Enterobacteriaceae without resuscitation -- MPN technique and colony-count technique.

ISO 8523 Microbiology -- General guidance for the detection of Enterobacteriaceae with pre-enrichment.

European Pharmacopoeia 7.0

VIOLET RED BILE AGAR WITH LACTOSE (VRBL) ISO 4832

CAT. 1093

Selective medium for the detection and enumeration of coliforms in dairy products, water and food

FORMULA IN g/l

Lactose	**10.00**	Bile Salts	**1.50**
Gelatin Peptone	**7.00**	Neutral Red	**0.03**
Sodium Chloride	**5.00**	Crystal Violet	**0.002**
Yeast Extract	**3.00**	Bacteriological Agar	**15.00**
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 41.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. DO NOT AUTOCLAVE. Cool to 45ºC and use immediately. If desired, the can also be dispensed and sterilized in the autoclave at 118ºC for 15 minutes, as Hartham demonstrated that media prepared only by boiling gave the same results as media autoclaved. The prepared medium should be stored at 8 - 15°C. The color of the medium is purple-red.

The dehydrated medium should be homogeneous, free-flowing and beige reddish in color. If there are any physical changes, discard the medium.

USES

VIOLET RED BILE AGAR WITH LACTOSE (VRBL), containing Bile and Violet Red dye, is based on MacConkey Agar (**Cat. 1052**) for the detection and enumeration of lactose-fermenting bacteria and the differentiation of coliforms or *Coli-aerogenes* group from non-lactose fermenting organisms in dairy products, water and foods.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Lactose is the fermentable carbohydrate providing carbon and energy. Bile salts and Crystal violet inhibit Gram-positive bacteria. Neutral red is a pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

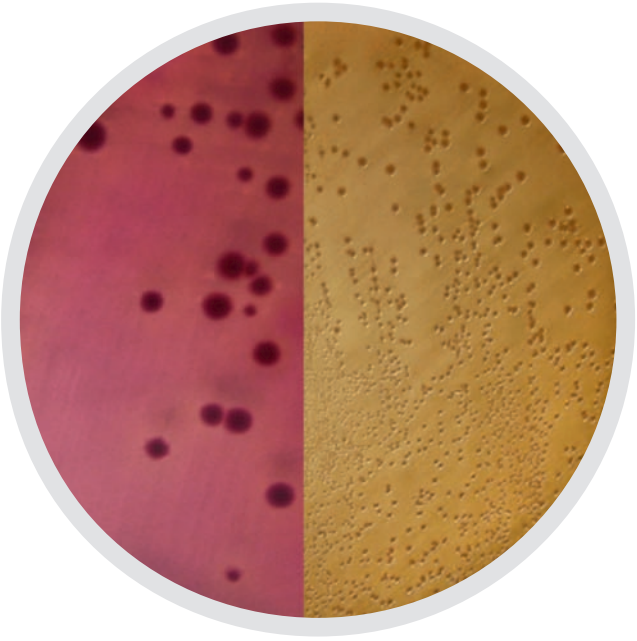
It is convenient to use the pour plate method by placing 1 ml of the desired dilution in a sterile Petri dish, adding 15 ml of the medium, cooled to 45 - 50°C, and rotating gently before allowing to solidify. Once solidified, pour a second layer of the medium to a depth of 5 mm. Allow to solidify. Incubate at temperatures of 35 ± 2°C for 18 - 24 hours.

ISO 4832 recommends the preparation of two dishes for the liquid product and/or from each dilution chosen. With a sterile pipette transfer 1 ml of liquid product or the appropriate dilutions to the centre of each dish. Use another sterile pipette to inoculate each dilution into the dishes. Pour about 15 ml of VRBL medium, at 44 °C to 47°C, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes should not exceed 15 min. Carefully mix the inoculum with the medium and allow the mixture to solidify with the Petri dishes standing on a cool horizontal surface.

Also prepare a control plate with of the medium for checking its sterility.

After complete solidification, pour about 4 ml of VRBL medium, at 44 °C to 47°C, onto the surface of the inoculated medium. Allow to solidify as described above. Invert the prepared dishes and incubate them in the incubator set at 30°C or 37°C (as agreed) for 24 h ± 2 h.

Lactose fermenters form red colonies with red-purple halos. Occasionally the cocci of the intestinal tract can develop as small, punctiform red colonies.



Escherichia coli ATCC 25922 | *Salmonella gallinarum* NCTC 9240

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours*.

Microorganisms	Growth	Colony Color	Inoculum (Cfu/ml)	Recovery Rate (%)
Escherichia coli ATCC 25922	Good	Purple	10^3 -10^5	≥ 30
Enterobacter aerogenes ATCC 13048	Good	Purple	10^3 -10^5	≥ 30
Salmonella gallinarum NCTC 9240	Good	Colorless	10^3 -10^5	≥ 30
Staphylococcus aureus ATCC 6538	Inhibited		$>10^5$	≤ 0.01
Enterococcus faecalis ATCC 29212	Inhibited			
Pseudonomas aeruginosa ATCC 27853	Good	Colorless - beige	10^2	≥ 80

*According to ISO 4832. Incubation at 30 or 37ºC and observed after 24 ± 2 h

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO 4832. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms - Colony-count technique.

Collins, J. Milk and Food Tech 18:169, 1955. Hartman, J. Milk and Food Tech 23:43. 1960

Speck, M.L. (ed) 1976. Compendium of Methods for the Microbiological Examination of Foods (APHA).

VIOLET RED BILE AGAR WITH LACTOSE & GLUCOSE (VRBLG) EUROPEAN PHARMACOPOEIA

CAT. 1144

Recommended for the detection and enumeration of Enterobacteria

FORMULA IN g/l

Glucose Monohydrate	**10.00**	Bile Salts	**1.50**
Lactose Monohydrate	**10.00**	Neutral Red	**0.03**
Gelatin Pancreatic Digest	**7.00**	Crystal Violet	**0.002**
Sodium Chloride	**5.00**	Bacteriological Agar	**15.00**
Yeast Extract	**3.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 51.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Cool to 45°C and use immediately. The medium can also be dispensed and sterilized in the autoclave at 118°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color of the medium is purple-red.

The dehydrated medium should be homogeneous, free-flowing and beige-reddish in color. If there are any physical changes, discard the medium.

USES

VIOLET RED BILE AGAR WITH LACTOSE AND GLUCOSE (VRBLG) is recommended by the European Pharmacopoeia for the selective isolation of lactose and glucose-fermenting Gram-negative bacteria.

This medium is used in the microbiological examination of non-sterile products, as a test for specified microorganisms mainly belonging to the Enterobacteriaceae group, but other types of organisms may be recovered, e.g. *Pseudomonas, Aeromonas*.

Gelatin pancreatic digest provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Lactose and Glucose are the energy source carbohydrates. Bile salts and Crystal violet inhibit Gram-positive bacteria. Neutral red is a pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Detection: The sample is prepared using Lactose Broth (**Cat. 1206**), homogenized and incubated at 35 ± 2°C for 2 - 5 hours, time enough to revive bacteria but not sufficient for growth. After mixing with Mossel EE Broth (**Cat. 1202**) in a 1:100 ml ratio and incubating at 35 ± 2°C for 18 - 24 hours, subculture onto VRBLG Agar to obtain selective isolation. Incubate at 35 ± 2ºC for 18 - 24 hours. The product passes the test if there is no growth of Gram-negative bacteria colonies on none of the plates.

Quantitative evaluation: Inoculate sample in Mossel EE Broth (**Cat. 1202**) (dilutions 0.1, 0.01, 0.001 ml) and incubate at 35 ± 2°C for 24 - 48 hours. Subculture on plates of VRBLG Agar to obtain selective isolation. Incubate at 35 ± 2ºC for 18 - 24 hours. Growth of well-developed red colonies of Gram-negative bacteria constitutes a positive result. Note the smallest quantity which gives a positive result and the largest quantity that gives a negative result. Refer to the appropriate reference for further identification tests.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony color
Escherichia coli ATCC 11775	Good	Red
Salmonella gallinarum NCTC 9240	Good	Red
Staphylococcus aureus ATCC 6538	Inhibited	

Microorganisms	Growth	Colony color
Shigella flexneri ATCC 29903	Good	Red
Streptococcus lactis ATCC 19435	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

EP

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Hitchins, A.D., P.A. Hartman, and E.C.D. Todd. 1992. Coliforms – Escherichia coli and its toxins, p. 325-369. In Vanderzant, C., and D.F. Splittstoesser (ed.) Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, DC.

VOGEL-JOHNSON AGAR

CAT. 1079

For the selective isolation of *Staphylococcus aureus* from clinical samples and foods

FORMULA IN g/l

Glycine	**10.00**	Lithium Chloride	**5.00**
D-Mannitol	**10.00**	Yeast Extract	**5.00**
Triptone	**10.00**	Phenol red	**0.025**
Dipotassium Phosphate	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 60 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add 6 ml of 3.5% Potassium Tellurite (**Cat. 5208**). Homogenize gently and dispense into Petri dishes. To prepare a less selective medium, only add 3 ml of 3.5%. Potassium Tellurite. The prepared medium should be stored at 8 - 15°C. The color is red, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

VOGEL-JOHNSON AGAR BASE is a selective and differential medium used for the early detection of *Staphylococcus aureus* by identifying the coagulase-positive and mannitol-fermenting strains. The medium is excellent for the detection of staphylococci carriers as well as studies of sanitary concern.

S. aureus reduce the potassium tellurite to the metal tellurium and result in the growth of black colonies. The fermentation of mannitol is indicated by the yellow zones around the black colonies and changes the red color of the medium to yellow. Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Mannitol is the fermentable carbohydrate providing carbon and energy Potassium tellurite, Lithium chloride and the high Glycine concentration inhibit most microorganisms other than staphylococci. Phenol red is the pH indicator. Dipotassium phosphate is a buffer. Bacteriological agar is the solidifying agent.

Vogel-Johnson Agar plates can be streaked heavily with a swab and incubated at 35 ± 2°C for 24 - 48 hours. Look for black colonies surrounded by a yellow zone. During the first 24 hours the majority of microorganisms, except for coagulase-positive staphylococci are totally or markedly inhibited. At 48 hours many coagulase-negative staphylococci, mannitol-positive and mannitol-negative, begin to appear. *Staphylococcus epidermidis*, mostly early inhibited, forms small grayish-black colonies without yellow zones. Coagulase-positive staphylococci form black colonies on the red medium. If they ferment mannitol, the colonies are surrounded by a yellow zone. Mannitol-negative organisms do not change the red color of the medium.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the additive added, after incubation at a temperature of 35 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Colony color
Escherichia coli ATCC 25922	Inhibited	
Proteus mirabilis ATCC 25933	Negative to poor	Black
Staphylococcus aureus ATCC 25923	Good	Black with yellow halos
Staphylococcus epidermidis ATCC 12228	Moderate	Translucid to black

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

United States Pharmacopoeia XXI (1985) Microbial limit tests. Rockville Md. Vogel R.A. Jonhson, M. 3. (1961) Pub. Hlth. Lab, 18, 131.

Zebovitz E. Evans, J.B. add Niven C.P. (1955) J. Bact. 70. 687.

WILKINS CHALGREN MEDIUM

CAT. 1503

For susceptibility testing as well as for the isolation and culture of anaerobic bacteria in general from clinical samples

FORMULA IN g/l

Tryptone	**10.00**	Sodium Pyruvate	**1.00**
Bacteriological Peptone	**10.00**	L-Arginine	**1.00**
Yeast Extract	**5.00**	Vitamin K1	**0.0005**
Sodium Chloride	**5.00**	Hemin	**0.005**
Dextrose	**1.00**	Bacteriological Agar	**15.00**
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 48 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium in plates should be stored at 8 - 15°C. The prepared medium in tubes should be stored at 2 - 8ºC. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

WILKINS CHALGREN MEDIUM was designed for use in the determination of minimum inhibitory concentrations (MIC) of antibiotics for anaerobic bacteria by the agar dilution method. It is also recommended for the isolation of anaerobic organisms from clinical specimens. It has the same performance in Petri dishes as in tubes.

It has the advantage over other media in that it does not need the addition of blood to obtain the satisfactory growth of clinically important anaerobic bacteria.

Tryptone and Bacteriological peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group and other growing factors to cultivate *Bacteroides melaninogenicus* and *Peptostreptococcus anaerobius*. Dextrose is the fermentable carbohydrate providing carbon and energy. L-Arginine provides amino acids for the growth of *Eubacterium lentum*. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium pyruvate acts as an energy source for asaccharolytic cocci such as *Veillonella* and to catalyze and degrade traces of hydrogen peroxide which affects the metabolism of anaerobes. Haemin and vitamin K1 are growth factors. Haemin is essential for the growth of *Bacteroides* species. Bacteriological agar is the solidifying agent.

Inoculate and incubate at a temperature of 35 ± 2°C during 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Bacteroides fragilis ATCC 25285	Good
Bacteroides melaninogenicus ATCC 25611	Good
Clostridium perfringens ATCC 13123	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Wilkins T.D. and Chalgren S. (1976) Antimicrob. Agents. Chemother., 10. 926-928.

Sutter V.L., Barry A.L., Wilkins T.D. and Zabransky R.J. (1 979) and Microb. Agents Chemother, 16. 495-502. Brown W.J. and Waatti P.E. (1980) Antimicrob. Agents Chemother., 17. 629-635.

WILKINS CHALGREN MEDIUM II

CAT. 1568

For the general development and microbial sensitivity test of anaerobic agents from clinical samples

FORMULA IN g/l

Tryptone	**10.00**	L-Arginine	**1.00**
Bacteriological Peptone	**10.00**	Sodium Pyruvate	**1.00**
Yeast Extract	**5.00**	Vitamin K1	**0.005**
Sodium Chloride	**5.00**	Hemin	**0.005**
Dextrose	**1.00**		
Final pH 7.1 ± 0.2 at 25ºC			

PREPARATION

Suspend 33 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add the desired antibiotics. The prepared medium should be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

WILKINS CHALGREN MEDIUM II is used in studies of antimicrobial susceptibilities with both the broth and the agar, standardizing by using identical nutrient formulation media.

This medium is also recommended to grow anaerobic microorganisms. It has the advantage over other media in that it does not need the addition of blood to obtain the satisfactory growth of clinically important anaerobic bacteria.

Yeast extract provides vitamins, particularly the B-group, and other growing factors to cultivate *Bacteroides melaninogenicus* and *Peptostreptococcus anaerobius*. Tryptone and Peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the carbohydrate energy source. L-Arginine provides amino acids for the growth of *Eubacterium lentum*. Sodium pyruvate acts as an energy source for saccharolytic cocci, such as *Veillonella*, and to catalyze and degrade traces of hydrogen peroxide which affects the metabolism of anaerobes. Hemin is essential for the growth of *Bacteroides spp.* Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Inoculate and incubate at a temperature of 35 ± 2°C and observe after 24 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth
Bacteroides fragilis ATCC 25285	Good
Bacteroides melaninogenicus ATCC 25611	Good
Clostridium perfringens ATCC 13123	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Hall, Jean F. (1971) J. Inst. Brewing 77, 513-516

WL DIFFERENTIAL AGAR

CAT. 1026

Selective medium used in the control of industrial fermention processes, especially in brewery

FORMULA IN g/l

Dextrose	**50.00**	Magnesium Sulfate	**0.125**
Tryptone	**5.00**	Bromocresol Green	**0.022**
Yeast Extract	**4.00**	Cycloheximide	**0.004**
Monopotassium Phosphate	**0.55**	Ferric Chloride	**0.0025**
Potassium Chloride	**0.425**	Manganese Sulfate	**0.0025**
Calcium Chloride	**0.125**	Bacteriological Agar	**20.00**

Final pH 5.5 ± 0.2 at 25ºC

PREPARATION

Suspend 80 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8 - 15°C. The color is blue.

The dehydrated medium should be homogeneous, free-flowing and beige with blue tint in color. If there are any physical changes, discard the medium.

CAUTION: This medium contains cycloheximide and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

WL DIFFERENTIAL AGAR (Wallerstein Laboratories) is a selective medium for the isolation and enumeration of microbial flora used together with WL Nutrient Agar (**Cat. 1086**) for the control of the manufacture of beer and other fermentation processes by yeasts. Both media are widely used in the industries of vinegar, bread yeasts, grape and wine-growing and distilled spirits. In the production of yeasts for the bakery and distillery industries, the pH of the media is adjusted to 6.5.

The medium doesn't allow the multiplication of yeast in fermentation liquids, which contain a microflora mix consisting of fungi and bacteria. When there are yeast present, bacteria can be detected.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Yeast extract is a source of vitamins, particularly of the B-group. Monopotassium phosphate is the buffer. Potassium, Calcium and Ferric chlorides all provide essential ions for the osmotic balance. Magnesium and Manganese sulfates are sources of divalent cations. Bromocresol purple is the pH indicator. Bacteriological agar is the solidifying agent. The addition of 0.004 grams of Cycloheximide converts the WL Nutrient formula into a differential medium, which inhibits the development of yeasts and molds while permitting the

notable proliferation of the bacteria present in the fermentation liquids and subsequent identification and enumeration.

Inoculate and incubate at a temperature of 30°C and observe after 24 - 48 hours. Time and temperature of incubation are important factors according to the type of yeast. In general, temperatures of 25°C with the beer yeasts and 30°C with the bread and other alcoholic fermentation yeasts are appropriate. The time of incubation varies from 2 to 7 days and up to 14 days, depending on the flora found.

Likewise, the atmosphere chosen for incubating the culture must be appropriate. The bread yeasts are incubated aerobically while the alcoholic fermentation yeasts are incubated anaerobically and in the presence of CO_2.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Lactobacillus fermentun ATCC 9338	Good
Saccharomyces cerevisiae ATCC 9763	Inhibited
Saccharomyces uvarum ATCC 9080	Inhibited
Proteus mirabilis ATCC 25933	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Green and Grey. Wallenstein, Lab. Comm. 13:357. 1950. Green and Grey. Wallenstein, Lab. Comm. 14:169, 1951. Applicable to bacteriological investigation in brewing Wallesstein Lab. Commus 13: 357.

WL NUTRIENT AGAR

CAT. 1086

For the determination of microbial flora in beer fermentation processes and manufacturing

FORMULA IN g/l

Dextrose	**50.00**	Magnesium Sulfate	**0.125**
Tryptone	**5.00**	Bromocresol Green	**0.022**
Yeast Extract	**4.00**	Ferric Chloride	**0.0025**
Monopotassium Phosphate	**0.55**	Manganese Sulfate	**0.0025**
Potassium Chloride	**0.425**	Bacteriological Agar	**15.00**
Calcium Chloride	**0.125**		
Final pH 5.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 75 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is grayish-blue.

The dehydrated medium should be homogeneous, free-flowing and clear toasted with blue tint in color. If there are any physical changes, discard the medium.

USES

WL NUTRIENT AGAR, based on the Green and Grey formulation, is recommended for the control of industrial fermentations, particularly the manufacturing of beer. With a pH of 5.5, true counts of beer yeasts can be made. With a pH of 6.5, the medium is ideal for bakery and distilled spirit yeasts.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Yeast extract is a source of vitamins, particularly of the B-group. Monopotassium phosphate is the buffer. Potassium, Calcium and Ferric chlorides all provide essential ions for the osmotic balance. Magnesium and Manganese sulfates are sources of divalent cations. Bromocresol green is the pH indicator. Bacteriological agar is the solidifying agent.

Both WL Nutrient (WLN) and WL Differential (WLD) formulae are used in conjunction, as with WLN Agar the bacteria may not be detected unless the number of yeast cells is very small. 1 plate WLN and 2 plates WLD must be used. .

- The WLN Agar plate is incubated aerobically for the total plate count of yeasts.

- One of the WLD Agar plates is incubated aerobically for acetic acid bacteria: *Flavobacterium, Proteus*, thermophilic bacteria and others

- The second WLD plate is incubated anaerobically for the investigation of lactic-acid bacteria and species of *Pediococcus*.

Inoculate and incubate at a temperature of 30°C and observe after 24 - 48 hours. All plates are incubated, in general, at 25°C in the case of beer, and at 30°C for bakery and malt alcoholic yeasts. Plates are incubated for 2 - 10 days and up to 2 weeks, according to the flora present. Counts are made at regular intervals during this period.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 24 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Moderate
Lactobacillus fermentun ATCC 9338	Moderate
Proteus mirabilis ATCC 25933	Moderate
Saccharomyces cerevisiae ATCC 9763	Good
Saccharomyces uvarum ATCC 9080	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Green, S.R. and P.P. Gray 1950. Paper read at American Society of Brewing Chemist Meeting. Wallerstein Lab. Commun 1 2:43. Green, S.R. and P.P. Gray 1950. A differential procedure applicable to bacteriological investigation in brewing. Wallersteia Lab. Commun 13:357.

MacFaddin J.D. 1985. media for isolation cultivation-identification-maintenance of medical bacteria, vol. 1. p. 854-856 Willians Wilkins, Baltimore, MD.

WORT BROTH

CAT. 1444

For the cultivation and enumeration of yeasts

FORMULA IN g/l

Malt Extract	**15.00**	Casein Peptone	**1.00**
D-Maltose	**12.50**	Dipotassium Phosphate	**1.00**
Dextrin	**2.50**	Ammonium Chloride	**1.00**
Final pH 4.8 ± 0.2 at 25ºC			

PREPARATION

Suspend 33 grams of the medium in one liter of distilled water. Add 2 - 3 ml of Glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

WORT BROTH is commonly used for the detection and enumeration of fungi, particularly yeasts in butter, syrups and other materials, especially in the soft drinks industry.

Yeasts grow well on media that contain dextrose or maltose, moreover if the reaction is acidic. This medium has been enriched with the addition of salts and other nutrients. Malt extract and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrin is a carbon source. Dipotassium phosphate is the buffer. Glycerol reduces the water activity from 0.999 to 0.95, thereby reducing bacterial growth.

The low pH inhibits the growth of most bacteria. Incubate at 30ºC and observed after 40 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with Glycerol added, after incubation, at a temperature of 30ºC and observed after 40 - 48 hours.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Saccharomyces cerevisiae ATCC 9763	Good
Saccharomyces uvarum ATCC 9080	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

J. Dairy Science, 16: 141,

RAPP Milchwiss, 29, 341-344 (1974)

XLD AGAR (XYLOSE LYSINE DESOXYCHOLATE AGAR) EUROPEAN PHARMACOPOEIA

CAT. 1080

For the isolation of enteropathogenic bacteria, especially from the genus of *Shigella* and *Salmonella*

FORMULA IN g/l

Lactose Monohydrate	**7.50**	Yeast Extract	**3.00**
Sucrose	**7.50**	Sodium Desoxycholate	**2.50**
Sodium Thiosulfate	**6.80**	Ferric Ammonium Citrate	**0.80**
Sodium Chloride	**5.00**	Phenol red	**0.08**
L-Lysine	**5.00**	Bacteriological Agar	**13.50**
Xylose	**3.50**		

Final pH 7.4 ± 0.2 at 25ºC

PREPARATION

Suspend 55.2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Dispense into appropriate containers. The prepared medium should be stored at 8 - 15°C. The color is reddish-orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

XLD AGAR was developed principally for isolating and differentiating Gram-negative enteric bacilli, particularly *Shigella* and *Salmonella*. It has been shown to be more effective than other enteric differential media.

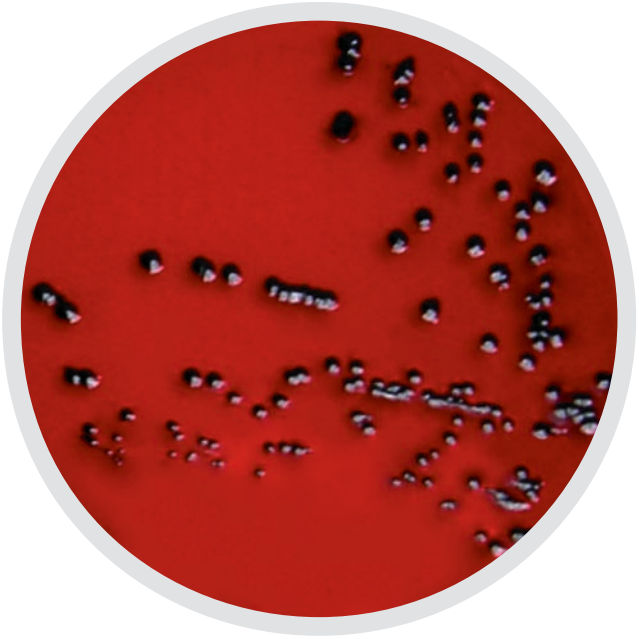
The reactions that take place are the degradation of the three fermentable carbohydrates: xylose, lactose and sucrose, with the production of acid, manifested in the color change from red to yellow. Sodium thiosulfate serves as a reactive substance, with Ferric ammonium citrate as an indicator of the formation of hydrogen sulfide under alkaline conditions. Lysine allows the *Salmonella* group to be differentiated from the non-pathogens since, without it, salmonellae would quickly ferment the xylose and be indistinguishable from non-pathogenic species. Once the salmonellae consume the xylose, lysine is attacked via the enzyme, lysine decarboxylase, with a reversion to an alkaline pH which is similar to the *Shigella* reaction. The bacteria that decarboxylate the L-Lysine to cadaverine are indentified by the presence of a purple- red color around the colonies due to the elevation of the pH. Phenol red is the pH indicator. Yeast extract is the source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium desoxycholate is the selective agent inhibiting Gram-positive microorganisms. Bacteriological Agar is the solidying agent.

The European Pharmacopoeia recommends to inoculate and incubate *Escherichia coli* (indicative) as well as *Salmonella* at 30 - 35ºC during 18 - 48 hours.

It also recommends in Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" to subculture in this medium after incubation in Rappaport Vassiliadis Salmonella Enrichment Broth (**Cat. 1414**), at 30 - 35ºC for 18 - 24 hours and incubate this medium at 30 - 35ºC for 18 - 48 hours.

Interpretation: The possible presence of *Salmonella* is indicated by the growth of well-developed red colonies, with or without black centers. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.



Salmonella typhimurium
ATCC 14028

CHARACTERISTICS OF COLONIES

ORGANISMS	COLONY CHARACTERISTICS
Arizona	Red and transparent with a black center
Citrobacter	Yellow and opaque. Can present a black center and clear edges
E. coli, Enterobacter, Serratia	Yellow and opaque. Zone of yellow precipitation around the colonies
Edwardsiella	Red with a black center and clear edges
Klebsiella	Large, yellow, pale, mucoid and opaque. Zone of yellow precipitation around the colonies
Proteus mirabilis and *P.vulgaris*	Yellow, transparent, with clear edges. Black center especially *P. mirabilis*
Proteus morganii and *P. rettgeri*	Red and transparent

ORGANISMS	COLONY CHARACTERISTICS
Salmonella	Red, transparent with black centers and, if H_2S is produced, yellow edges
Providencia and *Shigella*	Red and transparent

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30 - 35ºC and observed after 18 - 48 hours.

Microorganisms	Growth	Color Colony
Escherichia coli ATCC 25922	Moderate	Yellow (precipitate)
Escherichia coli ATCC 8739	Moderate	Yellow (precipitate)
**Salmonella typhimurium* ATCC 14028	Good	Clear Red (black center)
Shigella flexneri ATCC 12022	Good	Red
Staphylococcus aureus ATCC 25923	Inhibited	
Staphylococcus aureus ATCC 6538	Inhibited	

*According to European Pharmacopoeia Incubate at 30 - 35 ºC for 18 - 48 h

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

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Rollender, W. U. Beckford; R.D. Belsky, B. Krostoff (1969) Comparison of Xylose Lysine desoxycholate agar and MacConkey agar for the isolation of Salmonella and Shigella from clinical specimens (tech. Bull. Reg. Med. Tech, 39 (1) 8-p)

European Pharmacopoeia. 7.0

XLD AGAR (XYLOSE LYSINE DESOXYCHOLATE AGAR) ISO 6579

CAT. 1274

Selective medium for the isolation of *Salmonella* in food

FORMULA IN g/l

Lactose Monohydrate	**7.50**	Yeast Extract	**3.00**
Sucrose	**7.50**	Sodium Desoxycholate	**1.00**
Sodium Thiosulfate	**6.80**	Ferric Ammonium Citrate	**0.80**
Sodium Chloride	**5.00**	Phenol red	**0.08**
L-Lysine	**5.00**	Bacteriological Agar	**13.50**
Xylose	**3.75**		

Final pH 7.4 ± 0.2 at 25ºC

PREPARATION

Suspend 54 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVEARHEATING. DO NOT AUTOCLAVE. Cool to 45 - 50ºC and pour into Petri dishes as soon as it has cooled. The prepared medium should be stored at 8 - 15ºC. The color is red-orange.

The dehydrated medium should be homogeneous, free-flowing and pink in color. If there are any physical changes, discard the medium.

USES

XLD AGAR (XYLOSE LYSINE DESOXYCHOLATE AGAR) is prepared according to formulation of the ISO 6579 norm. It is recommended for the identification of *Salmonella* in food products, after pre-enrichment in a non-selective fluid medium (Buffered Peptone water **Cat. 1402**) and enrichment in a selective fluid medium (Muller Kauffmann Broth Base with Brilliant Green & Novobiocine (MKTTN) (**Cat. 1173**) and Rappaport Soy Broth (Vassiliadis) (**Cat. 1174**)

The reactions are the degradation of the three fermentable carboydrates: xylose, lactose, and sucrose, with the production of acid, manifested in the color change from red to yellow. Sodium thiosulfate serves as a reactive substance with Ferric ammonium citrate as an indicator of the formation of hydrogen sulfide under alkaline conditions. Lysine is included to enable the *Salmonella* group to be differentiated from the non-pathogens since, in its absence, salmonellae would quickly ferment the xylose, making it indistinguishable from non-pathogenic species. After the salmonellae finish all the xylose, the lysine is attacked through the enzyme lysine decarboxylase with a change to an alkaline pH, similar to the *Shigella* reaction. The bacteria that decarboxylate the L-Lysine to cadaverine are indentified by the presence of a purple red color around the colonies due to the elevation of the pH. Phenol red is the pH indicator. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium desoxycholate is the selective agent and is thus inhibitory to

Gram-positive microorganisms. Bacteriological Agar is the solidifying agent.

Inoculate and incubate at 37 ± 1°C for 24 ± 3 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37 ± 1°C and observed after 24 ± 3 hours.

Microorganisms	Growth	Color Colony
Escherichia coli ATCC 25922	Moderate	Yellow (precipitate)
Salmonella typhimurium ATCC 14028	Good	Clear red (black center)
Shigella flexneri ATCC 12022	Good	Red
Staphylococcus aureus ATCC 25923	Inhibited	

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

International Standard UNE-EN-ISO 6579. Food Microbiology for human consumption and Animal Feed. Horizontal Method for the detection of Salmonella spp.

XLT4 AGAR BASE

CAT. 1159

For the selective isolation of pathogenic Enterobacteria, especially *Salmonella*

FORMULA IN g/l

Lactose	**7.50**	Yeast Extract	**3.00**
Sucrose	**7.50**	Proteose Peptone	**1.60**
Sodium Thiosulfate	**6.80**	Ferric Ammonium Citrate	**0.80**
L-Lysine	**5.00**	Phenol red	**0.08**
Sodium Chloride	**5.00**	Bacteriological Agar	**18.00**
Xylose	**3.75**		

Final pH 7.4 ± 0.2 at 25ºC

PREPARATION

Suspend 59 grams of the medium in one liter of distilled water. Add 4.6 ml of XLT4 Supplement (**Cat. 6062**) (26 - 28% solution of 7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt; formerly Tergitol 4). Mix well and heat with frequent agitation until completely dissolved. Boil for one minute. AVOID OVERHEATING. DO NOT AUTOCLAVE. Distribute into sterile Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is orange- red.

The dehydrated medium should be homogeneous, free-flowing and pinkish-beige in color. If there are any physical changes, discard the medium.

USES

XLT4 AGAR BASE with Tergitol 4 supplement, was used in 1990 by Miller and Tate, is a highly selective medium for isolating *Salmonella* from competing bacteria such as *Proteus*. They reported isolation of non-typhi *Salmonella* from chicken and farm environmental drag-swab samples from heavily contaminated samples.

XLT4 Agar can be used clinically to screen stool samples for non-typhoid *Salmonella*.

The medium allows the optimum growth of *Salmonella*. Differentiation of *Salmonella* from other organisms in this medium is based on the fermentation of carbohydrates (Lactose, Xylose, Sucrose) with the resulting production of hydrogen sulfide. H_2S production is detected by the reaction of the iron salt, colonies appearing black or black-centered. Sodium thiosulfate and Ferric ammonium citrate are the H_2S indicators. The bacteria that decarboxylate the L-Lysine to cadaverine are identified by the presence of a purple-red color around the colonies due to the elevation of the pH. Phenol red is the pH indicator. Sodium Thiosulfate is also added as a source of inorganic sulfur. Yeast extract and Peptone are a nitrogen and amino acids source. Bacteriological agar is the solidifying agent. XLT4 supplement is added to inhibit the growth of non-*Salmonella* organisms.

Typical *Salmonella* colonies (H_2S-positive) appear black or black-centered with a yellow periphery after 18 - 48 hours of incubation at a temperature of 35 ± 2°C. Upon continued incubation, the colonies become entirely black or pink to red with black centers. Colonies of H_2S-negative *Salmonella* strains appear pink-yellow.

Most *Citrobacter* colonies are yellow without evidence of blackening. The growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. The growth of *Proteus, Pseudomonas* and *Yersinia enterocolitica* is markedly to completely inhibited. *Shigella* species are partially inhibited and colonies appear red.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with the supplement added after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth	Color Colony
Enterobacter aerogenes ATCC 13048	Moderate	Yellow
Escherichia coli ATCC 25922	Moderate	Yellow
Proteus mirabilis ATCC 14273	Inhibited	Yellow

Microorganisms	Growth	Color Colony
Salmonella typhimurium ATCC 14028	Good	Black center
Salmonella enteritidis ATCC 13076	Good	Black center
Shigella sonnei ATCC 11060	Inhibited	Red
Shigella flexneri ATCC 12022	Inhibited	Red

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Miller, R. G., and C. R. Tate. 1990. XLT4: A highly selective plating medium for the isolation of Salmonella. The Maryland Poultryman, April:2-7.

Tate, C. R., R. G. Miller, and E. T. Mallinson. 1992. Evaluation of two isolation and two non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. J. Food Prot. 55:964-967.

Dusch, H., and M. Altwegg. 1995. Evaluation of five new plating media for the isolation of Salmonella species. J. Clin. Microbiol. 33:802-804

YEAST EXTRACT AGAR FOR MOLDS

CAT. 1312

For the cultivation of yeast and molds from diverse materials, especially milk and dairy products

FORMULA IN g/l

Dextrose	**10.00**	Bacteriological Agar	**20.00**
Yeast Extract	**5.00**		
Final pH 6.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 35 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YEAST EXTRACT AGAR FOR MOLDS is suitable to cultivate molds and yeasts from milk and dairy products.

Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Dextrose is the carbohydrate energy source. Bacteriological agar is the solidifying agent. The high dextrose concentration makes this medium selective for yeasts and molds. For increased selectivity, acidify the medium or add antibiotics with the aseptic technique. It is not recommended to heat the acidified agar medium.

The inoculation method can be carried out either by flooding or on the surface, depending on the purpose for which the medium is intended. Incubation time is 7 days at a temperature of 28°C and in an aerobic environment.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 28°C and observed after 7 days.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Staphylococcus aureus ATCC 25923	Good
Candida albicans ATCC 10231	Good
Aspergillus brasiliensis ATCC 16404	Good
Penicillium spp.	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Cooke, W.B. and A. R. Brazis. 1968. Occurrence of molds and yeasts in dairy products. Mycopathol. Mycol. Appl. 35:281 -289. Overcase, W.W. and D:J. Weakley. 1969. An aureomycin-rose Bengal agar for enumeration of yeast and mold in cottage cheese. International Dairy Federation. Standard Method ISO/DIS 6611.

Koburger, J.A.. 1970. Fungi in foods: 1. Effect of inhibitor and incubation temperature on enumeration. J. Milk Food Technol. 33:433-434.

YEAST EXTRACT AGAR ISO 6222

CAT. 1049

For the enumeration of a wide spectrum of bacteria, yeasts and molds in water

FORMULA IN g/l

Tryptone	**6.00**	Bacteriological Agar	**15.00**
Yeast Extract	**3.00**		
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 24 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave

at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YEAST EXTRACT AGAR ISO 6222 is a medium rich in nutrients that permits the recovery of a wide spectrum of bacteria, yeasts and molds. This medium is recommended by ISO 6222 for the plate count of microorganisms in all types of water, including for human consumption.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the water-soluble portion of hydrolyzed yeast and is a source of vitamins, particularly of the B-group. Bacteriological agar is the solidifying agent.

The norm ISO 6222 recommend this medium for the enumeration of culturable microorganisms in potable water at 36°C and 22°C.

Prepare decimal dilutions and make plate counts using the pour plate method. Incubate two series of plates, one at 36± 2°C for 44 ± 4 hours and the other plates at 22 ±2°C for 68 ± 4 hours.

Count the colonies present on plates containing less than 300 colonies. Enumeration can be done using the automated colony counters.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 36 ± 2°C and observed after 44 ± 4 hours, and at a temperature of 22 ± 2°C and observed after 68 ± 4 hours.

Microorganisms	Growth 36ºC	Growth 22ºC
Escherichia coli ATCC 25922	Good	Inhibited
Staphylococcus aureus ATCC 25923	Good	Inhibited
Candida albicans ATCC 10131	Inhibited	Good
Aspergillus brasiliensis ATCC 16404	Inhibited	Good
Penicillium spp.	Inhibited	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

ISO 6222 Water Quality. Enumeration of culturable micro-organisms- colony count by inoculation in a nutrient agar culture medium.

YEAST EXTRACT SOY AGAR

CAT. 1097

For the selective isolation of dermatophytes and other pathogenic fungi in clinical samples

FORMULA IN g/l

Dextrose	**40.00**	Chloramphenicol	**0.05**
Soy Peptone	**10.00**	Streptomycin	**0.03**
Yeast Extract	**5.00**	Bacteriological Agar	**17.00**

Final pH 6.6 ± 0.2 at 25ºC

PREPARATION

Suspend 72 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YEAST EXTRACT SOY AGAR is used for the selective isolation of dermatophytes and other pathogenic fungi in clinical samples. This medium is becoming a preferred method as the use of antibiotics for suppressing bacteria results in a better recovery of fungal cells, which are sensitive to an acid environment.

Yeast Extract Soy Agar is a modification of Sabouraud Medium and was formulated by Carmichael and Claus for the selective isolation of *Trychophyton verrucossum* as well as other fungi associated with contagious diseases. Yeast Extract Soy Agar contains Streptomycin and Chloramphenicol, antibiotics that inhibit bacterial growth but allow for the detection of pathogenic fungi.

Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological agar is the solidifying agent.

Inoculate plates with sample and incubate at 25 - 30°C for up to 2 - 5 days.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30°C and observed after 2 - 5 days.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Escherichia coli ATCC 25922	Inhibited

Microorganisms	Growth
Trychophyton mentagrophytes ATCC 9533	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Cooke, W.B., and A. R. Brazis. 1968. Occurrence of molds and yeasts in dairy products. Mycopathol. Mycol. Appl. 35: 281-289. International Dairy Federation. Standard Methods ISO/DIS 6611.

Beuchat, L.R. 1979. Comparison of acidified and antibiotic-supplemented potato dextrose agar from three manufactures for its capacity to recover fungi from foods. J. Food Prot. 42: 427-428.

YEAST EXTRACT SOY BROTH (TSB NON-ANIMAL SOURCE)

CAT. 1268

For general laboratory use and to cultivate fastidious microorganisms

FORMULA IN g/l

Soy Peptone	**11.00**	Dipotassium Phosphate	**2.50**
Yeast Extract	**8.80**	Dextrose	**2.50**
Sodium Chloride	**5.00**		
Final pH 7.3 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YEAST EXTRACT SOY BROTH, a general purpose medium, is similar to Trypticasein Soy Broth but with all its components of vegetable origin. It is a medium rich in nutrients for general use in Microbiological laboratories. It supports the abundant growth of fastidious microorganisms such as pneumococci, streptococci, Neisseriae etc.

Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dipotassium phosphate acts as a buffer system.

Incubate at 35 ± 2ºC and observed after 18 - 48 hours.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Bacillus subtilis ATCC 6633	Good
Candida albicans ATCC 10231	Good
Staphylococcus aureus ATCC 6538	Good
Aspergillus brasiliensis ATCC 16404	Good
Streptococcus pneumonie ATCC 6303	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Gibbons and McDonald. J. Bacteriol., 80:164. 1960. Havens and Benham. A. Med. Tech., 23:305. 1957.

Muey and Edward. Proc. Soc. Exper. Biol. and Med., 97:550. 1958. Steward and Kelly. J. Bacteriol., 77:101. 1959.

MacFaddin, J.D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 797. vol. 1. Williams & Wilkins, Baltimore, MD.

YEAST MOLD AGAR

CAT. 1194

For the cultivation of yeast and molds

FORMULA IN g/l

Dextrose	**10.00**	Malt Extract	**3.00**
Peptone	**4.00**	Bacteriological Agar	**20.00**
Yeast Extract	**3.00**		
Final pH 6.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 50º, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YEAST MOLD AGAR is a medium used for the isolation and cultivation of yeast, molds and aciduric microorganisms.

Peptone and Malt extract provides the carbon, protein and nutrient sources required for the growth of microorganisms. Malt extract is particularly suitable for yeasts and molds as it contains a high concentration of maltose (39 - 42%) and other saccharides as energy sources. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological agar is the solidifying agent. The high dextrose concentration and acidic pH make this medium selective for fungi. If desired, the pH of the medium can be adjusted to 3.0 - 4.0 in order to increase the selectivity of the medium. Antibiotics like chloramphenicol can also be added. Once the pH is adjusted, the agar must not be reheated.

Inoculate and incubate at 30ºC ± 2ºC for 48 - 72 hours.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 30ºC ± 2ºC and observed after 48 - 72 hours.

Microorganisms	Growth
Aspergillus brasiliensis ATCC 16404	Good
Candida albicans ATCC 10231	Good
Lactobacillus casei ATCC 7649	Moderate
Saccharomyces cerevisiae ATCC 9763	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Jong. S.S, and M.J.Edwars 1991, American Type Culture Collection Catalog of filamentog fungi 18 the. American type Collection, Rockville, MD.

YERSINIA SELECTIVE AGAR BASE ISO 10273

CAT. 1126

Selective medium for *Yersinia enterocolitica* used with supplement

FORMULA IN g/l

D-Mannitol	**20.00**	Sodium Desoxycholate	**0.50**
Enzymatic Digest of Gelatin	**17.00**	Neutral Red	**0.03**
Enzymatic Digest of Casein and Animal Tissues	**3.00**	Magnesium Sulfate Anhydrous	**0.005**
Yeast Extract	**2.00**	Crystal Violet	**0.001**
Sodium Pyruvate	**2.00**	Bacteriological Agar	**12.50**
Sodium Chloride	**1.00**		
Final pH 7.4 ± 0.2 at 25ºC			

PREPARATION

Suspend 29 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC and aseptically add one vial of Yersinia selective supplement (**Cat. 6033**), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8 - 15°C. The color is purple-red.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Yersinia Selective Supplement (CIN) Cat. 6033

(Composition: each vial for 500 ml)

Cefsulodin ..... 7.5 mg

Irgasan ..... 2.0 mg

Novobiocine ..... 1.25 mg

USES

Yersinia Selective Agar Base ISO 10273 is a selective and differential medium when used with supplement. The formula is based on CIN (Cefsulodin-Irgasan-Novobiocine) Agar of Schiemann, and is recommended by ISO 10273 for

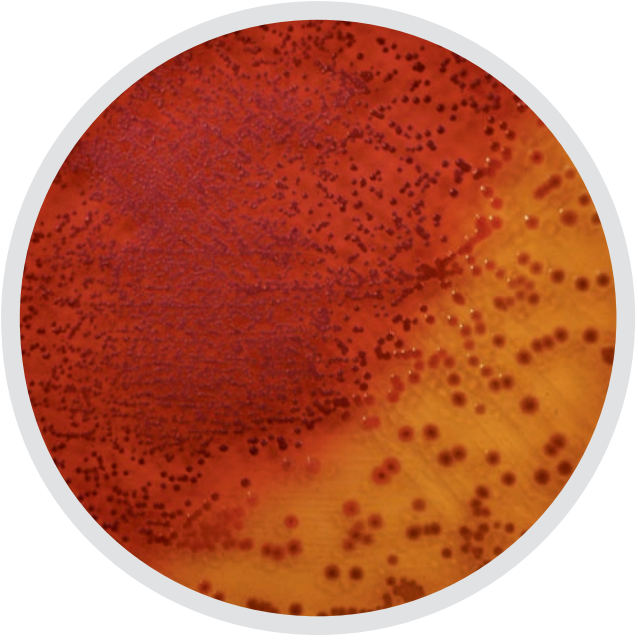
the isolation of *Yersinia enterocolitica* from a variety of clinical samples and food.

The accompanying flora is inhibited by the addition of antibiotics to the supplement. *Yersinia* growth is promoted by pyruvate as well as by the high nutrients content in the base. *Yersinia* degrades the mannitol in the medium to an acid; the colonies turning a red color due to the neutral red indicator.

D-Mannitol is the fermentable carbohydrate. Mannitol fermentation in the presence of neutral red produces a characteristic "bull's-eye" colony, colorless with a red center. Enzymatic digest of gelatin and Enzymatic digest of casein and animal tissues provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium pyruvate is added as a source of energy and as a protective substance in order to overcome oxygen toxicity biologically produced by the organisms. Selective inhibition of Gram-negative and Gram-positive organisms is obtained through crystal violet, sodium desoxycholate and Irgasan (triclosan). Cefsulodin and novobiocin improve the inhibition of normal enteric organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Magnesium sulphate is a magnesium ion required in a large variation of enzymatic reactions, including DNA replication. Neutral red is the pH indicator.

ISO 10273 norm recommends the use of this medium for the detection of presumptive pathogenic *Yersinia enterocolitica*, after incubation of the enrichment media PSB, at a temperature of 22ºC to 25 ºC for 48 to 72 hours or 5 days without agitation, and ITC medium at a temperature of 25 ºC for 48 hours. Inoculate and incubate at 30ºC for 24 - 48 hours.

Typical colonies of *Y. enterocolitica*, will appear colorless, with dark red centers, like bull's eye, surrounded by a transparent border.



Yersinia enterocolitica
ATCC 27729

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with Yersinia Selective Supplement (**Cat. 6033**) added, after incubation at a temperature of 30ºC and observed after 24 - 48 hours.

Microorganisms	Growth
Yersinia enterocolitica ATCC 27729	Good
Escherichia coli ATCC 25922	Inhibited

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

ISO

American Public Health Association: Compendium of Methods for the microbiological Examination of Foods.

Schiemann, D:A: Synthesis of a selective agar medium for Yersinia enterocolitica.- Canad.J.Microbiol,25 1298-1304

ISO 10273 Microbiology of food and animal stuffs. Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica

Molecular Biology Products

DEHYDRATED CULTURE MEDIA

LB AGAR (LENNOX)*

CAT. 1083

Recommended medium for the test of *Escherichia coli* in molecular genetics studies

FORMULA IN g/l

Tryptone	**10.00**	Sodium Chloride	**5.00**
Yeast Extract	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 35 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LB AGAR (LENNOX) is nutritionally rich medium developed by Lennox for the growth and maintenance of pure culture of recombinant strains of *E. coli*.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. If desired, antibiotics can also be added.

In LB Agar (Lennox) sodium chloride level contains is ten times higher than in Luria Agar (Miller's Modification) (**Cat. 1308**) and a half of the level found in Luria Agar (Miller's LB Agar) (**Cat. 1552**). This allows selecting the optimal salt concentration medium for a specific strain.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Escherichia coli ATCC 11775	Good
Escherichia coli ATCC 33694	Good
Escherichia coli ATCC 33849	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

*** See Composition Comparison for all LB/Luria Media Table**

BIBLIOGRAPHY

Atlas, R.M., L.C. Parks (1993) Handbook of Microbiological Media. CRC Press, Inc. London1

Lennox. 1955. Virology 1:190.

Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

LB BROTH (LENNOX)*

CAT. 1231

Recommended medium for the test of *Escherichia coli* in molecular genetics studies

FORMULA IN g/l

Tryptone	**10.00**	Yeast Extract	**5.00**
Sodium Chloride	**5.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 20 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is clear amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LB BROTH (LENNOX) is nutritionally rich medium developed by Lennox for the growth and maintenance of pure culture of recombinant strains of *E. coli*.

It is a rich growth medium that contains all the nutritional requirements for *E. coli* strains. In the formula, Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

LB Broth (Lennox) contains ten times the sodium chloride level of Luria Broth (Miller's Modification) (Cat. 1266) and a half of the level found in Luria Broth (Miller's LB Broth) (Cat. 1551). This allows selecting the optimal salt concentration medium for a specific strain.

Bacteria that contain plasmids tend to grow best in broth that has between 5 and 10 g of salt. For a faster growth, the medium can be supplemented with 0.1% glucose or 0.4% glycerol. Various cofactors may also need to be added to the broth if working with certain types of bacteriophages. For example, bacteriophage labmda requires an excess of magnesium in the broth to properly infect bacteria.

The cultivation in LB Broth allows the cells with an insert plasmid, to begin to express the genes on the transformed plasmid, including the antibiotic resistance gene. If the transformed *E. coli* are plated directly onto selective agar media (LB Agar containing antibiotic), fewer transformed colonies will appear per ml plated. Growing the transformed cells in LB broth will increase the number of transformed cells per ml.

To select the bacteria with the plasmid, it is necessary to subcultivate an inoculum from LB Broth to a LB Agar plate with the antibiotic added.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Escherichia coli ATCC 11775	Good
Escherichia coli ATCC 33694	Good
Escherichia coli ATCC 33849	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

*** See Composition Comparison for all LB/Luria Media Table**

BIBLIOGRAPHY

Atlas, R.M., L.C. Parks (1993) Handbook of Microbiological Media. CRC Press, Inc. London

Lennox. (1955). Virology 1:190.

Sambrook, Fritsch and Maniatis. (1989). Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

LURIA AGAR (MILLER'S LB AGAR)*

CAT. 1552

For *E. coli* in molecular genetics studies

FORMULA IN g/l

Tryptone	**10.00**	Yeast Extract	**5.00**
Sodium Chloride	**10.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 40 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LURIA AGAR (MILLER'S LB AGAR) is based on LB Medium as described by Miller for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.

It is nutritionally rich medium designed for growth of pure cultures of recombinant strains. *E. coli* grows faster because the Tryptone and Yeast extract supply essential growth factors such as nitrogen, carbon, sulfurs, minerals and vitamins, particularly B group and other metabolites that the microorganism would otherwise have to synthesize. Sodium Chloride supplies essential electrolytes for transport and osmotic balance.

If desired aseptically add 10 ml of sterile 20% glucose solution and mix thoroughly for a better growth.

In Luria Agar (Miller's LB Agar) sodium chloride level is five times higher than in LB Agar (Lennox) (**Cat. 1083**) and twenty times higher than in Luria Agar (Miller's Modification) (**Cat. 1308**). This allows selecting the optimal salt concentration medium for a specific strain.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Escherichia coli ATCC 11775	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

*** See Composition Comparison for all LB/Luria Media Table**

BIBLIOGRAPHY

Atlas, R.M., L.C.Parks (1993) Handbook of Microbiological Media. CRC Press, Inc. London

The condensed protocols from molecular cloning: a laboratory manual/ Joseph Sambrook, David W .Russell

LURIA AGAR (MILLER'S MODIFICATION)*

CAT. 1308

Recommended medium for *Escherichia coli* studies in molecular genetics

FORMULA IN g/l

Tryptone	**10.00**	Sodium Chloride	**0.50**
Yeast Extract	**5.00**	Bacteriological Agar	**15.00**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 30.5 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LURIA AGAR (Millers modification) is based on LB Medium as described by Miller. Its modification consists in a minimal concentration of sodium chloride. It is used for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures. It is used for strains in which the optimal concentration of salt is 0.5 g / l.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological Agar is the solidifying agent. If desired, antibiotics can also be added.

Luria Agar (Miller's Modification) contains ten times less sodium chloride level of LB Agar (Lennox) (**Cat. 1083**) and twenty times less found in Luria Agar (Miller's LB Agar) (**Cat. 1552**). This allows selecting the optimal salt concentration medium for a specific strain.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 23724	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

*** See Composition Comparison for all LB/Luria Media Table**

BIBLIOGRAPHY

Miller J. H.: Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972).

LURIA BROTH (MILLER'S LB BROTH)*

CAT. 1551

For *E. coli* in molecular genetics studies

FORMULA IN g/l

Tryptone	**10.00**	Yeast Extract	**5.00**
Sodium Chloride	**10.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 25 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LURIA BROTH (Miller's LB Broth) is based on LB Medium as described by Miller. It is suitable for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.

It is a nutritive rich medium designed for growth of pure cultures of recombinant strains. *E. coli* grows quicker because the Tryptone and Yeast extract supply essential growth factors such as nitrogen, carbon, sulfurs, minerals and vitamins, particularly

B-group and other metabolites that the microorganism, would otherwise, have to synthesize. Sodium Chloride supplies essential electrolytes for transport and osmotic balance.

In Luria Broth (Miller's LB Broth) sodium chloride level is five times higher than in LB Broth (Lennox) (**Cat. 1231**) and twenty times higher than in Luria Broth (Miller's Modification) (**Cat. 1266**). This allows selecting the optimal salt concentration medium for a specific strain.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Good
Escherichia coli ATCC 11775	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

*** See Composition Comparison for all LB/Luria Media Table**

BIBLIOGRAPHY

Atlas, R. M., L.C. Parks (1993) Handbook of Microbiological Media. CRC Press, Inc. London.

The condensed protocols from molecular cloning: a laboratory manual/ Joseph Sambrook, David W .Russell

LURIA BROTH (MILLER'S MODIFICATION)*

CAT. 1266

For *E. coli* studies in molecular genetics

FORMULA IN g/l

Tryptone	**10.00**	Sodium Chloride	**0.50**
Yeast Extract	**5.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 15.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

LURIA BROTH (MILLER'S MODIFICATION) is based on LB Medium as described by Miller. Its modification consists in a minimal concentration of sodium chloride. It is suitable for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures. This is a nutritionally rich media designed for growth of pure cultures of recombinant strains. *E. coli* grows more rapidly because they provide the cells with amino acids, nucleotide precursors, vitamins and other metabolites that the microorganism would otherwise have to synthesize.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. If desired, antibiotics can also be added.

In Luria Broth (Miller's Modification) sodium chloride level is ten times lower than in LB Broth (Lennox) (**Cat. 1231**) and twenty times lower than in Luria (Miller's LB Broth) (**Cat. 1551**). This allows selecting the optimal salt concentration medium for a specific strain.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 23724	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

*** See Composition Comparison for all LB/Luria Media Table**

BIBLIOGRAPHY

Miller J. H.: Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972).

COMPOSITION COMPARISON FOR ALL LB/LURIA MEDIA

INGREDIENTS IN g/l	CAT. No. 1083 *LB AGAR (LENNOX)*	CAT. No. 1552 *LURIA AGAR (MILLER'S LB)*	CAT. No. 1308 *LURIA AGAR (MILLER'S MODIFICATION)*	CAT. No. 1432 *sLB AGAR**	CAT. No. 1231 *LB BROTH (LENNOX)*	CAT. No. 1551 *LURIA BROTH (MILLER'S LB)*	CAT. No. 1266 *LURIA BROTH (MILLER'S MODIFICATION)*	CAT. No. 1163 *sLB BROTH***
Tryptone (Pancreatic Digest of Casein)	10	10	10	-	10	10	10	-
Yeast Extract	5	5	5	-	5	5	5	-
Sodium Chloride	5	10	0,5	-	5	10	0,5	-
Bacteriological Agar	15	15	15	-	-	-	-	-
pH	7	7	7	7	7	7	7	7
Grams/liter	35	40	30,5	55	20	25	15,5	40

Pack Sizes: bulk 5, 10, 25 & 50 Kg.

* Contains a blend of special peptones, yeast extract, salts and agar. ** Contains a blend of special peptones, yeast extract, and salts.

NZCYM BROTH

CAT. 1549

For the cultivation of recombinant *E. coli* strains

FORMULA IN g/l

Pancreatic Digest of Casein	**10.00**	Yeast Extract	**5.00**
Sodium Chloride	**5.00**	Magnesium Sulfate (Anhydrous)	**0.98**
Casaminoacids	**1.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 22 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

NZCYM BROTH is used as an enrichment medium for the cultivation of recombinant strains of *E. coli* and propagating bacteriophages developed by Blattner and colleagues.

E. coli grows fast in this enrichment medium, which supplies the amino acids, vitamins and other metabolites as nucleotide precursors and other factors that otherwise would be synthesized by the cell. Casein digest, Yeast extract and Casaminoacids provide the necessary nutrients and cofactors required for excellent growth of recombinant strains of *E. coli*. Magnesium sulfate is the magnesium ions font required in a big variation of enzymatic reactions, including DNA replication.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 23724	Good
Escherichia colli ATCC 33526	Good
Escherichia coli ATCC 53868	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Blattner, F. R., B. G Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies. 1977. Charon phages: Safer derivatives of bacteriophage Lambda for DNA cloning. Science 196:161.

The condensed protocols from molecular cloning: a laboratory manual/ Joseph Sambrook, David W .Russell

RM BASE AGAR MEDIUM

CAT. 1542

Solid medium for the maintenance and propagation of the promoter PL in the *E. coli* strains GI724, GI826 and GI698

FORMULA IN g/l

Casaminoacids	**20.00**	Sodium Chloride	**0.50**
Disodium Phosphate	**6.00**	Magnesium Chloride	**0.095**
Monopotassium Phosphate	**3.00**	Bacteriological Agar	**15.00**
Ammonium Chloride	**1.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 45.6 grams of the dehydrated medium in 900 ml of distilled water, add 20 ml of 50% glycerol and adjust to a final volume of 1000 ml. Mix well. Heat with frequent agitation until complete dissolution. Sterilize in the autoclave at 121 °C for 15 minutes. Add 1 ml of 100 µg/ml of ampicillin under sterile conditions and mix well. Cool to 45 - 50°C and pour into Petri dishes. The prepared medium must be stored at 8 - 15ºC. The color is amber.

The dehydrated medium should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the medium.

USES

RM BASE AGAR MEDIUM is used for the maintenance and propagation of the promoter PL in *E. coli* strains GI724, GI826 and GI698 and to increase the yield of plasmid for sequencing positive clones. These strains have the gene Lambda cI repressor, under the control of the promoter tryptophane inducible, trp. This medium has low tryptophane levels.

Casaminoacids provide the necessary nutrients and cofactors required for excellent growth of recombinant strains of *E. coli*. Due to its higher degree of digestion, casaminoacids are an excellent source of free aminoacids. Phosphates act as a buffer system. Ammonium chloride and magnesium sulfate provide essential ions. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. To promote growth it may require the addition of glucose.

RM solid medium is used for the isolation of recombinant strains.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC GI724	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

La Vallie, E, R. et al.(1 992) Bio/Technology 11: 187-193. Mieschendahl, M. et al.(1 996) Bio/Technology 4: 802-808.

RM BASE MEDIUM

CAT. 1538

Liquid medium for the maintenance and propagation of the promoter PL in the *E. coli* strains GI724, GI826 and GI698

FORMULA IN g/l

Casaminoacids	**20.00**	Ammonium Chloride	**1.00**
Disodium Phosphate	**6.00**	Sodium Chloride	**0.50**
Monopotassium Phosphate	**3.00**	Magnesium Chloride	**0.095**
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 30.6 grams of the dehydrated medium in 900 ml of distilled water, add 20 ml of 50% glycerol and adjust to a final volume of 1000 ml. Mix well. Heat with frequent agitation until complete dissolution. Distribute in appropriate containers and sterilize in the autoclave at 121 °C for 15 minutes. Add 1 ml/liter of 100 µg/ml of ampicillin under sterile conditions and mix well. The prepared medium must be stored at 2 - 8°C. The color is amber.

The dehydrated medium should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the medium.

USES

RM BASE MEDIUM is used for the maintenance and propagation of the promoter PL in *E. coli* strains GI724, GI826 and GI698 and to increase the yield of plasmid for sequencing positive clones. These strains have the gene Lambda cI repressor, under the control of the promoter tryptophane inducible, trp. This medium has low tryptophane levels.

Casaminoacids provides the necessary nutrients and cofactors required for excellent growth of recombinant strains of *E. coli*. Due to its higher degree of digestion, casaminoacids are an excellent source of free aminoacids. Phosphates act as a buffer system. Ammonium chloride and magnesium sulfate provide essential ions. Sodium chloride supplies essential electrolytes for transport and osmotic balance. To promote growth it may require the addition of glucose.

RM liquid medium is used for the massive growth of recombinant strains.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC GI724	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

La Vallie, E, R. et al.(1 992) Bio/Technology 11: 187-1 93. Mieschendahl, M. et al.(1 996) Bio/Technology 4: 802-808.

sLB AGAR

CAT. 1432

Medium designed to increase bacterial growth and leads to high yields of low copy plasmids and extra high yields of high copy plasmids

FORMULA IN g/l

Special Peptone Mixture	Salts
Yeast Extract	Bacteriological Agar
Final pH 7.0 ± 0.2 at 25ºC	

PREPARATION

Suspend 55 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

sLB AGAR is a new medium to complete the sLB product line and is the ideal medium to be used along with sLB Broths (**Cat. 1163, Cat. 1199**) , which have been especially designed to give high yields of low copy plasmids and extremely high yields of high copy plasmids, or with the rest of molecular biology brothers. A higher number of colonies are obtained, so a better recuperation of plasmid can be achieved.

The special peptone mixture, yeast extract, agar and salts supply essential growth factors such as nitrogen, carbon, sulfurs, minerals and vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes: Sodium ions for transport and osmotic balance. Bacteriological agar is the solidifying agent.

CELL GROWING TEST

To make this test it must be prepared a cell culture by inoculating a 15 ml tube of sLB Broth with *E. coli* sample. This tube is incubated at 37ºC for 24 hours without aeration. A 10 ul of an aliquot of a 104 cells/ml was distributed in the sLB agar plates using a glass "hockey stick" spreader. The inoculated plates were incubated at 37ºC overnight.

After the incubation the colonies were counted and two colonies were measured its diameter.

Microorganisms	Plate 1	Plate 2	Range among
Average Colony Diameter in mm	1.70	3.80	1.0 - 4.0 mm
Escherichia coli ATCC C600	410	480	400 - 500 mm

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol. 1. Greene Publishing Associates, Inc., Brooklyn, N.Y.

sLB BROTH

CAT. 1163

Medium designed to increase bacterial growth and leads to high yields of low copy plasmids and extra high yields of high copy plasmids

FORMULA IN g/l

Special Peptone Mixture	Salts
Yeast Extract	
Final pH 7.0 ± 0.2 at 25ºC	

PREPARATION

Suspend 40.0 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in tubes and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color is amber, slightly opalescent.

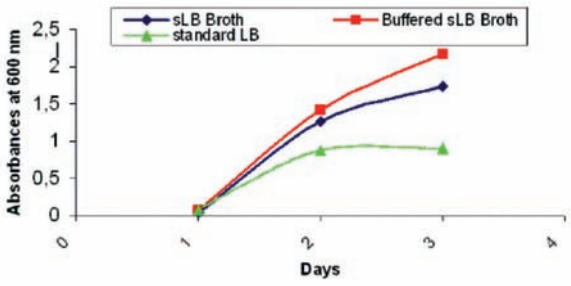
The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

sLB BROTH has been formulated to significantly increase cellular density when compared to the traditional LB Broth. In the standard LB Broth, *E. coli* cells reach an abrupt stationary phase upon consumption of nutrients contained in the medium. Cell multiplication is stopped and some cell death and plasmid loss occurs.

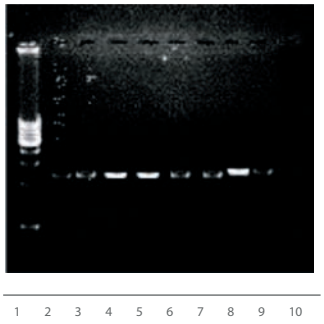
Peptone mixture, Yeast extract and salts allow recombinant *E. coli* cells to have a higher growth. At the end of the log phase replication continues, thus obtaining higher DNA plasmid yields. sLB Broth cultures have shown cell stability up to 3 days without cell death, being this one a more convenient medium that eliminates the need of constant attention.

Fig 1: *E. coli* DH5's growth during 3 days at 37°C.



E. coli's growth is higher in sLB and buffered sLB Broths than in standard LB after 3 days in the media at 37ºC. Extracted pUC1 9 plasmid DNA after 3 days incubation in different media is shown in the following picture.

Fig 2: pUC1 9 Plasmid DNA extracted from *E. coli* DH5_ cells after growth in different broths during 3 days.



Lane 1: DNA Ladder 1Kb.

Lanes 2 & 3: LB Broth Brand A

Lanes 4 & 5: Buffered sLB

Lanes 6 & 7: LB Broth Brand B

Lanes 8 & 9: sLB

Lane 10: Negative Control

CELL GROWTH AND PLASMID YIELD TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C and observed after 24, 48 and 72 hours.

Microorganisms Growth (Absorbance at 600 nm)	Day 1	Day 2	Day 3
Escherichia coli DH5 a	0.047	1.262	1.738

Cell pellet weight / 30 ml: 0.12 grams (range among 0.1 - 0.3 grams)

TSP Yield per 1.5 ml: 10.03 µg (usually up to 15 µg per 1.5 ml of broth)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols inmolecular biology, vol. 1. Greene Publishing Associates, Inc., Brooklyn, N.Y.

sLB BROTH (BUFFERED)

CAT. 1199

Medium designed to increase bacterial growth and leads to high yields of low copy plasmids and extra high yields of high copy plasmids

FORMULA IN g/l

Special Peptone Mixture	Salts
Yeast Extract	
Final pH 7.0 ± 0.2 at 25ºC	

PREPARATION

Suspend 54.8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense in tubes and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 4°C. The color is amber, slightly opalescent.

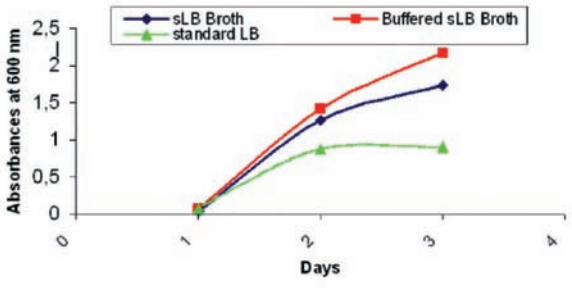
The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

sLB BROTH (BUFFERED) has been designed to increase bacterial growth and leads to high yields of low copy plasmids and extra high yields of high copy plasmids. The medium pH is stabilized using a biological buffer system. In the standard LB Broth, *E. coli* cells reach an abrupt stationary phase upon consumption of nutrients contained in the medium. Cell multiplication is stopped and some cell death and plasmid loss occurs.

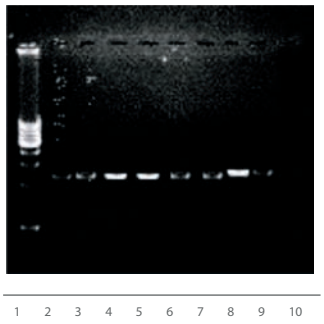
Peptone mixture, Yeast extract and salts allow recombinant *E. coli* cells to have a higher growth. At the end of the log phase replication continues, thus obtaining higher DNA plasmid yields. sLB Broth cultures have shown cell stability up to 3 days without cell death, being this one a more convenient medium that eliminates the need of constant attention.

Fig 1: *E. coli* DH5's growth during 3 days at 37°C.



E. coli's growth is higher in sLB and buffered sLB Broths than in standard LB after 3 days in the media at 37ºC. Extracted pUC1 9 plasmid DNA after 3 days incubation in different media is shown in the following picture.

Fig 2: pUC1 9 Plasmid DNA extracted from *E. coli* DH5_ cells after growth in different broths during 3 days.



Lane 1: DNA Ladder 1Kb.

Lanes 2 & 3: LB Broth Brand A

Lanes 4 & 5: Buffered sLB

Lanes 6 & 7: LB Broth Brand B

Lanes 8 & 9: sLB

Lane 10: Negative Control

CELL GROWTH AND PLASMID YIELD TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 37°C and observed after 24, 48 and 72 hours.

Microorganisms Growth (Absorbance at 600 nm)	Day 1	Day 2	Day 3
Escherichia coli DH5 a	0.064	1.423	2.160

Cell pellet weight / 30 ml: 0.19 grams (range among 0.1 - 0.3 grams)

TSP Yield per 1.5 ml: 13.07 µg (usually up to 15 µg per 1.5 ml of broth)

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols inmolecular biology, vol. 1. Greene Publishing Associates, Inc., Brooklyn, N.Y.

SOB MEDIUM

CAT. 1541

For the cultivation of recombinant strains of *E.coli*

FORMULA IN g/l

Tryptone	**20.00**	Sodium Chloride	**0.50**
Yeast Extract	**5.00**	Magnesium Chloride	**0.96**
Potassium Chloride	**0.186**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 26.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SOB MEDIUM is a nutrient rich medium for the preparation and transformation of competent cells. The transformation requires perforation of the bacteria to allow the introduction of alien DNA inside the cell. In order to survive this process the competent cells need an isotonic rich medium. SOB medium is used in the final stage of the transformation and it can be prepared by adding aseptically 20 ml of a sterile 20% glucose solution to the sterile medium. This addition supplies a carbon and energy source that *E. coli* uses to repair the perforation as well as for replication.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride and Potassium chloride supplies essential electrolytes for transport and osmotic balance. Magnesium sulfate is a source of magnesium ions.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 53868	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Josep Sambrook, David W .Russell. The condese protocols from molecular cloning a laboratory manual.

TERRIFIC BROTH

CAT. 1246

Medium used with glycerol for the cultivation of recombinant strains of *E. coli*.

FORMULA IN g/l

Yeast Extract	**24.00**	Dipotassium Phosphate	**12.54**
Tryptone	**12.00**	Monopotassium Phosphate	**2.31**
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 50.8 grams of the medium in 900 ml of distilled water. Mix well. Add 4 ml of glycerol and adjust to a final volume of 1000 ml. and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TERRIFIC BROTH is a medium which supports a high cell density and, in the case is formulated for optimum growth of *E. coli*, maintains growth in the logarithmic phase for a long time. As a result it yields a greater number of recombinant proteins and plasmic DNA. In some circumstances it substitutes LB Broth (Lennox) (**Cat. 1231**) used in genetic studies.

Tryptone provide nitrogen, vitamins, minerals and amino acids essential for growth Yeast extract is source of vitamins, particularly the B-group. Potassium phosphates act as a buffer system to prevent cell death.

The source of carbohydrates and carbon is glycerol that is not fermented to acetic acid as glucose and does not lead to confusing results.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 23724	Good
Escherichia coli ATCC 33694	Good
Escherichia coli ATCC 33849	Good
Escherichia coli ATCC 53868	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Josep Sambrook, David W. Russell. The condese protocols from molecular cloning: a laboratory manual.

TERRIFIC BROTH (MODIFIED)

CAT. 1548

Medium for use with glycerol in the culture of recombinant *E. coli* strains

FORMULA IN g/l

Tryptone (pancreatic digest of casein)	**12.00**	Dipotassium phosphate	**9.40**
Yeast Extract	**24.00**	Monopotassium phosphate	**2.20**
Final pH 7.2 ± 0.2 at 25ºC			

PREPARATION

Suspend 47.6 grams of the medium in 900 ml of distilled water. Mix well. Add 4 ml of glycerol and adjust to a final volume of 1000 ml. and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is amber.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

TERRIFIC BROTH (MODIFIED) is a medium which supports a high cell density and, in the case is formulated for optimum growth of *E. coli*, maintains growth in the logarithmic phase for a long time. As a result it yields a greater number of recombinant proteins and plasmic DNA. In some circumstances it substitutes LB Broth (Lennox) (**Cat. 1231**) used in genetic studies.

Tryptone provide nitrogen, vitamins, minerals and amino acids essential for growth Yeast extract is source of vitamins, particularly the B-group. Potassium phosphates act as a buffer system to prevent cell death.

The source of carbohydrates and carbon is glycerol that is not fermented to acetic acid as glucose and does not lead to confusing results.

The formulation of this medium has been modified from the terrific broth (**Cat. 1246**) with a different concentration of biological buffer.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35ºC ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 23724	Good
Escherichia coli ATCC 33694	Good
Escherichia coli ATCC 33849	Good
Escherichia coli ATCC 53868	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

SAMBROC, J., E.F. FRITSCH, T. MANIATIS (1989) MOLECULAR CLONING

YEAST NITROGEN BASE W/O ADDED AMINO ACIDS AND WITH AMMONIUM SULFATE

CAT. 1545

For yeast classification based on amino acids and carbohydrates requirements

FORMULA IN g/l

Ammonium Sulfate	**5.00**	Yeast Nitrogen Base*	**1.70**
Final pH 5.4 ± 0.2 at 25ºC			

*YEAST NITROGEN BASE FORMULA PER LITER

Monopotassium Phosphate	**1.0 gr**	Calcium Pantothenate	**0.4 mg**
Magnesium Sulfate	**0.5 gr**	Manganese Sulfate	**0.4 mg**
Calcium Chloride	**0.1 gr**	Ferric Chloride	**0.2 mg**
Sodium Chloride	**0.1 gr**	Sodium Molibdate	**0.2 mg**
Inositol	**2.0 mg**	Riboflavin	**0.2 mg**
Boric Acid	**0.5 mg**	P-Aminobenzoic Acid	**0.2 mg**
Zinc Sulfate	**0.4 mg**	Potassium Iodide	**0.1 mg**
Thiamide Hydrochloride	**0.4 mg**	Copper Sulfate	**0.04 mg**
Niacin	**0.4 mg**	Folic Acid	**0.002 mg**
Pyridoxine Hydrochloride	**0.4 mg**	Biotin	**0.002 mg**

PREPARATION

Prepare a 10X solution by dissolving 6.7 grams of the medium in 100 ml of distilled water with 5 grams of dextrose, or the equivalent amount of another carbohydrate, and 5 - 10 mg of the desired amino acid. Mix well. Heat with frequent agitation until complete dissolution. DO NOT BOIL. DO NOT AUTOCLAVE. Sterilize the solution by filtration. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution to 4.5 ml of distilled water. Swirl to mix solution before inoculation. The prepared medium should be stored at 2 - 8°C. The color is colorless.

The dehydrated medium should be homogeneous, free flowing and off-white in color. If there are any changes physically, discard the medium.

USES

YEAST NITROGEN BASE w/o amino acids and with ammonium sulfate is used for classifying yeasts based on carbon and nitrogen requirements and is prepared according to the formulas of Wickerharm and Burkholder. The medium contains all the essential vitamins and inorganic salts needed to cultivate yeasts, except for the amino acids and carbohydrate sources. Ammonium sulfate is included as a readily available nitrogen source for nitrogen assimilation.

This medium is used in many applications for the study of yeast in molecular biology as is useful for the determination of aminoacids and carbohydrate utilization.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30°C and observed after 2 - 5 days.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Kloeckera apiculata ATCC 9774	Good
Saccharomyces pastorianus ATCC 9080	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Shadomy, S., and Espinel Ingroff, A. 1980. Susceptibility Testing with Antifungal Drugs, p. 647-653. In E. H. Lennete, A. Balows, W. J. Hausler, Jr., and J.P. Truant, Manual of Clinical Microbiology, 3rd Ed., American Society for Microbiology, Washington, D.C. U.S. Dept. Agric. Tech. Bull. No. 1029,1951.

YEAST NITROGEN BASE W/O ADDED AMINO ACIDS AND W/O AMMONIUM SULFATE

CAT. 1553

For yeast classification based on carbon and nitrogen requirements

FORMULA IN g/l

Yeast Nitrogen Base*	1.70
Final pH 4.5 ± 0.2 at 25ºC	

*YEAST NITROGEN BASE FORMULA PER LITER

Monopotassium Phosphate	1.0 gr	Calcium Pantothenate	0.4 mg
Magnesium Sulfate	0.5 gr	Manganese Sulfate	0.4 mg
Calcium Chloride	0.1 gr	Ferric Chloride	0.2 mg
Sodium Chloride	0.1 gr	Sodium Molibdate	0.2 mg
Inositol	2.0 mg	Riboflavin	0.2 mg
Boric Acid	0.5 mg	P-Aminobenzoic Acid	0.2 mg
Zinc Sulfate	0.4 mg	Potassium Iodide	0.1 mg
Thiamide Hydrochloride	0.4 mg	Copper Sulfate	0.04 mg
Niacin	0.4 mg	Folic Acid	0.002 mg
Pyridoxine Hydrochloride	0.4 mg	Biotin	0.002 mg

PREPARATION

Prepare a 10X solution by dissolving 1.7 grams of the medium in 100 ml of distilled water with 5 grams of dextrose, or the equivalent amount of another carbohydrate, and 5 - 10 mg of the desired amino acid. Mix well. Heat with frequent agitation until complete dissolution. DO NOT BOIL. DO NOT AUTOCLAVE. Sterilize the solution by filtration. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution to 4.5 ml of distilled water. Swirl to mix solution before inoculation. The prepared medium should be stored at 2 - 8°C. The color is colorless.

The dehydrated medium should be homogeneous, free flowing and off-white in color. If there are any changes physically, discard the medium.

USES

YEAST NITROGEN BASE w/o amino acids and w/o ammonium sulfate is used for classifying yeasts based on carbon and nitrogen requirements and is prepared according to the formulas of Wickerharm and Burkholder. The medium contains all the essential vitamins and inorganic salts needed to cultivate yeasts, except for the amino acids and carbohydrate sources.

This medium is used in many applications for the study of yeast in molecular biology as is useful for the determination of aminoacids and carbohydrate utilization.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 - 30°C and observed after 2 - 5 days.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Kloeckera apiculata ATCC 9774	Good
Saccharomyces pastorianus ATCC 9080	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Shadomy, S., and Espinel Ingroff, A.1980. Susceptibility Testing with Antifungal Drugs, p. 647-653. In E. H. Lennete, A. Balos, W. J. Hausler, Jr., and J.P. Truant, Manual of Clinical Microbiology, 3rd Ed., American Society for Microbiology, Washington, D.C. U.S. Dept. Agric. Tech. Bull. No.1029,1951.

YP AGAR BASE MEDIUM

CAT. 1513

For maintaining and developing yeast in molecular biology procedures

FORMULA IN g/l

Peptone	20.00	Bacteriological Agar	20.00
Yeast Extract	10.00		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 50 grams of the dehydrated medium in 900 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to room

temperature and aseptically add 100 ml of sterile dextrose at 20%. Mix well and dispense into appropriate containers. The prepared medium must be stored at 8 - 15°C. The color is clear amber, slightly opalescent

The dehydrated medium should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the medium.

USES

YP AGAR BASE MEDIUM is used for the maintenance and the development of yeast in molecular biology procedures.

YP Agar is also used to cultivate *Saccharomyces cerevisiae* and other yeasts. Yeasts grow well on a medium containing only a minimal amount of glucose and salts. This medium contains glucose (with the addition of dextrose after autoclaving), salts and proteins, which favours the growth of *Saccharomyces cerevisiae* and reduces growing times. Yeast extract is the source of vitamins, particularly the B-group essential for bacterial growth. Peptone provides nitrogen, vitamins, minerals and amino acids. Bacteriological agar is the solidifying agent.

Saccharomyces cerevisiae has a genome of 14 Mb containing 6000 genes arranged in 16 chromosomes, which have been completely sequenced, and thus, is a species type in microbiology and genetics studies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 ± 2°C and observed after 42 - 48 hours.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Saccharomyces cerevisiae ATCC 18790	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Guide to yeast genetics and molecular biology. (1991) Ed. Christine Guthrie & Gerald Fink. Methods in Enzymology vol. 194.

Current protocols in Molecular Biology. Eds. Ausubel, F. M. Brent, R., Kingston, R, E., Moore, D. D., Seidman, J. G., Smith J. A., and Struhl, K.13,01.-13.2.10. The Yeast Genome Directory (1997, May 29) Nature Supp. to volume 387

The condensed protocols from molecular cloning: a laboratory manual/ Joseph Sambrook, David W .Russell

YP BASE MEDIUM

CAT. 1511

For maintaining and developing yeast in molecular biology procedures

FORMULA IN g/l

Peptone	**20.00**	Yeast Extract	**10.00**
Final pH 6.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 30 grams of the medium in 900 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes Cool to room temperature and aseptically add 100 ml. of sterile dextrose at 20%. Mix well and dispense intro appropriate containers. The prepared medium stored at 2 - 8°C. The color of the prepared medium is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YP BASE MEDIUM is used for the maintenance and the development of yeast in molecular biology procedures.

YP Base Medium is also used to cultivate *Saccharomyces cerevisiae* and other yeasts. Yeasts grow well on a medium containing only a minimal amount of glucose and salts. This medium contains glucose (with the addition of dextrose after autoclaving), salts and proteins, which favors the growth of *Saccharomyces cerevisiae* and reduces growing times. Yeast extract is the source of vitamins, particularly the B-group essential for bacterial growth. Peptone provides nitrogen, vitamins, minerals and amino acids.

Saccharomyces cerevisiae has a genome of 14 Mb containing 6000 genes arranged in 16 chromosomes, which have been completely sequenced, and thus, is a species type in microbiology and genetics studies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 ± 2°C and observed after 42 - 48 hours.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Saccharomyces cerevisiae ATCC 18790	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Guide to yeast genetics and molecular biology. (1991) Ed. Christine Guthrie & Gerald Fink. Methods in Enzymology vol. 194.

Current protocols in Molecular Biology. Eds. Ausubel, F. M. Brent, R., Kingston, R, E., Moore, D. D., Seidman, J. G., Smith J. A., and Struhl, K.13,01.-13.2.10. The Yeast Genome Directory (1997, May 29) Nature Supp. to volume 387.

Joseph Sambrook, David W .Russell. The condensed protocols from molecular cloning: a laboratory manual.

YPD AGAR

CAT. 1546

For maintaining and developing yeast in molecular biology procedures

FORMULA IN g/l

Peptone	**20.00**	Yeast Extract	**10.00**
Dextrose	**20.00**	Bacteriological Agar	**20.00**
Final pH 6.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 70 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118ºC for 15 minutes. Cool to 45 - 50ºC, mix well and dispense into plates. The prepared medium should be stored at 8 - 15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YPD Agar is used for maintaining and developing yeast in molecular microbiology procedures. The formula is the same as in YP Agar Base Medium (**Cat. 1513**) but with the dextrose added.

YPD Agar is also used to cultivate *Saccharomyces cerevisiae* and other yeasts. Yeasts grow well on a medium containing only a minimal amount of glucose and salts. This medium contains glucose, salts and proteins, which favors the growth of *Saccharomyces cerevisiae* and reduces growing times. Yeast extract is the source of vitamins, particularly the B-group essential for bacterial growth. Peptone provides nitrogen, vitamins, minerals and amino acids. Bacteriological agar is the solidifying agent.

Saccharomyces cerevisiae has a genome of 14 Mb containing 6000 genes arranged in 16 chromosomes, which have been completely sequenced, and thus, is a species type in microbiology and genetics studies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 ± 2°C and observed after 42 - 48 hours.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Saccharomyces cerevisiae ATCC 18790	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Guide to yeast genetics and molecular biology. (1991) Ed. Christine Guthrie & Gerald Fink. Methods in Enzymology vol. 194.

Current protocols in Molecular Biology. Eds. Ausubel, F. M. Brent, R., Kingston, R, E., Moore, D. D., Seidman, J. G., Smith J. A., and Struhl, K.13,01.-13.2.10. The Yeast Genome Directory (1997, May 29) Nature Supp. to volume 387.

The condensed protocols from molecular cloning: a laboratory manual/ Joseph Sambrook, David W. Russell

YPD BROTH

CAT. 1547

For maintaining and developing yeast in molecular biology procedures

FORMULA IN g/l

Peptone	**20.00**	Yeast Extract	**10.00**
Dextrose	**20.00**		
Final pH 6.5 ± 0.2 at 25ºC			

PREPARATION

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Avoid overheating. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

YPD BROTH is used for the maintenance and the development of yeast in molecular biology procedures. The formula is the same as in YP Base Medium (**Cat. 1511**) but with the dextrose added.

YPD Broth is also used to cultivate *Saccharomyces cerevisiae* and other yeasts. Yeasts grow well on a medium containing only a minimal amount of glucose and salts. This medium contains glucose, salts and proteins, which favors the growth of *Saccharomyces cerevisiae* and reduces growing times. Yeast extract is the source of vitamins, particularly the B-group essential for bacterial growth. Peptone provides nitrogen, vitamins, minerals and amino acids.

Saccharomyces cerevisiae has a genome of 14 Mb containing 6000 genes arranged in 16 chromosomes, which have been completely sequenced, and thus, is a species type in microbiology and genetics studies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 25 ± 2°C and observed after 42 - 48 hours.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Saccharomyces cerevisiae ATCC 18790	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Guide to yeast genetics and molecular biology. (1991) Ed. Christine Guthrie & Gerald Fink. Methods in Enzymology vol. 194.

Current protocols in Molecular Biology. Eds. Ausubel, F. M. Brent, R., Kingston, R, E., Moore, D. D., Seidman, J. G., Smith J. A., and Struhl, K.13,01.-13.2.10. The Yeast Genome Directory (1997, May 29) Nature Supp. to volume 387.

Joseph Sambrook, David W. Russell. The condensed protocols from molecular cloning: a laboratory manual.

2xYT MEDIUM

CAT. 1507

For the cultivation of recombinant strains of *E. coli* and for growth of filamentous bacteriophages

FORMULA IN g/l

Tryptone	**16.00**	Sodium Chloride	**5.00**
Yeast Extract	**10.00**		
Final pH 7.0 ± 0.2 at 25ºC			

PREPARATION

Suspend 31 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2 - 8°C. The color of the prepared medium is clear amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

2xYT MEDIUM is a nutritive medium optimized for the growth and maintenance of M13 phages and other filamentous bacteriophages. It is also suitable for growth of recombinant strain of *E. coli*.

Tryptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The components of the 2xYT Medium include nitrogen and other growth factors that allow bacteriophages to reproduce in large quantities without weakening the host. *E. coli* grows faster in this enriched medium, as it contains amino acids, precursors of nucleotides, vitamins and other metabolisms which otherwise the cell itself would have to synthesize.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth
Escherichia coli ATCC 23724	Good
Escherichia coli ATCC 33694	Good
Escherichia coli ATCC 33849	Good

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Principles Of Virology: Molecular Biology, Pathogenesis And Control Of Animal Viruses.

J.S. Flint, L.W. Enquist, V.Racaniello y A.M. Shalka. 2004. 2ª ed. ASM PRESS, Washington, D.C.

Joseph Sambrook, David W .Russell. The condensed protocols from molecular cloning: a laboratory manual.

AGAR, PEPTONES AND OTHER

INGREDIENTS

AMERICAN BACTERIOLOGICAL AGAR

CAT. 1802 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium*, *Gracilaria* and *Pterocladia* types. American Bacteriological Agar is a gelling agent used in the preparation of culture media and other bacteriological applications. The main advantage of this agar is the absence of inhibitors, which could interfere in the growth of microorganisms. It has excellent transparency, high hysteresis and very reliable reproducibility. Each batch produced is thoroughly tested for biological performance against a battery of known bacterial cultures in order to ensure proper growth characteristics and absence of inhibitors. American Agar type gives a lower strength than the European version. It is used in concentrations from 1.2% to 1.6%.

CHEMICAL CHARACTERISTICS

Appearance	**White cream powder**
Moisture	**Less than 10%**
Ashes	**≤ 6.5%**
Gel strength (1.5%, Nikan)	**600 – 750 g/cm²**
pH (1.5%) before autoclaving	**7.0 ± 0.4**
pH (1.5%) after autoclaving	**6.5 ± 0.4**
Melting point (1.5%)	**85 ± 5°C**
Gelling point (1.5%)	**35 ± 3°C**
Transparency (1.5%)	**≤ 5 NTU**
Colorimetry (absorbance) 430 nm	**≤ 0.200**
Particle size	**95 % Over sieve 60**

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated American Bacteriological Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

EUROPEAN BACTERIOLOGICAL AGAR

CAT. 1800 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium*, *Gracilaria* and *Pterocladia* types. European Bacteriological Agar is a gelling agent used in the preparation of culture media and other bacteriological applications. The main advantage of this agar is the absence of inhibitors, which could interfere in microorganisms' growth. It has excellent transparency, high hysteresis and very reliable reproducibility. Each batch produced is thoroughly tested for biological performance against a battery of known bacterial cultures in order to ensure proper growth characteristics and absence of inhibitors. European Agar type gives a higher gel strength than the American version. It is used in concentrations from 1.2% to 1.6%.

CHEMICAL CHARACTERISTICS

Appearance	**White cream powder**
Moisture	**Less than 10%**
Ashes	**≤ 4.5%**
Gel strength (1.5%, Nikan)	**800 – 1100 g/cm²**
pH (1.5%) before autoclaving	**7.0 ± 0.4**
pH (1.5%) after autoclaving	**6.5 ± 0.4**
Melting point (1.5%)	**85 ± 5°C**
Gelling point (1.5%)	**35 ± 3°C**
Transparency (1.5%)	**≤ 5 NTU**
Colorimetry (absorbance) 430 nm	**≤ 0.200**
Particle size	**95 % Over sieve 60**

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated European Bacteriological Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

BACTERIOLOGICAL AGAR HS (HIGH STRENGTH)

CAT. 1809 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium, Gracilaria and Pterocladia* types. High Strength Bacteriological Agar is a gelling agent specially designed for the preparation of culture media and other bacteriological applications, in which it is necessary to reduce the agar content maintaining the desired gelling properties. The main advantage of this agar is the high gel strength (typical 1,000 g/cm^2).

As other Conda's Bacteriological Agar types, it has absence of inhibitors, excellent transparency, high hysteresis and reliable reproducibility. It can be used for preparing media such as Thioglycollate fluid Media.

CHEMICAL CHARACTERISTICS

Appearance	White Cream Powder
Moisture	Less than 18%
Ashes	≤ 4.5%
Gel Strength [1.5%, Nikan]	900 – 1100 g/cm^2
pH [1.5] after autoclaving	6 - 8
Melting Point [1.5%]	86 - 98ºC
Gelling Point [1.5%]	35 ± 3ºC
Transparency [1.5%]	≤ 5 NTU
Particle Size	95 % Over Sieve 60

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Bacteriological Agar Hs (High Strength) should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

BACTERIOLOGICAL FLAKE AGAR

CAT. 1805 AGARS

DESCRIPTION

This Bacteriological Agar is high in purity and quality. Its particular manufacturing process gives this agar special physical and chemical characteristic making it ideal to prepare culture media for Molecular Biology applications such as bacterial culture, plasmids research, etc.

In addition it has the advantage of not producing any dust while adding it to the media formulations, and thus reducing any environmental contamination.

CHEMICAL CHARACTERISTICS

Appearance	White Cream Flake
Moisture	Less than 10%
Ashes	≤ 6.5%
Gel Strength [1.5%, Nikan]	900 - 1000 g/cm^2
pH [1.5] after autoclaving	6 - 8
Melting Point [1.5%]	88 - 96ºC
Gelling Point [1.5%]	33 to 38ºC
Transparency [1.5%]	≤ 7 NTU

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Bacteriological Flake Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

INDUSTRIAL AGAR

CAT. 1804 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium, Gracilaria and Pterocladia* types. The marked application increase in the use of agar within the food industry (for example, tin can produce, sweets, pastries, ice creams, etc) is widely spread because of its properties as a dispersing, stabilizing, thickening and gelling agent. It is widely used as a replacement of pectin and being a vegetable gelatin of marine origin, it is the perfect substitute for animal gelatin, having ten times more jellification power. Other applications can be in the use of techniques for the micro propagation of plants.

CHEMICAL CHARACTERISTICS

Appearance	White cream powder
Moisture	Less than 8%
Ashes	≤ 5%
Gel strength (1.5%, Nikan)	≤ 850 g/cm^2
pH (1 .5%) before autoclaving	7.2 ± 0.4
pH (1.5%) after autoclaving	7.0 ± 0.4
Melting point (1.5%)	85 - 90°C
Gelling point (1.5%)	34 - 38°C
Transparency (1 .5%)	≤ 7 NTU
Colorimetry (absorbance) 430 nm	≤ 0.200
Particle size	95 % Over sieve 60

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Industrial Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PHARMACEUTICAL AGAR

CAT. 1816 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium, Gracilaria* and *Pterocladia* types. This is a pharmaceutical quality agar that follows the specifications of the European Pharmacopoeia and American Pharmacopoeia (USP). It is specifically designed for all kind of applications in the pharmaceutical industry for medicinal and cosmetic preparations such as suspensions, reconstituting suspensions, emulsions, sprays, tablets, capsules and creams.

CHEMICAL CHARACTERISTICS

Appearance	White cream powder
Moisture	Less than 20%
Ashes	≤ 4,5%
Gel strength (1.5%, Nikan)	≤ 750 g/cm^2
pH (1.5%) before autoclaving	7.2 ± 0.4
pH (1.5%) after autoclaving	7.0 ± 0.4
Melting point (1.5%)	85 ± 5°C
Gelling point (1.5%)	35 ± 3°C
Transparency (1.5%)	≤ 15 NTU
Colorimetry (absorbance) 430 nm	≤ 0.250
Particle size	95 % Over sieve 60
Water absortion	≤75 cm^3

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Pharmaceutical Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PLANT PROPAGATION AGAR (PPA)

CAT. 1812 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium, Gracilaria* and *Pterocladia* types. This agar is impurity free and recommended for the commercial micro-propagation of ornamental, succulent and woody plant species as well as in vitro genetic engineering in the plant research field. This agar has a very high gel strength, ≥ 1.000 g/cm² which allows usage at very low concentrations in typical applications ranging from 0.5 to 0.6% or higher concentrations when used with other hydrocolloids. The product is clear and exhibits excellent transparency aiding in identifying visual contamination by bacteria or molds that could interfere in the development of plant cultures.

CHEMICAL CHARACTERISTICS

Appearance	White cream powder
Moisture	< 18%
Ashes	2.5% - 3.5%
Gel strength (1.5%, Nikan)	≥ 1000 g/cm²
pH (1 .5%) before autoclaving	7.2 ± 0.4
pH (1.5%) after autoclaving	7.0 ± 0.4
Melting point (1.5%)	92 ± 2°C
Gelling point (1.5%)	34 ± 2°C
Transparency (1.5%)	≤ 7 NTU
Colorimetry (absorbance) 430 nm	≤ 0.200
Particle size	95% Over sieve 80

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Plant Propagation Agar (PPA) should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PURIFIED AGAR

CAT. 1806 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium, Gracilaria* and *Pterocladia* types. This agar is highly purified with a very low ash content for use in microbiology and biochemistry. It is subjected to rigid tests which guarantee its excellent performance in biochemical, bacteriological and mycological applications. It can be used in special studies such as yeast assimilation and vitamin assays.

CHEMICAL CHARACTERISTICS

Appearance	White cream powder
Moisture	≤ 10%
Ashes	≤ 1.6%
Gel strength (1.5%, Nikan)	700 -1200 g/cm²
pH (1.5%) before autoclaving	7.0 ± 0.4
pH (1.5%) after autoclaving	6.8 ± 0.4
Melting point (1.5%)	80 - 95°C
Gelling point (1.5%)	32 - 37.5°C
Transparency (1.5%)	≤ 5 NTU
Colorimetry (absorbance) 430 nm	≤ 0.100
Particle size	95% over sieve 60

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Purified Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

V AGAR

CAT. 1815 AGARS

DESCRIPTION

This type of agar is specially designed to add to Microbiological Cultured Media, that once prepared they need a "better adhesion" to the packaging material that contains them. Due to its meticulous manufacturing process it gains special characteristics as for viscosity that provides to the gel a degree of elasticity that helps to avoid the " rigidness" effect to the gel and thus, it's possible breakage. This advantage is important above all when the media has to be prepared in thin layers and the sinerisis of the agar (water evaporation) can affect the gel in a greater measure.

CHEMICAL CHARACTERISTICS

Appearance	White Cream Powder
Mositure	Maximum 18%
Ashes	3.0 - 4.5%
Gel Strength [1.5%, Nikan]	900 - 1000 g/cm^2
pH [1.5] after autoclaving	6 - 8
Melting Point [1.5%]	86 - 98ºC
Gelling Point [1.5%]	32 to 38ºC
Transparency [1.5%]	≤ 30 NTU
Viscosity	15 at 50 cps

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated V Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

VITRO AGAR

CAT. 1808 AGARS

DESCRIPTION

Agar is a natural hydrocolloid extracted from several species of red algae, mainly the *Gelidium*, *Gracilaria* and *Pterocladia* types. This agar was developed especially for in vitro cell culture. Due to its physical-chemical characteristics (color, transparency, degree of purity) and, above all, its high gel strength (approximately 1000 g/cm^2, which allows usage levels as low as 0.4 - 0.5%), this agar is recommended for micro propagation techniques (initiation, propagation, radiation, etc.). This product is strictly controlled and designed to give high yields in large industrial operations for growing tissue culture plants (ornamentals, horticulture, woody plants, etc.).

CHEMICAL CHARACTERISTICS

Appearance	White cream powder
Moisture	Less than 5%
Ashes	≤ 6.5%
Gel strength [1.5%, Nikan]	≥ 1000 g/cm^2
pH [1 .5%] before autoclaving	7.2 ± 0.4
pH [1.5%] after autoclaving	7.0 ± 0.4
Melting point [1 .5%]	85 ± 5°C
Gelling point [1.5%]	35 ± 3°C
Transparency [1.5%]	≤ 7 NTU
Colorimetry [absorbance] 430 nm	≤ 0.200
Particle size	95% Over sieve 80

MICROBIOLOGICAL TEST

Standard plate count	Less than 3000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Less than 3 cfu/g
E.coli	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Vitro Agar should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

ACID CASEIN PEPTONE (H)

CAT. 1604 PEPTONES

DESCRIPTION

ACID CASEIN PEPTONE (H) is an acid hydrolysate of casein. The agent in a complete or total acid hydrolysis is commonly hydrochloric acid (6-8 N). Acid hydrolysis is a crude process that cleaves all peptide bonds. Acid hydrolysis results commonly to a total hydrolysis of protein to amino acids. The process destroys glutamine, asparagines, tryptophan, cysteine, serine, threonine, lysine, aspartic acid, proline racemises amino acids and completely destroys vitamins.

As this peptone is free from vitamins, it is used for the determination of vitamin content by microbiological methods. It has a good solubility and clarity when dissolved.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.5%	**5.40%**
Total Nitrogen (TN)	Minimum 5.0%	**8.80%**
AN/TN Ratio	N/A	**61.40%**
Loss on drying	Maximum 4.0%	**< 3.00%**
Ash	Maximum 45%	**33.00%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.015%**
Magnesium	**0.005%**
Potassium	**0.035%**
Sodium	**12.25%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**1.70**	Histidine	**1.40**	Proline	**5.80**
Arginine	**2.00**	Isoleucine	**2.90**	Serine	**2.80**
Aspartic acid	**5.30**	Leucine	**5.00**	Threonine	**2.00**
Cystine	**0.20**	Lysine	**4.60**	Tryptophan	**< 1.00**
Glutamic acid	**13.70**	Methionine	**1.50**	Tyrosine	**1.70**
Glycine	**1.00**	Phenylalanine	**2.00**	Valine	**3.60**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Acid Casein Peptone (H) should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

BACTERIOLOGICAL OX BILE

CAT. 1710 PEPTONES

DESCRIPTION

BACTERIOLOGICAL OX BILE is prepared by a low temperature dehydration process. It is used as a selective inhibitory agent in culture media such as Brilliant Green Bile Broth 2%.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
pH (2% solution)	6.0 - 8.5	**8.30**
Moisture	Maximum 6%	**3.10%**
Cholic acid	Minimum 45%	**47.00%**
Solubility (2% solution)	Clear without sediment, without fat and without particles on surface	
Color	Yellowish-green	

GROWTH SUPPORTING PROPERTIES

Brilliant Green Bile Broth 2%	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Bacteriological Ox Bile should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

BACTERIOLOGICAL PEPTONE

CAT. 1616 PEPTONES

DESCRIPTION

BACTERIOLOGICAL PEPTONE is a high quality hydrolysate produced by the enzymatic digestion of animal tissues. Enzymatic digestion produces amino acids, including essential amino acids and peptides, the enzymes normally used are trypsin and pepsin.

It is widely used in culture media and has been used extensively in the producition of toxins, vaccines and other biological products.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 2.6%	**3.00%**
Total Nitrogen (TN)	Minimum 12%	**15.55%**
AN/TN Ratio		**19.20%**
Loss on drying	Maximum 6.0%	**3.20%**
Ash	Maximum 15%	**4.70%**
pH (2% solution)	6.5 - 7.5	**6.90**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.023%**
Magnesium	**0.013%**
Potassium	**0.25%**
Sodium	**1.40%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**7.95**	Histidine	**0.93**	Proline	**11.71**
Arginine	**7.21**	Isoleucine	**1.41**	Serine	**3.51**
Aspartic acid	**6.42**	Leucine	**3.02**	Threonine	**1.90**
Cystine	**0.14**	Lysine	**3.69**	Tryptophan	**0.09**
Glutamic acid	**9.93**	Methionine	**0.92**	Tyrosine	**0.75**
Glycine	**20.71**	Phenylalanine	**1.94**	Valine	**2.40**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Bacteriological Peptone should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

BEEF EXTRACT

CAT. 1700 PEPTONES

DESCRIPTION

BEEF EXTRACT is prepared from very low fat bovine skeletal muscle and marrow, which is spinal column and tendons free. Beef Extract is highly nutritious and is used in preparing microbiological culture media. It is recommended for use in culture media for the bacteriological examination of water, milk and other materials.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.9%	**4.10%**
Total Nitrogen (TN)	Minimum 10.0%	**12.48%**
AN/TN Ratio	N/A	**32.90%**
Loss on drying	Maximum 6%	**2.50%**
Ash	Maximum 16%	**9.40%**
pH (2% solution)	6.5 - 7.5	**6.80**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.011%**
Magnesium	**0.019%**
Potassium	**2.60%**
Sodium	**1.60%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.28**	Histidine	**2.08**	Proline	**6.91**

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Arginine	**3.22**	Isoleucine	**3.91**	Serine	**4.37**
Aspartic acid	**6.60**	Leucine	**6.50**	Threonine	**3.56**
Cystine	**0.35**	Lysine	**5.98**	Tryptophan	**0.97**
Glutamic acid	**15.86**	Methionine	**1.63**	Tyrosine	**1.68**
Glycine	**2.29**	Phenylalanine	**3.58**	Valine	**4.85**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Beef Extract should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

BILE SALTS N°3

CAT. 1706 PEPTONES

DESCRIPTION

BILE SALTS N° 3 is a mixture of bile extracts especially prepared for use in selective media such as MacConkey Agar (**Cat. 1052**) and Salmonella Shigella Agar (**Cat. 1064**). It is an excellent inhibitor of Gram-positive bacteria such as streptococci and staphylococci.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Appearance	White powder	**White powder**
Sodium Cholate	45 - 55%	**49.30%**
Sodium Desoxycholate	45 - 55%	**49.40%**
Loss on drying	Maximum 5%	**4.10%**
pH (2% solution)	7.5 - 9.0	**8.1**
Solubility (2% solution)	Clear	**Clear, no precipitate**
Heavy metals	—	**< 20 ppm**

GROWTH SUPPORTING PROPERTIES

MacConkey Agar, Salmonella Shigella Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Bile Salts N° 3 should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

CASEIN CC PEPTONE

CAT. 1603 PEPTONES

DESCRIPTION

CASEIN CC PEPTONE is a pancreatic digest of casein enriched with the enzymatic digest of animal tissue. Pancreatic digestion produces a balanced mixture of amino acids, including essential amino acids, in optimal ration and low molecular peptides. Enzymatic digestion produces amino acids, including essential amino acids and peptides, the enzymes normally used are trypsin and pepsin. It is a superior source of nutrients widely used in pharmaceutical and veterinary industries.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.8%	**4.15%**
Total Nitrogen (TN)	Minimum 10%	**13.10%**
AN/TN Ratio	N/A	**31.67**
Loss on drying	Maximum 6%	**3.15%**
Ash	Maximum 15%	**6.80%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.02%**
Magnesium	**0.0069%**
Potassium	**1.80%**
Sodium	**2.20%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**2.94**	Histidine	**2.39**	Proline	**8.62**
Arginine	**3.36**	Isoleucine	**4.44**	Serine	**5.02**
Aspartic acid	**6.28**	Leucine	**7.60**	Threonine	**3.96**
Cystine	**0.41**	Lysine	**6.63**	Tryptophan	**0.92**
Glutamic acid	**17.90**	Methionine	**2.31**	Tyrosine	**1.85**
Glycine	**1.88**	Phenylalanine	**4.13**	Valine	**5.50**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Casein Peptone CC should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

CASEIN PEPTONE

CAT. 1602 PEPTONES

DESCRIPTION

CASEIN PEPTONE is a pancreatic digest of casein. Pancreatic digestion produces a balanced mixture of amino acids, including essential amino acids, in optimal ration and low molecular peptides. In many cases this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

It can be used in the production of toxins, vaccines, enzymes, in fermentation applications and microbiological culture media, especially in blood-containing media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.9%	**4.20%**
Total Nitrogen (TN)	Minimum 10.0%	**13.13%**
AN/TN Ratio	N/A	**32.00%**
Loss on drying	Maximum 6.0%	**3.30%**
Ash	Maximum 15.0%	**6.00%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.019%**
Magnesium	**0.0079%**
Potassium	**1.30%**
Sodium	**2.10%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**2.91**	Histidine	**2.38**	Proline	**8.65**
Arginine	**3.30**	Isoleucine	**4.45**	Serine	**5.08**
Aspartic acid	**6.99**	Leucine	**7.62**	Threonine	**3.91**
Cystine	**0.44**	Lysine	**6.60**	Tryptophan	**0.95**
Glutamic acid	**18.74**	Methionine	**2.32**	Tyrosine	**1.86**
Glycine	**1.86**	Phenylalanine	**4.11**	Valine	**5.51**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeast and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Casein Peptone should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

CASEIN PEPTONE N°2

CAT. 1620 PEPTONES

DESCRIPTION

CASEIN PEPTONE N°2 is a pancreatic digest of casein. Pancreatic digestion produces a balanced mixture of amino acids, including essential amino acids in an optimal ration and low molecular peptides. In many cases this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

It can be used in the production of toxins, vaccines, enzymes and microbiological culture media, especially in blood-containing media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.9%	**4.20%**
Total Nitrogen (TN)	Minimum 10%	**13.49%**
AN/TN Ratio	N/A	**31.10%**
Loss on drying	Maximum 6%	**3.30%**
Ash	Maximum 15%	**6.40%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.019%**
Magnesium	**0.0057%**
Potassium	**0.86%**
Sodium	**2.20%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**2.74**	Histidine	**2.44**	Proline	**9.11**
Arginine	**3.29**	Isoleucine	**4.52**	Serine	**5.14**
Aspartic acid	**6.99**	Leucine	**7.70**	Threonine	**3.95**
Cystine	**0.49**	Lysine	**6.73**	Tryptophan	**1.00**
Glutamic acid	**19.23**	Methionine	**2.45**	Tyrosine	**1.91**
Glycine	**1.73**	Phenylalanine	**4.18**	Valine	**5.59**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Casein Peptone nº2 should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

CASEIN PEPTONE N°3

CAT. 1718 PEPTONES

DESCRIPTION

CASEIN PEPTONE Nº3 is a pancreatic digest of casein containing all the amino acids found in casein as well as larger peptide fractions. Its unique properties of digestion and high phosphate content make it the hydrolysates of choice of many organisms.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.9%	**4.00%**
Total Nitrogen (TN)	Minimum 10.0%	**13.20%**
AN/TN Ratio	N/A	**30.00%**
Loss on drying	Maximum 6.0%	**3.20%**
Ash	Maximum 15.0%	**8.00%**
pH (2% solution)	6.5 - 7.5	**7.1**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.0185%**
Magnesium	**0.009%**

	Typical Analysis
Potassium	**0.60%**
Sodium	**3.50%**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Casein Peptone nº3 should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

CASEIN PEPTONE HS (HIGH SOLUBILITY)

CAT. 1622 PEPTONES

DESCRIPTION

CASEIN PEPTONE HS (HIGH SOLUBILITY) is a high quality hydrolysate produced by pancreatic digest, using a special process to increase solubility. It is a rich source with high concentrations of amino acids used in the production of toxins, vaccines, enzymes and other biological products.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 5.5%	**5.90%**
Total Nitrogen (TN)	Minimum 10%	**12.26%**
AN/TN Ratio	N/A	**48.10%**
Loss on drying	Maximum 6%	**3.00 %**
Ash	Maximum 7%	**6.20 %**
pH (2% solution)	6.5 - 7.5	**6.6**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.039 %**
Magnesium	**0.0098%**
Potassium	**0.48%**
Sodium	**2.90%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**2.44**	Histidine	**2.20**	Proline	**7.90**
Arginine	**2.99**	Isoleucine	**3.99**	Serine	**4.53**
Aspartic acid	**5.98**	Leucine	**6.52**	Threonine	**5.87**
Cystine	**0.30**	Lysine	**7.82**	Tryptophan	**0.94**
Glutamic acid	**16.36**	Methionine	**1.46**	Tyrosine	**1.23**
Glycine	**1.69**	Phenylalanine	**3.51**	Valine	**4.96**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Casein Peptone HS (High Solubility) should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

CASEIN PEPTONE TT

CAT. 1722 PEPTONES

DESCRIPTION

CASEIN PEPTONE TT is a peptone containing all the amino acids found in casein as well as large peptide fractions. It is an excellent nutrient for use in culture media for producing toxins. Casein Peptone TT was developed specifically to achieve maximal toxin production.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.90%	**4.30%**
Total Nitrogen (TN)	Minimum 10.00%	**12.10%**
AN/TN Ratio	N/A	**35.00%**
Loss on drying	Maximum 6.00%	**3.80%**
Ash	Maximum 15.00%	**8.00%**
pH (2% solution)	6.5 - 7.5	**6.9**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.023%**
Magnesium	**0.028%**
Potassium	**0.09%**
Sodium	**2.30%**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Casein Peptone TT should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

GELATIN PEPTONE

CAT. 1606 PEPTONES

DESCRIPTION

GELATIN PEPTONE is a pancreatic digest of porcine skin. Pancreatic digestion produces a balanced mixture of amino acids, including essential amino acids in an optimal ration and low molecular peptides. It is low in fermentable carbohydrates and is used in culture media, especially for non-fastidious microorganisms. It is also used in fermentation studies.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 2.7%	**3.50%**
Total Nitrogen (TN)	Minimum 10%	**15.48%**
AN/TN Ratio	N/A	**22.60%**
Loss on drying	Maximum 6 %	**3.00%**
Ash	Maximum 15 %	**4.20%**
pH (2% solution)	6.5 - 7.5	**6.9**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.018%**
Magnesium	**0.01%**
Potassium	**1.10%**
Sodium	**0.97%**

AMINO ACIDS

	Total (g/100g)		Total (g/100g)		Total (g/100g)
Alanine	**7.89**	Histidine	**0.89**	Proline	**11.46**
Arginine	**7.16**	Isoleucine	**1.39**	Serine	**3.45**
Aspartic acid	**6.34**	Leucine	**2.84**	Threonine	**1.87**
Cystine	**0.13**	Lysine	**3.61**	Tryptophan	**0.09**
Glutamic acid	**9.58**	Methionine	**0.85**	Tyrosine	**0.71**
Glycine	**20.60**	Phenylalanine	**1.88**	Valine	**2.31**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Gelatin Peptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

HEART INFUSION

CAT. 1714 PEPTONES

DESCRIPTION

Heart Infusion is a specially formulated preparation of dried infusion from bovine hearts. It is supplied as a light, fine powder for use in microbiological culture media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3%	**3.50%**
Total Nitrogen (TN)	Minimum 10%	**11.0%**
AN/TN Ratio	N/A	**31.80%**
Loss on drying	Maximum 6%	**3.50%**
Ash	Maximum 15%	**10.10%**
pH (2% solution)	6.5 - 7.5	**7.0**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.009%**
Magnesium	**0.02%**
Potassium	**1.40%**
Sodium	**3.60%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.20**	Histidine	**2.13**	Proline	**7.04**
Arginine	**3.55**	Isoleucine	**3.91**	Serine	**4.54**
Aspartic acid	**6.05**	Leucine	**6.68**	Threonine	**3.12**
Cystine	**0.31**	Lysine	**6.01**	Tryptophan	**0.87**
Glutamic acid	**15.97**	Methionine	**1.75**	Tyrosine	**1.61**
Glycine	**2.50**	Phenylalanine	**3.66**	Valine	**4.73**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Heart Infusion should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

LACTALBUMIN HYDROLYSATE

CAT. 1626 PEPTONES

DESCRIPTION

LACTALBUMIN HYDROLYSATE is the enzymatically hydrolyzed protein portion of milk whey. It is a mixture of peptides, amino acids and carbohydrates simple and complex. It is used for preparing bacterial, insect and mammalian cell culture media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 4.8%	**5.30%**
Total Nitrogen (TN)	Minimum 10%	**12.40%**
AN/TN Ratio	N/A	**42.70%**
Loss on drying	Maximum 6%	**4.40%**
Ash	Maximum 15%	**5.30%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.078%**
Magnesium	**0.027%**
Potassium	**0.83%**
Sodium	**2.10%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.20**	Histidine	**2.02**	Proline	**7.37**
Arginine	**1.36**	Isoleucine	**4.30**	Serine	**4.72**
Aspartic acid	**7.59**	Leucine	**6.52**	Threonine	**4.58**
Cystine	**0.76**	Lysine	**6.68**	Tryptophan	**1.17**

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Glutamic acid	**17.12**	Methionine	**1.60**	Tyrosine	**0.97**
Glycine	**1.83**	Phenylalanine	**3.08**	Valine	**5.07**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Lactalbumin Hydrolysate should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

MALT EXTRACT

CAT. 1708 PEPTONES

DESCRIPTION

MALT EXTRACT is prepared by successive purifications, removing all enzymatic activity. In solution, it has a very light color. It is particularly well suited for culturing yeasts and molds, allowing the sporulation of molds such as Aspergillus and Penicillium. It has high carbohydrate content and should not be heated in excess to avoid the darkening of the medium. Malt Extract provides carbon, protein and nutrients in culture media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)		**0.40%**
Total Nitrogen (TN)		**1.30%**
AN/TN Ratio	N/A	**30.77%**
Sugar content	> 95%	**96.00%**
Loss on drying	Maximum 6%	**2.50%**
Sulfuric ash	Maximum 3.5%	**1.40%**
pH (5% solution)	4.5 - 5.5	**5.0**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.014%**
Magnesium	**0.073%**
Potassium	**0.0005%**
Sodium	—

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**0.40**	Histidine	**0.60**	Proline	**0.60**
Arginine	**0.50**	Isoleucine	**0.50**	Serine	**0.40**
Aspartic acid	**0.90**	Leucine	**0.60**	Threonine	**0.40**
Cystine	—	Lysine	**0.60**	Tryptophan	—
Glutamic acid	**1.60**	Methionine	**0.20**	Tyrosine	**0.30**
Glycine	**0.40**	Phenylalanine	**0.70**	Valine	**0.60**

GROWTH SUPPORTING PROPERTIES

Malt Extract Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Malt Extract should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

MEAT PEPTONE

CAT. 1600 PEPTONES

DESCRIPTION

MEAT PEPTONE is an enzymatic digest of animal tissue. Meat Peptones are proteins from animal sources that have been hydrolyzed, or broken down into amino acids and peptides, to provide nitrogen for microorganisms. Meat peptones can be tailored to specific nutritive needs of microorganisms by controlling the quality and origin of the protein, the quality and source of the enzyme used to digest the protein, and the method used for hydrolysis, concentration and drying the peptone. It can be incorporated into a variety of liquid and solid culture media formulations for the cultivation of fastidious and non-fastidious microorganisms.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.4%	**3.70%**
Total Nitrogen (TN)	Minimum 10%	**12.33%**
AN/TN Ratio	N/A	**30.00%**
Loss on drying	Maximum 6%	**2.70%**
Ash	Maximum 15%	**9.20%**
pH (2% solution)	6.5 - 7.5	**6.9**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.072%**
Magnesium	**0.029%**
Potassium	**2.70%**
Sodium	**2.50%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**5.62**	Histidine	**1.31**	Proline	**6.29**
Arginine	**4.08**	Isoleucine	**2.63**	Serine	**2.95**
Aspartic acid	**5.61**	Leucine	**4.50**	Threonine	**2.46**
Cystine	**0.37**	Lysine	**4.30**	Tryptophan	**0.59**
Glutamic acid	**11.62**	Methionine	**0.85**	Tyrosine	**1.11**
Glycine	**8.37**	Phenylalanine	**2.61**	Valine	**3.50**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Meat Peptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

MEAT LIVER PEPTONE

CAT. 1703 PEPTONES

DESCRIPTION

Meat liver peptone is a mixture of meat peptone and liver peptone. It can be used in some culture media as a superior source of nutrients.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 4.20%	**4.30%**
Total Nitrogen (TN)	Minimum 11.00%	**11.70%**
AN/TN Ratio	N/A	**0.36%**
Loss on drying	Maximum 6.00%	**3.90%**
Ash	Maximum 15.00%	**11.20%**
pH (2% solution)	6.5 - 7.5	**7.1**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.03%**
Magnesium	**0.025%**
Potassium	**0.18%**
Sodium	**1.37%**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Meat Liver Peptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PEPTONIZED MILK

CAT. 1628 PEPTONES

DESCRIPTION

Peptonized Milk is a pancreatic digest of fat-free milk which is used primarily in culture media for the isolation and growth of lactobacilli and streptococci in dairy products.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 1.9%	**2.40%**
Total Nitrogen (TN)	Minimum 6%	**7.41%**
AN/TN Ratio	N/A	**32.40%**
Loss on drying	Maximum 6%	**4.30%**
Ash	Maximum 10%	**8.40%**
pH (2% solution)	6.5 - 7.5	**6.7**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.43%**
Magnesium	**0.061%**
Potassium	**1.20%**
Sodium	**1.90%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**1.56**	Histidine	**1.29**	Proline	**4.81**
Arginine	**1.71**	Isoleucine	**2.43**	Serine	**2.73**
Aspartic acid	**3.86**	Leucine	**4.25**	Threonine	**2.18**
Cystine	**0.28**	Lysine	**3.35**	Tryptophan	**0.52**
Glutamic acid	**10.01**	Methionine	**1.02**	Tyrosine	**1.20**
Glycine	**1.07**	Phenylalanine	**2.18**	Valine	**2.98**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Peptonized Milk should be homogeneous, free flowing and clear beige in color. If there are any changes physically, discard the product.

POLYPEPTONE

CAT. 1610 PEPTONES

DESCRIPTION

POLYPEPTONE is a mixture of peptones made up of equal parts of pancreatic digest of casein and peptic digest of animal tissue. It Includes the high content of amino acids and small polypeptides characteristic of peptic digest of animal tissue. Polypeptone provides nitrogen, amino acids and vitamins in microbiological culture. It can be used in general culture media as a superior source of nutrients. The growth of some microorganisms may be better than when the individual peptones are used.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.7%	**4.10%**
Total Nitrogen (TN)	Minimum 10%	**13.12%**
AN/TN Ratio	N/A	**31.25%**
Loss on drying	Maximum 6%	**3.40%**
Ash	Maximum 15%	**8.80%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.03%**
Magnesium	**0.014%**
Potassium	**1.60%**
Sodium	**2.12%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**4.05**	Histidine	**1.81**	Proline	**8.21**

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Arginine	**3.76**	Isoleucine	**3.44**	Serine	**4.33**
Aspartic acid	**8.83**	Leucine	**5.99**	Threonine	**3.31**
Cystine	**0.43**	Lysine	**5.50**	Tryptophan	**0.80**
Glutamic acid	**15.97**	Methionine	**1.81**	Tyrosine	**1.42**
Glycine	**5.70**	Phenylalanine	**3.34**	Valine	**4.37**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Polypeptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PORK BRAIN HEART INFUSION

CAT. 1712 PEPTONES

DESCRIPTION

Pork Brain Heart Infusion is a specially formulated preparation of dried extract of pork hearts and pork brains. It is supplied as a light, yellow-brown fine powder. It is a suitable substitute for Bovine Brain Heart Infusion in selected microbiological culture media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3%	**3.30%**
Total Nitrogen (TN)	Minimum 10%	**11.81%**
AN/TN Ratio	N/A	**27.90%**
Loss on drying	Maximum 6%	**3.50%**
Ash	Maximum 15%	**9.20%**
pH (2% solution)	6.5 - 7.5	**7.2**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.020%**
Magnesium	**0.012%**
Potassium	**2.11%**
Sodium	**4.09%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.47**	Histidine	**1.72**	Proline	**6.25**
Arginine	**3.31**	Isoleucine	**3.35**	Serine	**3.80**
Aspartic acid	**8.78**	Leucine	**5.80**	Threonine	**6.27**
Cystine	**0.50**	Lysine	**5.40**	Tryptophan	**0.87**
Glutamic acid	**15.04**	Methionine	**1.65**	Tyrosine	**1.49**
Glycine	**3.45**	Phenylalanine	**3.15**	Valine	**4.21**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Pork Brain Heart Infusion should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PORK HEART INFUSION

CAT. 1716 PEPTONES

DESCRIPTION

Pork Heart Infusion is a specially formulated preparation of dried infusion from pork hearts supplied as a light fine powder. It is a substitute for Bovine Heart Infusion in selected microbiological culture media.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3%	**3.50%**
Total Nitrogen (TN)	Minimum 10%	**11.67%**
AN/TN Ratio	N/A	**30.00%**
Loss on drying	Maximum 6%	**4.00%**
Ash	Maximum 15%	**10.50%**
pH (2% solution)	6.5 - 7.5	**7.1**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.018%**
Magnesium	**0.012%**
Potassium	**2.36%**
Sodium	**3.96%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.32**	Histidine	**0.05**	Proline	**5.84**
Arginine	**3.08**	Isoleucine	**3.39**	Serine	**3.81**
Aspartic acid	**8.77**	Leucine	**5.83**	Threonine	**3.33**
Cystine	**0.54**	Lysine	**5.43**	Tryptophan	**0.91**
Glutamic acid	**14.77**	Methionine	**1.65**	Tyrosine	**1.44**
Glycine	**2.55**	Phenylalanine	**3.15**	Valine	**4.24**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Pork Heart Infusion should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PORK MEAT PEPTONE

CAT. 1624 PEPTONES

DESCRIPTION

Pork Meat Peptone is an enzymatic hydrolysate of porcine animal tissue. It can be substituted for Meat Peptone (Bovine) in culture media formulations.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.4%	**3.70%**
Total Nitrogen (TN)	Minimum 10%	**13.08%**
AN/TN Ratio	N/A	**28.30%**
Loss on drying	Maximum 6%	**2.70%**
Ash	Maximum 15%	**9.50%**
pH (2% solution)	6.5 - 7.5	**6.9**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.023%**
Magnesium	**0.020%**
Potassium	**1.66%**
Sodium	**2.65%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**5.38**	Histidine	**1.24**	Proline	**7.37**
Arginine	**4.55**	Isoleucine	**2.45**	Serine	**3.26**
Aspartic acid	**8.56**	Leucine	**4.32**	Threonine	**2.58**
Cystine	**0.29**	Lysine	**4.37**	Tryptophan	**0.52**
Glutamic acid	**12.38**	Methionine	**1.19**	Tyrosine	**1.22**
Glycine	**9.85**	Phenylalanine	**2.48**	Valine	**3.30**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Pork Meat Peptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PROTEOSE PEPTONE

CAT. 1609 PEPTONES

DESCRIPTION

Proteose Peptone is an enzymatic digestion of animal tissues. It is commonly used in the preparation of culture media for the production of toxins, and in the fermentation industry for starter cultures. It is a highly nutritious source for the growth of a wide range of microorganisms.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.4%	**4.30%**
Total Nitrogen (TN)	Minimum 10%	**12.57%**
AN/TN Ratio	N/A	**34.20%**
Loss on drying	Maximum 6%	**3.00%**
Ash	Maximum 10%	**7.80%**
pH (2% solution)	6.5 - 7.5	**6.7**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.024%**
Magnesium	**0.023%**
Potassium	**1.40%**
Sodium	**2.70%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.49**	Histidine	**1.98**	Proline	**7.11**
Arginine	**3.54**	Isoleucine	**3.66**	Serine	**4.30**
Aspartic acid	**6.50**	Leucine	**6.68**	Threonine	**3.46**
Cystine	**0.38**	Lysine	**5.81**	Tryptophan	**0.80**
Glutamic acid	**15.51**	Methionine	**1.64**	Tyrosine	**1.59**
Glycine	**3.41**	Phenylalanine	**3.53**	Valine	**4.82**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Proteose Peptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PROTEOSE PEPTONE N° 3

CAT. 1607 PEPTONES

DESCRIPTION

Proteose Peptone N° 3 is a high quality hydrolysate produced by the enzymatic digestion of animal tissues. It is widely used in culture media and has been used extensively in the manufacture of toxins, vaccines, enzymes and other biological products. This product provides nitrogen in a form that is readily available for bacterial growth.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.4%	**4.35%**
Total Nitrogen (TN)	Minimum 10.0%	**12.42%**
AN/TN Ratio	N/A	**35.02%**
Loss on drying	Maximum 6%	**3.20%**
Ash	Maximum 10%	**8.20%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.024%**
Magnesium	**0.02%**
Potassium	**2.20%**
Sodium	**2.40%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**3.48**	Histidine	**1.99**	Proline	**6.95**
Arginine	**3.29**	Isoleucine	**3.83**	Serine	**4.30**
Aspartic acid	**6.69**	Leucine	**6.50**	Threonine	**3.57**
Cystine	**0.47**	Lysine	**5.95**	Tryptophan	**0.95**
Glutamic acid	**16.14**	Methionine	**1.77**	Tyrosine	**1.58**
Glycine	**2.90**	Phenylalanine	**3.56**	Valine	**4.89**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Proteose Peptone nº3 should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

SOY PEPTONE

CAT. 1608 PEPTONES

DESCRIPTION

Soy Peptone is a papaic/pancreatic digest of defatted soybean meal. It is used in culture media for the cultivation of a wide variety of microorganisms including bacteria and fungi. It is an excellent source of vitamins and carbohydrates.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 2.2%	**3.00%**
Total Nitrogen (TN)	Minimum 7%	**12.54%**
AN/TN Ratio	N/A	**23.90%**
Loss on drying	Maximum 6%	**2.80%**
Ash	Maximum 15%	**8.70%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.026%**
Magnesium	**0.012%**
Potassium	**1.50%**
Sodium	**3.00%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**4.73**	Histidine	**0.94**	Proline	**6.88**
Arginine	**4.31**	Isoleucine	**1.56**	Serine	**2.81**
Aspartic acid	**5.94**	Leucine	**2.82**	Threonine	**1.78**
Cystine	**0.34**	Lysine	**3.08**	Tryptophan	**0.19**
Glutamic acid	**9.98**	Methionine	**0.73**	Tyrosine	**1.06**
Glycine	**11.38**	Phenylalanine	**1.83**	Valine	**2.09**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Soy Peptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

TRYPTONE

CAT. 1612 PEPTONES

DESCRIPTION

Tryptone is a pancreatic digest of casein containing all amino acids found in casein as well as larger peptide fractions. This product is recommended for preparing media where enzymatic hydrolyzed casein is desired. It is an excellent nutrient for use in culture media for producing antibiotics, toxins, enzymes and other biological products. This product is widely used in the pharmaceutical and veterinary industries and the diagnostic culture media industry.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 3.9%	**4.20%**
Total Nitrogen (TN)	Minimum 10%	**13.31%**
AN/TN Ratio	N/A	**31.70%**
Loss on drying	Maximum 6%	**3.30%**
Ash	Maximum 15%	**6.00%**
pH (2% solution)	6.5 - 7.5	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.019%**
Magnesium	**0.0065%**
Potassium	**0.95%**
Sodium	**2.10%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**2.87**	Histidine	**2.29**	Proline	**8.65**
Arginine	**3.31**	Isoleucine	**4.48**	Serine	**5.08**
Aspartic acid	**6.52**	Leucine	**7.63**	Threonine	**3.91**
Cystine	**0.40**	Lysine	**6.51**	Tryptophan	**1.05**
Glutamic acid	**18.70**	Methionine	**2.35**	Tyrosine	**1.86**
Glycine	**1.79**	Phenylalanine	**4.09**	Valine	**5.51**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Tryptone should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

TRYPTOSE

CAT. 1614 PEPTONES

DESCRIPTION

Tryptose is a mixed enzymatic hydrolysate with distintictive nutritional properties. It is an excellent sole source of nitrogen, demonstrating a superiority over meat peptone in this regard. It is used to grow many fastidious microorganisms such as *Brucella, Streptococcus*, and *Neisseria*.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 2.9%	**4.40%**
Total Nitrogen (TN)	Minimum 10%	**13.40%**
AN/TN Ratio	N/A	**32.50%**
Loss on drying	Maximum 6%	**3.20%**
Ash	Maximum 15%	**9.70%**
pH (2% solution)	6.5 - 7.5	**7.4**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.001%**
Magnesium	**0.022%**
Potassium	**0.679%**
Sodium	**3.410%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**4.45**	Histidine	**< 0.01**	Proline	**6.33**
Arginine	**4.65**	Isoleucine	**0.34**	Serine	**4.09**
Aspartic acid	**6.34**	Leucine	**3.67**	Threonine	**3.55**
Cystine	**0.44**	Lysine	**4.64**	Tryptophan	**0.62**
Glutamic acid	**13.92**	Methionine	**1.92**	Tyrosine	**2.21**
Glycine	**2.84**	Phenylalanine	**7.52**	Valine	**1.93**

GROWTH SUPPORTING PROPERTIES

Peptone Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Tryptose should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

YEAST EXTRACT

CAT. 1702 PEPTONES

DESCRIPTION

Yeast Extract is a concentrate of the water- soluble portion of Saccharomyces cerevisiae cells that have been autolyzed. . It is rich in vitamins (especially B-complexes) amino acids and other growth factors. It is used in many microbiological culture media formulations as an excellent growth source.

Yeast extract is considered a non-animal product and is used extensively for many non-animal formulations of bacterial, fungal, mammalian and insect cell culture.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen (AN)	Minimum 4.5%	**5.40%**
Total Nitrogen (TN)	Minimum 10%	**10.70%**
AN/TN Ratio	N/A	**50.46%**
Loss on drying	Maximum 5%	**3.30%**
Ash	Maximum 15%	**9.50%**
pH (2% solution)	6.0 - 7.2	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.10%**
Magnesium	**0.10%**
Potassium	**5.70%**
Sodium	**0.30%**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**8.70**	Histidine	**2.0**	Proline	**4.0**
Arginine	**5.00**	Isoleucine	**5.60**	Serine	**4.70**
Aspartic acid	**9.70**	Leucine	**7.60**	Threonine	**4.40**
Cystine	**0.80**	Lysine	**8.0**	Tryptophan	**1.20**
Glutamic acid	**16.10**	Methionine	**1.30**	Tyrosine	**2.30**
Glycine	**4.90**	Phenylalanine	**3.80**	Valine	**5.80**

GROWTH SUPPORTING PROPERTIES

Yeast Extract Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Yeast Extract should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

YEAST EXTRACT, MICRO-GRANULATED

CAT. 1732 PEPTONES

DESCRIPTION

Yeast Extract, micro-granulated is a water soluble extract of selected autolyzed yeast cells. It is rich in vitamins, especially B-complexes, amino acids and other growth factors. It is used in many microbiological culture media formulations as an excellent growth source.

CHEMICAL CHARACTERISTICS

	Specifications	Typical Analysis
Amino Nitrogen [AN]	Minimum 4.5%	**5.40%**
Total Nitrogen [TN]	Minimum 10%	**10.70%**
AN/TN Ratio	N/A	**50.46%**
Loss on drying	Maximum 5%	**3.30%**
Ash	Maximum 15%	**9.50%**
pH [2% solution]	6.0 - 7.2	**6.8**

ELEMENTAL PROFILE

	Typical Analysis
Calcium	**0.10**
Magnesium	**0.10**
Potassium	**5.70**
Sodium	**0.30**

AMINO ACIDS

Total (g/100g)		Total (g/100g)		Total (g/100g)	
Alanine	**8.70**	Histidine	**2.0**	Proline	**4.0**
Arginine	**5.00**	Isoleucine	**5.60**	Serine	**4.70**
Aspartic Acid	**9.70**	Leucine	**7.60**	Threonine	**4.40**
Cystine	**0.80**	Lysine	**8.0**	Tryptophan	**1.20**
Glutamic acid	**13.10**	Methionine	**1.30**	Tyrosine	**2.30**
Glycine	**4.90**	Phenylalanine	**3.80**	Valine	**5.80**

GROWTH SUPPORTING PROPERTIES

Yeast Extract Agar	Good

MICROBIOLOGICAL TEST

Standard plate count	Less than 5000 cfu/g
Yeasts and molds	Less than 100 cfu/g
Coliforms	Negative
Salmonella	Negative

STORAGE

Once opened keep powdered medium closed to avoid hydration.

The dehydrated Yeast Extract, Micro-Granulated should be homogeneous, free flowing and beige in color. If there are any changes physically, discard the product.

PEPTONES, INFUSIONS, EXTRACTS AND RAW MATERIALS: TYPICAL ANALYSES

TYPICAL ANALYSIS		%						%				AMINO ACIDS (%)																			MICROBIOLOGICAL ANALYSIS				DIGESTION
PRODUCT	CAT. NO.	Amino Nitrogen (AN)	Total Nitrogen (TN)	AN/TN Ratio	Loss on drying	Ash	pH (2% solution)	Calcium	Magnesium	Potassium	Sodium	Alanine	Arginine	Aspartic acid	Cystine	Glutamic acid	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine	Standard plate count	Yeast and molds	Coliforms	Salmonella	
ACID CASEIN PEPTONE (H)	1604	5,40	8,80	61,40	3,00	33,00	6,8	0,015	0,005	0,035	12,25	1,70	2,00	5,30	0,20	13,70	1,00	1,40	2,90	5,00	4,60	1,50	2,00	5,80	2,80	2,00	<1,0	1,70	3,60	< 5000	< 100	Neg	Neg	Acid Hydrolysis
BACTERIOLOGICAL OX BILE (i)	1710	-	-	-	3,10	-	8,3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< 5000	< 100	Neg	Neg	Purification
BACTERIOLOGICAL PEPTONE	1616	3,00	15,55	19,20	3,20	4,70	6,9	0,023	0,013	0,25	1,40	7,95	7,21	6,42	0,14	9,93	20,71	0,93	1,41	3,02	3,69	0,92	1,94	11,71	3,51	1,90	0,09	0,75	2,40	< 5000	< 100	Neg	Neg	Enzymatic
BEEF EXTRACT	1700	4,10	12,48	32,90	2,50	9,40	6,8	0,011	0,019	2,60	1,60	3,28	3,22	6,60	0,35	15,86	2,29	2,08	3,91	6,50	5,98	1,63	3,58	6,91	4,37	3,56	0,97	1,68	4,85	< 5000	< 100	Neg	Neg	Extraction
BILE SALTS N° 3 (ii)	1706	-	-	-	4,10		8,1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< 5000	< 100	Neg	Neg	Purification
CASEIN C C PEPTONE	1603	4,15	13,10	31,67	3,15	6,80	6,8	0,020	0,007	1,80	2,20	2,94	3,36	6,28	0,41	17,90	1,88	2,39	4,44	7,60	6,63	2,31	4,13	8,62	5,02	3,96	0,92	1,85	5,50	< 5000	< 100	Neg	Neg	Enzymatic
CASEIN PEPTONE	1602	4,20	13,13	32,00	3,30	6,00	6,8	0,019	0,008	1,30	2,10	2,91	3,30	6,99	0,44	18,74	1,86	2,38	4,45	7,62	6,60	2,32	4,11	8,65	5,08	3,91	0,95	1,86	5,51	< 5000	< 100	Neg	Neg	Enzymatic
CASEIN PEPTONE N° 2	1620	4,20	13,49	31,10	3,30	6,40	6,8	0,019	0,006	0,86	2,20	2,74	3,29	6,99	0,49	19,23	1,73	2,44	4,52	7,70	6,73	2,45	4,18	9,11	5,14	3,95	1,00	1,91	5,59	< 5000	< 100	Neg	Neg	Enzymatic
CASEIN PEPTONE HS (High Solubility)	1622	5,90	12,26	48,10	3,00	6,20	6,6	0,039	0,010	0,48	2,90	2,44	2,99	5,98	0,30	16,36	1,69	2,20	3,99	6,52	7,82	1,46	3,51	7,90	4,53	5,87	0,94	1,23	4,96	< 5000	< 100	Neg	Neg	Enzymatic
GELATIN PEPTONE	1606	3,50	15,48	22,60	3,00	4,20	6,9	0,018	0,010	1,10	0,97	7,89	7,16	6,34	0,13	9,58	20,60	0,89	1,39	2,84	3,61	0,85	1,88	11,46	3,45	1,87	0,09	0,71	2,31	< 5000	< 100	Neg	Neg	Enzymatic
HEART INFUSION	1714	3,50	11,00	31,80	3,50	10,10	7,0	0,009	0,020	1,40	3,60	3,20	3,55	6,05	0,31	15,97	2,50	2,13	3,91	6,68	6,01	1,75	3,66	7,04	4,54	3,12	0,87	1,61	4,73	< 5000	< 100	Neg	Neg	Extraction
LACTALBUMIN HYDROLYSATE	1626	5,30	12,40	42,70	4,40	5,30	6,8	0,078	0,027	0,83	2,10	3,20	1,36	7,59	0,76	17,12	1,83	2,02	4,30	6,52	6,68	1,60	3,08	7,37	4,72	4,58	1,17	0,97	5,07	< 5000	< 100	Neg	Neg	Enzymatic
MALT EXTRACT (iii)	1708	0,40	1,30	30,77	2,50	-	5,0	0,014	0,073	0,001	-	0,40	0,50	0,90	-	1,60	0,40	0,60	0,50	0,60	0,60	0,20	0,70	0,60	0,40	0,40	-	0,30	0,60	< 5000	Neg	Neg	Neg	Enzymatic
MEAT PEPTONE	1600	3,70	12,33	30,00	2,70	9,20	6,9	0,072	0,029	2,70	2,50	5,62	4,08	5,61	0,37	11,62	8,37	1,31	2,63	4,50	4,30	0,85	2,61	6,29	2,95	2,46	0,59	1,11	3,50	< 5000	< 100	Neg	Neg	Enzymatic
PEPTONIZED MILK	1628	2,40	7,41	32,40	4,30	8,40	6,7	0,430	0,061	1,20	1,90	1,56	1,71	3,86	0,28	10,01	1,07	1,29	2,43	4,25	3,35	1,02	2,18	4,81	2,73	2,18	0,52	1,20	2,98	< 5000	< 100	Neg	Neg	Enzymatic
POLYPEPTONE	1610	4,10	13,12	31,25	3,40	8,80	6,8	0,030	0,014	1,60	2,12	4,05	3,76	8,83	0,43	15,97	5,70	1,81	3,44	5,99	5,50	1,81	3,34	8,21	4,33	3,31	0,80	1,42	4,37	< 5000	< 100	Neg	Neg	Enzymatic
PORK BRAIN HEART INFUSION	1712	3,30	11,81	27,90	3,50	9,20	7,2	0,020	0,012	2,11	4,09	3,47	3,31	8,78	0,50	15,04	3,45	1,72	3,35	5,80	5,40	1,65	3,15	6,25	3,80	6,27	0,87	1,49	4,21	< 5000	< 100	Neg	Neg	Extraction
PORK HEART INFUSION	1716	3,50	11,67	30,00	4,00	10,50	7,1	0,018	0,012	2,36	3,96	3,32	3,08	8,77	0,54	14,77	2,55	0,05	3,39	5,83	5,43	1,65	3,15	5,84	3,81	3,33	0,91	1,44	4,23	< 5000	< 100	Neg	Neg	Extraction
PORK MEAT PEPTONE	1624	3,70	13,08	28,30	2,70	9,50	6,9	0,023	0,020	1,66	2,65	5,38	4,55	8,56	0,29	12,38	9,85	1,24	2,45	4,32	4,37	1,19	2,48	7,37	3,26	2,58	0,52	1,22	3,30	< 5000	< 100	Neg	Neg	Enzymatic
PROTEOSE PEPTONE	1609	4,30	12,57	34,20	3,00	7,80	6,7	0,024	0,023	1,40	2,70	3,49	3,54	6,50	0,38	15,51	3,41	1,98	3,66	6,68	5,81	1,64	3,53	7,11	4,30	3,46	0,80	1,59	4,82	< 5000	< 100	Neg	Neg	Enzymatic
PROTEOSE PEPTONE N°3	1607	4,35	12,42	35,02	3,20	8,20	6,8	0,024	0,020	2,20	2,40	3,48	3,29	6,69	0,47	16,14	2,90	1,99	3,83	6,50	5,95	1,77	3,56	6,95	4,30	3,57	0,95	1,58	4,89	< 5000	< 100	Neg	Neg	Enzymatic
SOY PEPTONE	1608	3,00	12,54	23,90	2,80	8,70	6,8	0,026	0,012	1,50	3,00	4,73	4,31	5,94	0,34	9,98	11,38	0,94	1,56	2,82	3,08	0,73	1,83	6,88	2,81	1,78	0,19	1,06	2,09	< 5000	< 100	Neg	Neg	Enzymatic
TRYPTONE	1612	4,22	13,31	31,70	3,30	6,00	6,8	0,019	0,007	1,30	2,10	2,87	3,31	6,52	0,40	18,70	1,79	2,29	4,48	7,63	6,51	2,35	4,09	8,65	5,08	3,91	1,05	1,86	5,51	< 5000	< 100	Neg	Neg	Enzymatic
TRYPTOSE	1614	4,40	13,40	32,50	3,20	9,70	7,4	0,00	0,022	0,68	3,41	4,45	4,65	6,34	0,44	13,92	2,84	<0,01	0,34	3,67	4,64	1,92	7,52	6,33	4,09	3,55	0,62	2,21	1,93	< 5000	< 100	Neg	Neg	Enzymatic
YEAST EXTRACT	1702	5,40	10,70	50,46	3,30	9,50	6,8	0,100	0,100	5,70	0,30	8,70	5,00	9,70	0,80	16,10	4,90	2,00	5,60	7,60	8,00	1,30	3,80	4,00	4,70	4,40	1,20	2,30	5,80	< 5000	< 100	Neg	Neg	Autolysis

NOTES	ADDITIONAL CHEMICAL CHARACTERISTICS (%)			
BACTERIOLOGICAL OX BILE (i)	Cholic acid	47,0		
BILE SALTS N° 3 (ii)	Sodium cholate	49,3	Sodium Desoxycholate	49,4
MALT EXTRACT (iii)	Sugar content	96,0	Sulfuric ash	1,4

SUPPLEMENTS AND

ADDITIVES

AMPICILLIN SUPPLEMENT (AEROMONAS)

CAT. 6052

Selective supplement for the isolation of *Aeromonas hydrophila*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Sodium Ampicillin	**2.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of AEROMONAS AGAR BASE (RYAN) (**Cat. 1370**) autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
AMPICILLIN SUPPLEMENT (AEROMONAS)	6052	10 Vials. Each vial for 500 ml of medium
AEROMONAS AGAR BASE (RYAN)	1370	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of AEROMONAS AGAR BASE (**Cat. 1370**) from type cultures after incubation at a temperature of 30 - 35°C and observed after 24 hours.

Microorganisms	Growth	Colony Color
Aeromonas hydrophila ATCC 7966	Good	Green with black centre
Pseudomonas aeruginosa ATCC 27853	Good	Blue- green
Pseudomonas aeruginosa ATCC 9270	Good	Blue- green
Escherichia coli ATCC 25922	Inhibited	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Ryan N. (1985) Personal communication.

Rogol M., Sechter I., Grinberg L., Gerichter Ch. B. (1992) J. Med. Microbiol. 12. 229-231.

BACILLUS CEREUS SUPPLEMENT

CAT. 6021

Selective supplement for the isolation and enumeration of *Bacillus cereus*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Polymyxin B Sulfate	**50.000 IU**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 450 ml of BACILLUS CEREUS SELECTIVE AGAR BASE (**Cat. 1124**). Also can add to for BACILLUS CEREUS AGAR BASE (MYP) ISO 7932 (**Cat. 1343**) autoclaved, cooled to 50°C and with 50 ml of EGG YOLK EMULSION (**Cat. 5152**) added. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
BACILLUS CEREUS SUPPLEMENT	6021	10 Vials. Each vial for 500 ml of medium
BACILLUS CEREUS SELECTIVE AGAR BASE	1124	500 g
BACILLUS CEREUS AGAR BASE (MYP) ISO 7932	1343	500 g
EGG YOLK EMULSION	5152	100 ml

MICROBIOLOGICAL TEST

The following results were obtained in the performance of BACILLUS CEREUS AGAR BASE (**Cat. 1124** and **Cat. 1343**), with egg yolk emulsion, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 40 hours.

Microorganisms	Growth	Colony Color	Precipitation
**Bacillus cereus* ATCC 11778	Good	Red	+
Bacillus subtilis ATCC 6051	Good	Yellow	-
Proteus mirabilis ATCC 29906	Inhibited	Colorless	-
Staphylococcus aureus ATCC 6538	Inhibited	Yellow	+

* Incubation at 30°C for 18 - 24 hours according to ISO 7932.

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Mossel, D. A. A., Koopman, M. J. and Jongerius, E. 1 967. " Enumeration of Bacillus cereus in foods ". Appl. Microbiol.1 5:650-653.

BORDETELLA SUPPLEMENT

CAT. 6015

Selective supplement for isolation of *Bordetella spp.*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Cephalexin	**20 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of BORDET-GENGOU AGAR BASE (**Cat. 1107**), autoclaved, cooled to 50°C and with 5 - 10% defibrinated sterile blood added. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
BORDETELLA SUPPLEMENT	6015	10 Vials. Each vial for 500 ml of medium
BORDET-GENGOU AGAR BASE	1107	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of BORDET GENGOU AGAR BASE (**Cat. 1107**), with 5% defibrinated sterile sheep blood added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 48 - 72 hours.

Microorganisms	Growth
Bordetella bronchiseptica ATCC 4617	Good
Bordetella pertussis ATCC 8467	Good
Bordetella parapertussis ATCC 15311	Good

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, M.D.

Marcon, M.J. 1995. Bordetella, p. 566-573. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

BRUCELLA SUPPLEMENT

CAT. 6017

Selective supplement for the isolation of *Brucella spp.*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Nystatin	**50.000 IU**
Bacitracin	**12.500 IU**
Polymyxin B	**2.500 IU**
Cycloheximide	**50 mg**
Vancomycin	**10 mg**
Nalidixic Acid	**2.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of 1:1 solution of methanol / sterile distilled water. Incubate at 37°C for 10 - 15 minutes. Mix until completely dissolved and aseptically add to 500 ml of BRUCELLA AGAR BASE (**Cat. 1012**) or COLUMBIA AGAR BASE (**Cat. 1104**), autoclaved, cooled to 50°C and, if desired, with both 5 - 10% inactivated horse serum and 1 - 5% sterile dextrose solution added. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
BRUCELLA SUPPLEMENT	6017	10 Vials. Each vial for 500 ml of medium
BRUCELLA AGAR BASE	1012	500 g
COLUMBIA AGAR BASE	1104	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of BRUCELLA AGAR BASE (**Cat. 1012**) and COLUMBIA AGAR BASE (**Cat. 1104**) from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere and observed after 24 hours.

Microorganisms	Growth
Brucella abortus ATCC 4315	Good
Brucella melitensis ATCC 4309	Good
Brucella suis ATCC 4314	Good

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Moyer, N.P., and L.A. Holcomb (1995). Brucella, p. 549-555. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.). Manual for clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Vanderzant, C., and D.F. Splittstoesser (ed.) (1992). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

BURKHOLDERIA CEPACIA SUPPLEMENT

CAT. 6032

Selective supplement for the isolation of *Burkholderia cepacia*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Polymyxin B	**75.000 IU**
Ticarcillin	**50 mg**
Gentamicin	**2.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of BURKHOLDERIA CEPACIA AGAR BASE (**Cat. 1347**) autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
BURKHOLDERIA CEPACIA SUPPLEMENT	6032	10 Vials. Each vial for 500 ml of medium
BURKHOLDERIA CEPACIA AGAR BASE	1347	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium BURKHOLDERIA CEPACIA AGAR BASE (**Cat. 1347**) from type cultures after incubation at a temperature of 37°C and observed after 48 -72 hours.

Microorganisms	Growth
Burkholderia cepacia ATCC 25608	Good
Pseudomonas aeruginosa ATCC 27853	Inhibited (partial to complete)

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Bahame, J. B. and Schroth, M. N. [1989): Spatial-temporal colonization pattems of a rhizobacterium on underground organs of potato Phytopatology. Vol.77:1 093-1100.

Barelmann, I.; Meyer, I.M.; Taraz, K. and Budzikiewicz, D. [1996): Cepacia chelin, a new catecholate siderophore from Burkholderia [Pseudomonas) cepacia. Z Natarfosch. Vol. 51: 627-630.

Bashan, Y. and Holguin, Gina. [1998): Proposal for the Division of plant growth–promoting rhizobacteria into two classifications: biocontrol-PGPB [plant growth–promoting bacteria) and PGPB. Soil Biol Biochem. Vol. 30[8-9): 1225-1228.

CCDA SUPPLEMENT (CAMPYLOBACTER BLOOD FREE)

CAT. 6053

Selective supplement for the isolation of *Campylobacter jejuni* and *Campylobacter coli*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Cephoperazone	**16 mg**
Amphotericin B	**5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of CAMPYLOBACTER AGAR BASE (BLOOD FREE) (**Cat. 1129**) autoclaved and cooled to 50°C. Homogenize gently and dispense into Petri dishes.

PRODUCT	CAT.	PACK SIZE
CCDA SUPPLEMENT (CAMPYLOBACTER BLOOD FREE)	6053	10 Vials. Each vial for 500 ml of medium
CAMPYLOBACTER AGAR BASE BLOOD FREE (CCDA)	1129	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of CAMPYLOBACTER AGAR BASE BLOOD FREE (CCDA) (**Cat. 1129**) from type cultures after incubation at a temperature of 42°C and observed after 24 - 48 hours.

Microorganisms	Growth
Campylobacter jejuni ATCC 29428	Good
Campylobacter coli ATCC 33559	Good
Escherichia coli ATCC 25922	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Bolton F.J. Hutchinson D.N. y Cioste D. (1984) clin. Microbiol. 19,169-171 Bolton E.J., Roberstson L. (1982) J. Clin Parth 35, 462-67

CEFOXITIN MRSA SUPPLEMENT

CAT. 6069

For the detection of Methicillin resistant *Staphylococcus aureus*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Cefoxitin	**2 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution. Aseptically add to 500 ml of MRSA AGAR (**Cat. 1423**) or to 500 ml of MRSA AGAR, MODIFIED CHROMOGENIC (**Cat. 1498**), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
CEFOXITIN MRSA SUPPLEMENT	6069	10 vials
MRSA AGAR	1423	500 g
MRSA AGAR, MODIFIED CHROMOGENIC	1498	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of MRSA AGAR (**Cat. 1423**) and MRSA AGAR, MODIFIED CHROMOGENIC (**Cat. 1498**) from type cultures, with the supplement added, after incubation at a temperature of 37ºC ± 2ºC and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Staphylococcus aureus ATCC 25923	Inhibited	-
Staphylococcus aureus ATCC 43300	Good	Blue
Escherichia coli ATCC 25922	Inhibited	-

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Hutchison, M.J., Edwards, G.F.S., Morrison, D., Evaluation of chromogenic MRSA Reference Laboratory presented at the 2005 Institute of BioMedical

CLOSTRIDIUM DIFFICILE SUPPLEMENT

CAT. 6061

For the isolation of *Clostridium difficile*

FORMULA PER VIAL (Each vial for 500 ml of medium)

D-Cycloserine	**125 mg**
Cefoxitin	**4.0 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of Clostridium Difficile Agar Base (**Cat. 1447**). Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
CLOSTRIDIUM DIFFICILE SUPPLEMENT	6061	10 Vials. Each vial for 500 ml of medium
CLOSTRIDIUM DIFFICILE AGAR BASE	1447	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of CLOSTRIDIUM DIFFICILE AGAR BASE (**Cat. 1447**) from type cultures, adding 7% defibrinated Horse Blood, after incubation at a temperature of 35ºC - 37ºC and observed after 18 - 48 hours.

Microorganisms	Growth
Clostridium difficile ATCC 11204	Good
Staphylococcus aureus ATCC 25923	Inhibited
Escherichia coli ATCC 25922	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

George W. L., Sutter V.L., Goldstein E.C.J., Ludwig S.L and Finegold S.M (1978) Lancet. i. 802-803

Hall I. and O'Toole E. (1935) am. J. Dis. Child. 49. 390.

Keighley M.R.B, Burdon D.W., Alexander Williams J. et al (1978) Lancet ii. 1165-1167.

CLOSTRIDIUM PERFRINGENS SUPPLEMENT (TSC) ISO 7937

CAT. 6020

Selective supplement for the enumeration of *Clostridium perfringens*

FORMULA PER VIAL (Each vial for 500 ml of medium)

D-Cycloserine	**200 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of TSC. AGAR BASE (**Cat. 1029**), autoclaved, cooled to 50°C and. If desired, 25 ml of Egg Yolk Emulsion (**Cat. 5152**) can be added (Not indicated in ISO 7937). Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
CLOSTRIDIUM PERFRINGENS SUPPLEMENT (TSC)	6020	10 Vials. Each vial for 500 ml of medium
TSC AGAR BASE	1029	500 g
EGG YOLK EMULSION	5152	100 ml

MICROBIOLOGICAL TEST

The following results were obtained in the performance of TSC AGAR BASE (**Cat. 1029**) from type cultures with the Egg Yolk Emulsion (**Cat. 5152**) added after incubation at a temperature of 37°C and observed after 20 ± 2 hours under anaerobic conditions.

Microorganisms	Growth	Colony Color
Clostridium perfringens ATCC 13124	Good	Black

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Hauschild, A.H.W., Hilsheimer, R., and Griffith, D.W. 1974. Enumeration of faecal Clostridium perfringens spores in eggyolk - free Tryptose – Sulfite – Cycloserine Agar. Appl. Microb., 27:527-530.

NF T 90-415: October 1985. Water testing. Detection and enumeration of the spores of sulfite-reducing anaerobies and of sulfite-reducing Clostridia. General mehod by the standing tube technique.

CNA (STAPH/STREP) SUPPLEMENT

CAT. 6016

Selective supplement for the isolation of staphylococci, streptococci and pneumococci

FORMULA PER VIAL (Each vial for 500 ml of medium)

Nalidixic Acid	**7.5 mg**	Colistin Sulfate	**5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of COLUMBIA AGAR BASE (**Cat. 1104**), autoclaved, cooled to 50°C and, if desired, with 5 - 10% defibrinated sterile blood added. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
CNA (STAPH/STREP) SUPPLEMENT	6016	10 Vials. Each vial for 500 ml of medium
COLUMBIA AGAR BASE	1104	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of COLUMBIA AGAR BASE (**Cat. 1104**), with 5% defibrinated sterile sheep blood added, from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Growth with 5% sheep blood	Hemolysis
Proteus mirabilis ATCC 12453	Inhibited	Inhibited	–
Staphylococcus aureus ATCC 25923	Good	Good	Beta
Streptococcus pneumoniae ATCC 6305	Good	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Good	Beta

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Ellner, P.D. Stoessel, C.J. Drakeford, E. & Vasi, F. (1966), "A new culture medium for medical bacteriology" Am.J. clin. Path., 45,502-504.

FECAL COLIFORMS SUPPLEMENT

CAT. 6023

Supplement for isolation of fecal coliforms

FORMULA PER VIAL (Each vial for 500 ml of medium)

Rosolic Acid	**50 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of 1% solution of 0.2N NaOH. Mix gently until complete dissolution and aseptically add to 500 ml of FECAL COLIFORMS AGAR BASE (**Cat. 1127**) or FECAL COLIFORMS BROTH BASE (**Cat. 1121**) autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
FECAL COLIFORM SUPPLEMENT	6023	10 Vials. Each vial for 500 ml of medium
FECAL COLIFORM AGAR BASE	1127	500 g
FECAL COLIFORM BROTH BASE	1121	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the FECAL COLIFORMS AGAR BASE (**Cat. 1127**) from type cultures after incubation at both temperatures of 44.5 ± 0.5°C and 35 ± 2°C and observed after 24 ± 2 hours using the membrane - filter technique.

Microorganisms	Growth 44.5°C	Growth 35°C	Colony Color
Escherichia coli ATCC 25922	Good	Good	Blue
Salmonella typhymurium ATCC 14028	Inhibited	Good	Grey
Shigella flexneri ATCC 12022	Inhibited	Good	Grey
Enterococcus faecalis ATCC 19433	Inhibited	Inhibited	-

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Geldreich, Clark and Kabler, 1963. USPHS, HEW. Personal Communication.

Geldreich, Clark, Huff and Bert, 1965. Journal of American Water Works Association, 57:208.

FERRIC AMMONIUM CITRATE SUPPLEMENT

CAT. 6050

Supplement for the detection of *Listeria monocytogenes*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Ferric Ammonium Citrate	**250 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of LISTERIA FRASER BROTH BASE (ISO 11290-1) (**Cat. 1182**) and LISTERIA HALF FRASER BROTH BASE (ISO 11290-1) (**Cat. 1183**), autoclaved and cooled to 50ºC. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
FERRIC AMMONIUM CITRATE SUPPLEMENT	6050	10 Vials. Each vial for 500 ml of medium
LISTERIA FRASER BROTH BASE	1182	500 g
LISTERIA HALF FRASER BROTH BASE	1183	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, added with LISTERIA HALF FRASER BROTH BASE (ISO 11290-1) (**Cat. 1183**) (**Cat. 1182**) from type cultures after incubation at a temperature of 30ºC, in aerobic conditions and observed after 24 ± 2 hours.

Microorganisms	Growth
Listeria monocytogenes ATCC 19111	Good
Enterococcus faecalis ATCC 29212	Null

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

ISO NORMATIVE 11290-1 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method

Fraser J.A and Sperber W.H (1988) McClain D. and Lee W.H (1988)

FRASER LISTERIA SELECTIVE SUPPLEMENT ISO 11290-1

CAT. 6001

Selective supplement for isolation of *Listeria monocytogenes* and other species of *Listeria* from food products

FORMULA PER VIAL (Each vial for 500 ml of medium)

VIAL A		VIAL B	
Ferric Ammonium Citrate	**250 mg**	Acryflavine hydrochloride	**12.5 mg**
		Nalidixic Acid	**10 mg**

PREPARATION

Aseptically reconstitute each of the 2 vials with 5 ml of sterile distilled water. Mix gently until complete dissolution. Aseptically add both vials to 500 ml of LISTERIA ENRICHMENT BROTH BASE FRASER (**Cat. 1120**), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
FRASER LISTERIA SELECTIVE SUPPLEMENT	6001	2 x 5 Vials. Each pair of vials for 500 ml of medium
LISTERIA ENRICHMENT BROTH BASE FRASER	1120	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LISTERIA ENRICHMENT BROTH BASE FRASER (**Cat. 1120**) from type cultures after incubation at a temperature of 37± 2°C in aerobic conditions and observed after 26 ± 2 hours.

Microorganisms	Growth	Esculin reaction
Listeria monocytogenes ATCC 19117	Good	+
Enterococcus faecalis ATCC 29212	Null	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Fraser J.A. and Sperber W.H (1988) McClain D. and Lee W.H (1988).

ISO 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

HALF FRASER LISTERIA SELECTIVE SUPPLEMENT ISO 11290-1

CAT. 6002

Selective supplement for isolation of *Listeria monocytogenes* and other species of *Listeria* from food products

FORMULA PER VIAL (Each vial for 500 ml of medium)

VIAL A		VIAL B	
Ferric Ammonium Citrate	**250 mg**	Acryflavine Hydrochloride	**6.25 mg**
		Nalidixic Acid	**5 mg**

PREPARATION

Aseptically reconstitute each of the 2 vials with 5 ml of sterile distilled water. Mix gently until complete dissolution. Aseptically add both vials to 500 ml of LISTERIA ENRICHMENT BROTH BASE HALF FRASER (**Cat. 1120**), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
HALF FRASER LISTERIA SELECTIVE SUPPLEMENT	6002	2 x 5 Vials. Each pair of vials for 500 ml of medium
LISTERIA ENRICHMENT BROTH BASE FRASER	1120	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LISTERIA ENRICHMENT BROTH BASE FRASER (**Cat. 1120**) from type cultures after incubation at a temperature of 37 ± 2°C in aerobic conditions and observed after 26 ± 2 hours.

Microorganisms	Growth	Esculin reaction
Listeria monocytogenes ATCC 19117	Good	+
Enterococcus faecalis ATCC 29212	Null	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Fraser J.A. and Sperber W.H (1988) McClain D. and Lee W.H (1988).

ISO 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

ITC SUPPLEMENT ISO 10273

CAT. 6051

Selective supplement for the isolation of *Yersinia enterocolitica*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Irgasan	**0.5 mg**
Ticarcillin	**0.5 mg**
Potassium chlorate	**500 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of ITC BROTH BASE (IRGASAN TICARCILLIN BROTH AND POTASSIUM CHLORATE) (ISO 10273) (**Cat.1361**) autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
ITC SUPPLEMENT (ISO 10273)	6051	10 Vials. Each vial for 500 ml of medium
ITC BROTH BASE (IRGASAN TICARCILLIN BROTH AND POTASSIUM CHLORATE) (ISO 10273)	1361	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of ITC BROTH BASE (**Cat. 1361**) from type cultures in the performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 18 - 48 hours.

Microorganisms	Growth
Yersinia enterocolitica ATCC 23715	Good
Yersinia enterocolitica ATCC 9610	Good
Escherichia coli ATCC 25922	Inhibited
Bacillus cereus ATCC 11778	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

American Public Health Association Compendium of Methods for the microbiological Examination of Foods.

Schiemann, DA: Synthesis of a selective agar medium for Yersinia enterocolitica. - Canad. J . Microbiol ,25 1 298 -130 4

International Standard ISO 10273 Microbiology of food and animal stuffs. Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica

LCAT SUPPLEMENT

CAT. 6012

Selective supplement for pathogen *Neisseria* isolation

FORMULA PER VIAL (Each vial for 500 ml of medium)

Trimethoprim	**3.25 mg**	Lincomycin	**0.5 mg**
Colistin Sulfate	**3 mg**	Amphotericin B	**0.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix until complete dissolution and aseptically add to 250 ml of GC AGAR BASE (**Cat. 1106**) autoclaved, cooled to 50°C and with 250 ml Sterile 2% hemoglobin solution and reconstituted POLYENRICHMENT SUPPLEMENT (**Cat. 6011**) added. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
LCAT SUPPLEMENT	6012	10 Vials. Each vial for 500 ml of medium
GC AGAR BASE	1106	500 g
POLYENRICHMENT SUPPLEMENT	6011	2 x 5 Vials. Each pair of vials for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of GC AGAR BASE (**Cat. 1106**), with hemoglobin to 2 % and both supplements, from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere and observed after 18 - 24 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13077	Good
Neisseria gonorrhoeae ATCC 49981	Good
Entrerococcus faecalis ATCC 19433	Inhibited
Escherichia coli ATCC 25922	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Thayer, J.D., and A. Lester. 1971. Transgrow, a medium for transport and growth Neisseria gonorrhoeae and Neisseria meningiditidis. HSMHA Health Service Rep., 86:30.

MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, M.D.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.).1 995. Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.

LEGIONELLA BCYE GROWTH SUPPLEMENT

CAT. 6022

Growth supplement for isolation of *Legionella spp.*

FORMULA PER VIAL (Each vial for 90 ml of medium)

Buffer ACES	**1 g**
Potassium Hydroxide	**200 mg**
Alpha-Ketoglutarate	**100 mg**
L-Cysteine	**40 mg**
Ferric Pyrophosphate	**25 mg**

PREPARATION

Aseptically reconstitute 1 vial with 10 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 90 ml of LEGIONELLA CYE AGAR BASE (**Cat. 1311**), autoclaved and cooled to 50°C. If further selectivity is desired, add 1 ml of 1 vial of LEGIONELLA GVPC SUPPLEMENT (**Cat. 6025**), previously reconstituted with 5 ml of sterile water/acetone 1:1 (see Preparation of Cat. 6025). Mix well and distribute into sterile containers.

Note: The apparent lack of uniformity (white - beige to reddish - amber) is due to slight temperature differences that the vials are subject to depending on their position within the trays during the lyophilisation process. This does not affect content or optimum performance.

PRODUCT	CAT.	PACK SIZE
LEGIONELLA BCYE GROWTH SUPPLEMENT	6022	10 Vials. Each vial for 90 ml of medium
LEGIONELLA CYE AGAR BASE	1311	500 g
LEGIONELLA GVPC SUPPLEMENT	6025	10 Vials. Each vial for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LEGIONELLA CYE AGAR BASE (**Cat. 1311**), with both supplements, from type cultures after incubation at a temperature of 36 ± 2°C and observed after 24 - 72 hours and up to 10 days.

Microorganisms	Growth	Colony Color
Legionella pneumophila ATCC 33153	Good	White
Escherichia coli ATCC 25922	Inhibited	–
Staphylococcus epidermidis ATCC 12228	Inhibited	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

ISO 11731 water quality- Detection and enumeration of Legionella

LEGIONELLA GVPC SUPPLEMENT

CAT. 6025

Selective supplement for isolation of *Legionella spp.*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Polymyxin B Sulfate	**39.600 IU**
Glycine	**1.5 g**
Cycloheximide	**40 mg**
Vancomycin	**0.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water/acetone 1:1. Mix gently until complete dissolution and aseptically add to 500 ml of LEGIONELLA CYE AGAR BASE (**Cat. 1311**), autoclaved and cooled to 50°C and adding 5 vials of LEGIONELLA BCYE GROWTH SUPPLEMENT (**Cat. 6022**) to it. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
LEGIONELLA GVPC SUPPLEMENT	6025	10 Vials. Each vial for 500 ml of medium
LEGIONELLA CYE AGAR BASE	1311	500 g
LEGIONELLA BCYE GROWTH SUPPLEMENT	6022	10 Vials. Each vial for 90 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LEGIONELLA CYE AGAR BASE (**Cat. 1311**), with both supplements, from type cultures after incubation at a temperature of 36 ± 2°C and observed after 24 - 72 hours and up to 10 days.

Microorganisms	Growth	Colony Color
Legionella pneumophila ATCC 33153	Good	White
Escherichia coli ATCC 25922	Inhibited	–
Staphylococcus epidermidis ATCC 12228	Inhibited	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

ISO 11731 water quality- Detection and enumeration of Legionella

LISTERIA CHROMOGENIC SELECTIVE SUPPLEMENT ISO 11290-1

CAT. 6040

Selective supplement for the isolation, detection and enumeration of *Listeria monocytogenes*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Cycloheximide	**50 mg**
Polymyxin B Sulfate	**38.500 IU**
Ceftazidime	**10 mg**
Nalidixic Acid Sodium Salt	**10 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water/acetone 1:1. Mix gently until complete dissolution and aseptically add to 500 ml of LISTERIA CHROMOGENIC AGAR BASE (ISO 11290-1) (**Cat.1345**) autoclaved and cooled to 50°C after adding 1 vial of LISTERIA LIPASE C Supplement (ISO 11290-1) (**Cat. 6031**). Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
LISTERIA CHROMOGENIC SELECTIVE SUPPLEMENT (ISO 11290-1)	6040	10 Vials. Each vial for 500 ml of medium
LISTERIA CHROMOGENIC AGAR BASE (ISO 11290-1)	1345	500 g
LISTERIA LIPASE C SUPPLEMENT (ISO 11290-1)	6031	10 Vials. Each vial for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LISTERIA CHROMOGENIC AGAR BASE (**Cat. 1345**) from type cultures, adding both supplements, after incubation at a temperature of 37 °C and observed after 24 ± 3 hours. Incubate negative ones a further 24 ± 3 hours.

Microorganisms	Growth	Colony Color	Halo
Listeria monocytogenes ATCC 19111	Good	Blue	+
Listeria monocytogenes ATCC 13932	Good	Blue	+
Listeria innocua ATCC 33090	Good	Blue	-
Enterococcus faecalis ATCC 19433	Inhibited	–	–
Escherichia coli ATCC 25922	Inhibited	–	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June

ISO 11290-1:2004 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method.

LISTERIA LIPASE C SUPPLEMENT ISO 11290-1

CAT. 6031

Selective supplement for the isolation, enumeration and detection of *Listeria monocytogenes*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Lipase C substrate	**10 ml**

PREPARATION

Aseptically add 1 vial to 500 ml of LISTERIA CHROMOGENIC AGAR BASE (ISO 11290) (**Cat. 1345**) autoclaved and cooled to 50 - 70°C, and 1 vial of LISTERIA CHROMOGENIC SELECTIVE SUPPLEMENT (ISO 11290-1) (**Cat. 6040**), previously reconstituted with 5 ml of sterile distilled water/acetone 1:1. Mix well and distribute into sterile containers. We recommend adding the supplement to the base medium at a temperature higher than normal (around 70ºC) and shake it strongly.

PRODUCT	CAT.	PACK SIZE
LISTERIA LIPASE C SUPPLEMENT (ISO 11290-1)	6031	10 Vials. Each vial for 500 ml of medium
LISTERIA CHROMOGENIC AGAR BASE (ISO 11290-1)	1345	500 g
LISTERIA CHROMOGENIC SELECTIVE SUPPLEMENT (ISO 11290-1)	6040	10 Vials. Each vial for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LISTERIA CHROMOGENIC AGAR BASE (**Cat. 1345**) from type cultures, adding both supplements, after incubation at a temperature of 37°C and observed after 24 ± 3 hours. Incubate negative ones a further 24 ± 3 hours.

Microorganisms	Growth	Colony Color	Halo
Listeria monocytogenes ATCC 19111	Good	Blue	+
Listeria monocytogenes ATCC 13932	Good	Blue	+
Listeria innocua ATCC 33090	Good	Blue	-
Enterococcus faecalis ATCC 19433	Inhibited	–	–
Escherichia coli ATCC 25922	Inhibited	–	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June

ISO 11290-1:2004 Horizontal method for the detection and enumeration of Listeria monocytogenes Part 1: Detection Method.

m-CP SUPPLEMENT

CAT. 6073

For the enumeration and isolation of *Clostridium perfringens*

FORMULA IN g/l

VIAL A		VIAL B	
D-Cycloserine	**200 mg**	Phenoftalein difosfate	**50 mg**
Polimixine-B-Sulfate	**12.5 mg**	**VIAL C**	
Indoxil-β-D-glucopiranoside	**30.0 mg**	$FeCl3.6H_2O$	**45 mg**

PREPARATION

Aseptically reconstitute each vial (A, B and C) separately with 2 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of CLOSTRIDIUM PERFRINGENS AGAR BASE (m-CP) (**Cat. 1132**) autoclaved and cooled to 50°. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
m-CP SUPPLEMENT	6073	3 x 10 vials. Each three vials for 500 ml of medium
CLOSTRIDIUM PERFRINGENS AGAR BASE (m-CP)	1132	500 g

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of CLOSTRIDIUM PERFRINGENS AGAR BASE (m-CP) (**Cat. 1132**), with the respective supplements added, after incubation at a temperature of 44± 1°C and observed after 21 ± 3 hours.

Microorganisms	Growth	Colony Color
Clostridium perfringens	Good	Opaque yellow or a color change to pink or red after 20 - 30 seconds exposure to ammonium hydroxide vapors

STORAGE

Once opened keep powdered medium closed to avoid hydration.

BIBLIOGRAPHY

Armon, R., and Payment, P., 1988, A modified m-CP medium for enumerating Clostridium perfringens from water samples: Canadian Journal of Microbiology, v.34. p.78-79.

Bisson, J.W., and Cabelli, V.J., 1979, Membrane filter enumeration method for Clostridium perfringens: Applied and Environmental Microbiology, v. 37. no.1. p. 55-66.

O.G.A. SUPPLEMENT

CAT. 6018

Selective supplement for the isolation of yeasts and molds

FORMULA PER VIAL (Each vial for 500 ml of medium)

Oxytetracycline	**50 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of OGA MEDIUM (OXYTETRACYCLINE GLUCOSE AGAR BASE) (**Cat. 1527**), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
OGA SUPPLEMENT	6018	10 Vials. Each vial for 500 ml of medium
OGA MEDIUM (Oxytetracycline Glucose Agar Base)	1527	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of OGA MEDIUM (**Cat. 1527**) from type cultures after incubation at a temperature of 20 - 25°C and observed after 5 - 7 days.

Microorganisms	Growth
Candida albicans ATCC 10231	Good
Penicillum spp	Good
Aspergillus brasiliensis ATCC 16404	Good
Escherichia coli ATCC 25922	Inhibited
Pseudomonas aeruginosa ATCC 27853	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Mossel, D.A.A., A.M.C.Kleynen-Semmeling, H.M. Vincentie, H. Beerens, and M. Catsaras. 1970. Oxytetracycline Glucose Yeast Extract Agar for selective enumeration of moulds and yeasts in foods and clinical material. J. Appl. Bacteriol. 33:454-457.

OXFORD LISTERIA SELECTIVE SUPPLEMENT ISO 11290-1

CAT. 6003

Selective supplement for isolation of *Listeria monocytogenes* and other species of *Listeria* from food

FORMULA PER VIAL (Each vial for 500 ml of medium)

Cycloheximide	**200 mg**	Acryflavine Hydrochloride	**2.5 mg**
Colistin Sulfate	**10 mg**	Cefotetan	**1 mg**
Fosfomycin	**5 mg**		

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of 1:1 v/v sterile distilled water/acetone. Mix gently until complete dissolution. Aseptically add to 500 ml of LISTERIA AGAR BASE OXFORD (**Cat. 1133**), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
OXFORD LISTERIA SELECTIVE SUPPLEMENT	6003	10 Vials. Each vial for 500 ml of medium
LISTERIA AGAR BASE OXFORD	1133	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LISTERIA AGAR BASE OXFORD (**Cat. 1133**) from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Listeria monocytogenes ATCC 19117	Good	Brown-grey with black center and black halo
Staphylococcus aureus ATCC 25923	Inhibited	White
Escherichia coli ATCC 25922	Null	–
Enterococcus faecalis ATCC 29212	Null	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Curtis, G.D.W., Mitchell, R.G., King, A.F., Griffin E.J.A selective medium for the isolation of Listeria monocytogenes. Letters in Appl. Microbiol.8.95-98

ISO 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

PALCAM LISTERIA SELECTIVE SUPPLEMENT ISO 11290-1

CAT. 6004

Selective supplement for isolation of *Listeria monocytogenes* and other species of *Listeria* from food products

FORMULA PER VIAL (Each vial for 500 ml of medium)

Ceftazidime	**10 mg**	Acryflavine Hydrochloride	**2.5 mg**
Polymyxin B Sulfate	**5 mg**		

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of 1:1 v/v sterile distilled water/acetone. Mix gently until complete dissolution. Aseptically add to 500 ml of LISTERIA AGAR BASE PALCAM (**Cat.**

1141), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
PALCAM LISTERIA SELECTIVE SUPPLEMENT	6004	10 Vials. Each vial for 500 ml of medium
LISTERIA AGAR BASE PALCAM	1141	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of LISTERIA AGAR BASE PALCAM (**Cat. 1141**) from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24 - 48 hours.

Microorganisms	Growth	Colony Color
Listeria monocytogenes ATCC 19117	Good	Green-grey with black center and black halo
Staphylococcus aureus ATCC 25923	Null	–
Enterococcus faecalis ATCC 29212	Null	–

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

ISO 11290-1 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

POLYENRICHMENT SUPPLEMENT

CAT. 6011

Selective supplement for *Neisseria* and *Haemophilus* isolation

FORMULA PER VIAL (Each vial for 500 ml of medium)

VIAL A Polyenrichment Growth Supplement		**VIAL B** Polyenrichment Restoring Solution	
Glutamine	**100 mg**	Distilled Water	**5 ml**
Adenine	**10 mg**	Glucose	**0.5 g**
NAD	**2.50 mg**		
Cocarboxylase	**1 mg**		
Guanine	**0.30 mg**		
Ferric Nitrate	**0.20 mg**		
Aminobenzoic Acid	**0.13 mg**		
Vitamin B12	**0.10 mg**		
Thiamine Hydrochloride	**0.03 mg**		

PREPARATION

Aseptically reconstitute 1 vial of Polyenrichment Growth Supplement (vial A) with 1 vial of Polyenrichment Restoring Solution (vial B). Mix gently until complete dissolution and aseptically add to 500 ml of a mixture of 250 ml GC AGAR BASE (**Cat. 1106**) autoclaved, cooled to 50°C and 250 ml Sterile 2% haemoglobin solution. Mix well and distribute into sterile containers.

If desired, this supplement can also be added to COLUMBIA AGAR BASE (**Cat. 1104**), BLOOD AGAR BASE (**Cat. 1108**) or BRUCELLA AGAR BASE (**Cat. 1012**).

PRODUCT	CAT.	PACK SIZE
POLYENRICHMENT SUPPLEMENT	6011	2 x 5 Vials. Each pair of vials for 500 ml of medium
GC AGAR BASE	1106	500 g
COLUMBIA AGAR BASE	1104	500 g
BLOOD AGAR BASE	1108	500 g
BRUCELLA AGAR BASE	1012	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the mediums listed above, with a 2% hemoglobin solution from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere and observed after 40 - 48 hours.

Microorganisms	Growth
Haemophilus influenzae ATCC 19418	Good
Neisseria meningitidis ATCC 13090	Good
Neisseria gonorrhoeae ATCC 19424	Good
Streptococcus pneumoniae ATCC 6303	Good
Streptococcus pyogenes ATCC 19615	Good

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Murray, P.R., E.J. Baron, M.A. P faller, F.C. Tenover and R.H. Yolken (ed.) 1995 Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C

PRESTON CAMPYLOBACTER SUPPLEMENT

CAT. 6019

Selective supplement for isolation of *Campylobacter*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Polymyxin B Sulfate	**2.500 IU**
Cycloheximide	**50 mg**
Rifampicin	**5 mg**
Trimetoprim	**5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of 1:1 solution of acetone / sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of CAMPYLOBACTER AGAR BASE (PRESTON) (**Cat. 1131**) autoclaved, cooled to 50°C and with added 5 - 10% defibrinated blood. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
PRESTON CAMPYLOBACTER SUPPLEMENT	6019	10 Vials. Each vial for 500 ml of medium
CAMPYLOBACTER AGAR BASE (PRESTON)	1131	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of CAMPYLOBACTER AGAR BASE (PRESTON) (**Cat. 1131**), with sterile sheep defibrinated blood, from type cultures after incubation in microaerophilic atmosphere at a temperature of 42°C and observed after 24 - 48 hours.

Microorganisms	Growth
Campylobacter jejuni ATCC 29428	Good
Campylobacter coli ATCC 33559	Good
Escherichia coli ATCC 25922	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Bolton, F.J., and Robertson, L. 1982. A selective medium for isolating Campylobacter jejuni/coli. J. Of Clin. Pathol., 35 :462-467

Laisney, M.J., Colin, P., et Lahellec, C.1 988. Influence de differents milieux d'enrichissement et d'isolement sur la mise en evidence de Campylobacter sur les carcasses de canards. Sci. Aliments, n°8 hors serie IX, 209-211.

RPF SUPPLEMENT ISO–FDIS 6888-2

CAT. 6024

Selective supplement for identification and enumeration of positive coagulase *Staphylococcus*

FORMULA PER VIAL (Each vial for 100 ml of medium)

Rabbit Plasma	**2.5 ml**
Bovine Fibrinogen	**380 mg**
Trypsin Inhibitor	**2.5 mg**
Potassium Tellurite	**2.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 10 ml of sterile distilled water. Mix gently until complete dissolution and leave it for 10 minutes. Aseptically add to 90 ml of BAIRD PARKER AGAR BASE (RPF) **(Cat. 1319)**, autoclaved and cooled to 45 - 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
RPF SUPPLEMENT	6019	10 Vials. Each vial for 100 ml of medium
BAIRD PARKER AGAR BASE (RPF)	1319	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the BAIRD PARKER AGAR BASE (RPF) (**Cat. 1319**) from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color	Coagulase
Escherichia coli ATCC 25922	Inhibited	-	-
Proteus mirabilis ATCC 25933	Good	Brown	-
Staphylococcus aureus ATCC 25923	Good	Black	+
Staphylococcus epidermidis ATCC 12228	Good	Black	-

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

ISO/FDIS 6888-2 1998 (E) Microbiology of food and animal feeding stuffs, Horizontal method for enumeration of coagulase positive staphylococci (S. aureus and other species) Part 2: Technique using rabbit plasma fibrinogen agar medium.

TTC 1% SUPPLEMENT

CAT. 6030

Redox type indicator of microbial growth

FORMULA PER VIAL (Each vial for 500 ml of medium)

Triphenyl Tetrazolium Chloride (TTC)	**50 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of KF STREPTOCOCCAL AGAR (**Cat. 1034**) or 1.25 ml of TTC 1% Supplement to 500 ml of TTC CHAPMAN AGAR (**Cat. 1076**). Mix well and distribute into sterile containers. When the TTC is required to be added to another media, refer to the specific instructions of the medium for the quantity of TTC 1% supplement that should be added.

PRODUCT	CAT.	PACK SIZE
TTC 1% SUPPLEMENT	6030	10 vials
KF STREPTOCOCCAL AGAR	1034	500 g
TTC CHAPMAN AGAR	1076	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the KF STREPTOCOCCAL AGAR (**Cat. 1034**) from type cultures after incubation at a temperature of 35 ± 2°C and observed after 46 - 48 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Enterococcus faecalis ATCC 19433	Good	Red
Enterococcus faecalis ATCC 29212	Good	Red

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Kennor, G.A., Clark, H.F. and Kabler, P.W. (1961) J. Appl. Microbiol. 9: 15-20.

Slanetz, L.W. and Bartley, C.H. (1957) J. Bact. 74: 591-595.

Chapman, G.H. (1947) J. Bact. 53: 504.

Nordic Committee on Food Analysis (1968) Leaflet 68.

VCAT SUPPLEMENT

CAT. 6014

Selective supplement for isolation of *Neisseria*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Colistin Sulfate	**3.75 mg**	Vancomycin	**1 mg**
Trimethoprim	**1.5 mg**	Amphotericin B	**0.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of 1:1 solution of ethyl alcohol / sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of a mixture of 250 ml GC AGAR BASE (**Cat. 1106**) autoclaved, cooled to 50°C and 250 ml Sterile 2% haemoglobin solution, and reconstituted POLYENRICHMENT SUPPLEMENT (**Cat. 6011**) aseptically added. If desired, this supplement can also be added to 500 ml of COLUMBIA AGAR BASE (**Cat. 1104**).

PRODUCT	CAT.	PACK SIZE
VCAT SUPPLEMENT	6014	10 Vials. Each vial for 500 ml of medium
GC AGAR BASE	1106	500 g
POLYENRICHMENT SUPPLEMENT	6011	2 x 5 Vials. Each pair of vials for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of GC AGAR BASE (**Cat. 1106**), with hemoglobin to 2 % added, from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere and observed after 40 - 48 hours.

Microorganisms	Growth
Neisseria gonorrhoeae ATCC 19424	Good
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Thayer,J.D.,and J.e.Martin,Jr.1966.improved medium selective for cultivation of n. gonorrhoeae and N. meningitides. Public Health Rep.,81:559.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H.Yolken (ed.) 1995 Manual of Clinical Microbiology, 6th ed. American Society for microbiology, Washigton,D.C.

VCN SUPPLEMENT

CAT. 6013

Selective supplement for isolation of *Neisseria spp.*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Nystatin	**6250 IU**	Colistin	**3.75 mg**
Vancomycin	**1.5 mg**		

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of a mixture of 250 ml GC AGAR BASE (**Cat. 1106**) autoclaved, cooled to 50°C and 250 ml Sterile 2% haemoglobin solution, and reconstituted POLYENRICHMENT SUPPLEMENT (**Cat. 6011**) aseptically added. If desired, this supplement can also be added to COLUMBIA AGAR BASE (**Cat. 1104**).

PRODUCT	CAT.	PACK SIZE
VCN SUPPLEMENT	6013	10 Vials. Each vial for 500 ml of medium
GC AGAR BASE	1106	500 g
POLYENRICHMENT SUPPLEMENT	6011	2 x 5 Vials. Each pair of vials for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of GC AGAR BASE (**Cat. 1106**), with a sterile 2% hemoglobin solution added, from type cultures after incubation at a temperature of 35 ± 2°C, under 5 - 10% CO_2 atmosphere and observed after 40 - 48 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good
Pseudomonas aeruginosa ATCC 27853	Inhibited
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aureus ATCC 25923	Inhibited
Enterococcus faecalis ATCC 19433	Inhibited
Proteus mirabilis ATCC 25933	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Thayer,J.D.,and J.E.Martin, Jr.1966.improved medium selective for cultivation of N. gonorrhoeae and N. meningitides. Public Health Rep.,81:559.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H.Yolken (ed.) 1995 Manual of Clinical Microbiology, 6th ed. American Society for microbiology, Washigton,D.C.

VCNT SUPPLEMENT

CAT. 6026

Selective supplement for isolation of *Neisseria*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Nystatin	**6.250 IU**
Colistin Sulfate	**3.75 mg**
Trimethoprim	**2.5 mg**
Vancomycin	**1.5 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of a mixture of 250 ml GC AGAR BASE (**Cat. 1106**) autoclaved, cooled to 50°C and 250 ml Sterile 2% haemoglobin solution, and reconstituted POLYENRICHMENT SUPPLEMENT (**Cat. 6011**) aseptically added. Mix well and distribute into sterile containers. If desired, this supplement can also be added to 500 ml of COLUMBIA AGAR BASE (**Cat. 1104**).

PRODUCT	CAT.	PACK SIZE
VCNT SUPPLEMENT	6026	10 Vials. Each vial for 500 ml of medium
GC AGAR BASE	1106	500 g
POLYENRICHMENT SUPPLEMENT	6011	2 x 5 Vials. Each pair of vials for 500 ml of medium

MICROBIOLOGICAL TEST

The following results were obtained in the performance of GC AGAR BASE (**Cat. 1106**) and the POLYENRICHMENT SUPPLEMENT (**Cat. 6011**) added, from type cultures after incubation at a temperature of 35 ± 2°C under 5 - 10% CO_2 atmosphere and observed after 40 - 48 hours.

Microorganisms	Growth
Neisseria meningitidis ATCC 13090	Good
Neisseria gonorrhoeae ATCC 19424	Good
Haemophilus influenzae ATCC 19418	Good
Escherichia coli ATCC 25922	Inhibited
Staphylococcus aureus ATCC 25923	Inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

Thayer, J. D., and J.e.Martin,Jr.1 966. Improved medium selective for cultivation of N. gonorrhoeae and N.meningitides. Public Health Rep., 81:559.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H.Yolken (ed.) 1995 Manual of Clinical Microbiology, 6th ed. American Society for microbiology.

BIBLIOGRAPHY

Miller, R. G., and C. R. Tate. 1990. XLT4: A highly selective plating medium for the isolation of Salmonella.

The Maryland Poultryman, April:2-7. Tate, C. R., R. G. Miller, and E. T. Mallinson. 1992. Evaluation of two isolation and two non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples.

J. Food Prot. 55:964-967. Dusch, H. and M. Altwegg. 1995. Evaluation of five new plating media for the isolation of Salmonella species.

XLT4 SUPPLEMENT

CAT. 6062

For the selective isolation of pathogenic Enterobacteria,especially *Salmonella*

FORMULA PER FLASK

26 % solution of 7-ethyl-2-methyl- 4-undecanol hydrogen sulfate, sodium salt; formerly Tergitol4

PREPARATION

Aseptically add 4.6 ml to one liter of XLT4 AGAR BASE (**Cat. 1159**). Mix well and heat with frequent agitation until completely dissolved. Boil for one minute. AVOID OVERHEATING. DO NOT AUTOCLAVE. Distribute into sterile Petri dishes.

PRODUCT	CAT.	PACK SIZE
XLT4 SUPPLEMENT	6062	100 ml
XLT4 AGAR BASE	1159	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of XLT4 AGAR BASE (**Cat. 1159**) from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Enterobacter aerogenes ATCC 13048	Moderate	Yellow
Escherichia coli ATCC 25922	Moderate	Yellow
Proteus mirabilis ATCC 14273	Inhibited	Yellow
Salmonella typhimurium ATCC 14028	Good	Black center
Salmonella enteritidis ATCC 13076	Good	Black center
Shigella sonnei ATCC 11060	Inhibited	Red
Shigella flexneri ATCC 12022	Inhibited	Red

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

YERSINIA SELECTIVE (C.I.N.) SUPPLEMENT ISO 10273

CAT. 6033

Selective supplement for the isolation of *Yersinia enterocolitica*

FORMULA PER VIAL (Each vial for 500 ml of medium)

Cefsulodin	**7.50 mg**
Novobiocin	**1.25 mg**
Irgasan	**2.0 mg**

PREPARATION

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of Yersinia Selective Agar Base ISO 10273 (**Cat. 1126**), autoclaved and cooled to 50°C. Mix well and distribute into sterile containers.

PRODUCT	CAT.	PACK SIZE
YERSINIA SELECTIVE (C.I.N.) SUPPLEMENT (ISO 10273)	6033	10 Vials. Each vial for 500 ml of medium
YERSINIA SELECTIVE AGAR BASE (ISO 10273)	1126	500 g

MICROBIOLOGICAL TEST

The following results were obtained in the performance of YERSINIA SELECTIVE AGAR BASE (ISO 10273) (**Cat. 1126**), after incubation at a temperature of 30°C and observed after 18 - 24 hours or at 22 - 25°C and observed after 48 hours.

Microorganisms	Growth
Yersinia enterocolitica ATCC 27729	Good
Escherichia coli ATCC 25922	Inhibited or partially inhibited

STORAGE

When stored as indicated, supplements remain stable until the stated expiry date shown on the label.

BIBLIOGRAPHY

American Public Health Association Compendium of Methods for the microbiological Examination of Foods. Schiemann, DA: Synthesis of a selective agar medium for Yersinia enterocolitica. - Canad. J . Microbiol , 25 1 298 -130 4 International Standard ISO 10273 Microbiology of food and animal stuffs. Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica

Additives

KOVACS REAGENT

CAT. 5205

For the detection of *microbial indole*

USES

For the detection of indole, the microorganisms must be grown aerobically in a glucose-free environment with plenty of Tryptophan (UREA INDOLE BROTH - **Cat. 1227**; PEPTONE WATER - **Cat. 1403**; SIM MEDIUM - **Cat. 1514**; TRYPTOPHAN CULTURE BROTH - **Cat. 1237**; MIO MEDIUM - **Cat. 1510**; INDOLE NITRATE MEDIUM - **Cat. 1504**). When the microorganisms are capable of cleaving the tryptophan, they liberate indole that reacts with 4-dimethylaminobenzaldehyde to form a dark red dye.

EGG YOLK EMULSION

CAT. 5152

For the detection of *lecithinase*

USES

Egg Yolk Emulsion, sterilized and stabilized, for use in microbiology applications, ready for use with BACILLUS CEREUS SELECTIVE AGAR BASE (**Cat. 1124**) for identifying *Bacillus Cereus* and TSC AGAR BASE (**Cat. 1029**) for identifying *Clostridium perfringens*. In both cases, the principle is the detection of lecithinase.

Other media where the Egg Yolk Emulsion is useful: LISTERIA AGAR BASE PALCAM (**Cat. 1141**); MANNITOL SALT AGAR (**Cat. 1062**).

TELLURITE EGG YOLK EMULSION

CAT. 5129

For the detection and enumeration of *Staphylococcus*

USES

Tellurite Egg Yolk Emulsion, sterilized and stabilized, for use in microbiology applications, ready for use with BAIRD PARKER AGAR BASE (**Cat. 1100**) for selection of coagulase-positive *Staphylococci*. The addition of Egg yolk and Potassium tellurite helps to differentiate these microorganisms from others capable of growing in the base agar, through the detection of *lecithinase* and the formation of black colonies.

POTASSIUM TELLURITE 3.5% SOLUTION

CAT. 5208

For the detection of *Staphylococcus*, *Streptococci*, *Corynebacteria* and *Vibrio*

USES

Potassium Tellurite solution for use in microbiology applications as an inhibitor of most Gram-negative bacteria and those Gram-positive bacteria that cannot reduce Potassium tellurite. Ready for use with BAIRD PARKER AGAR BASE (**Cat. 1100**) and GIOLITTI-CANTONI BROTH (**Cat. 1232**) and other selective media for *Staphylococci, Streptococci, Corynebacteria* and *Vibrio*.

Potassium tellurite helps to differentiate these microorganisms from others capable of growing in the base agar, through the formation of black colonies as a result of reducing the Potassium tellurite to Tellurium.

PRODUCT

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ANNEX

Formula or instructions indicated in this manual don´t take precedence over the product label or quality control due to possible modifications after the manual publication.

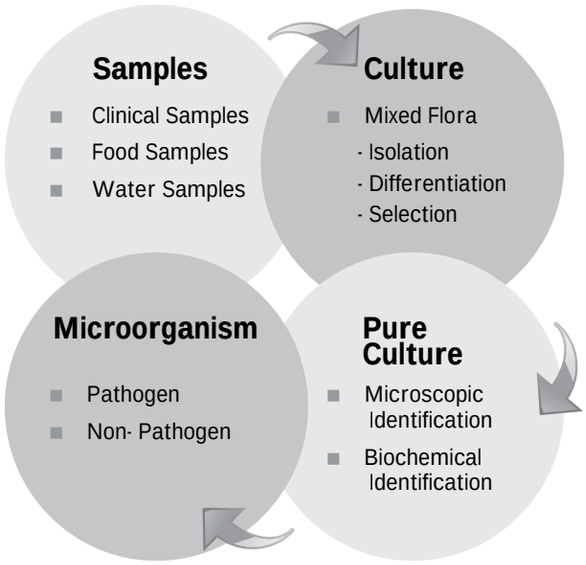
Culture Media Types & **Selection**

CLASIFICATION

The investigation of microorganisms has different phases that are designed to obtain a pure culture for confirmation testing. The differentiation of bacteria is important because microorganisms are always present in mixed populations.

ACCORDING TO USE

Media can be classified, depending on the intended use, as follows:



- **General media.** Allow the isolation of all strains present in the sample
- **Selective media.** Allow the growth of the target microorganism by inhibiting the accompanying flora. The inhibition is due to the addition of inhibiting elements.
- **Differential media.** They contain ingredients that allow the presumptive identification of specific genera or species from pure or mixed populations. Usually identification is made by the appearance of the colony
- **Biochemical identification.** Contain substances such as pH indicators that allow identification by color changes in the media
- **Chromogenic media.** These are media that allow the differentiation of microorganisms using chromogenic substrates
- **Other media.** Eg. Transportation media, Maintenance, Antibiotics test.

ACCORDING TO AGAR CONCENTRATION

Culture media according to agar concentration can be classified as follows:

SOLID MEDIUM (Contains Agar)			
On a plate			
Cultivation & Development	Morphological studies	Differentiation and Selection	Isolation and Identification
In a tube:			
Cultivation and Growth	Biochemical Identification	Transportation and Conservation	Motility
SEMISOLID MEDIUM (Low agar content)			
Motility	Biochemical Identification	Transportation and Conservation	
LIQUID MEDIUM (No agar)			
Count	Growth and Recovery	Biochemical Identification	

SELECTION

The choice of media is essential for a good investigation or enumeration of the microorganism. Before carrying out laboratory tests, the medium or media required must be ascertained. The medium is chosen based on the nutritional needs of the organism, depending also on the degree of selectivity or differentiation desired. Knowledge of the normal habitat of an organism is useful for choosing the culture medium because the nutritional requirements reflect their natural environment.

In the case of microbiological tests on food, the legislation for each country must be ascertained for each particular food type. This will either require enumeration or investigation of the organism and, depending on the type of test, the culture media used will vary.

Culture Media - **Groups of Microorganism**

Cat Nº	*Aeromonas*
	Isolation
1108	AEROMONAS AGAR BASE (RYAN)

Cat Nº	Anaerobic Bacteria
	Isolation/Cultivation
1000	ANAEROBIC AGAR
1142	LIVER AGAR
1242	LIVER BROTH
1066	SCHAEDLER AGAR
1218	SCHAEDLER BROTH
1516	THIOGLYCOLLATE MEDIUM WITHOUT INDICATOR USP
1503	WILKINS CHALGREN MEDIUM
1568	WILKINS CHALGREN MEDIUM II

Cat Nº	*Bacillus cereus*
	Enumeration/Isolation
1124	BACILLUS CEREUS SELECTIVE AGAR BASE (MYP)
	Confirmation
1328	BLOOD AGAR BASE Nº 2 ISO 7932

Cat Nº	*Bacteroides fragilis*
	Cultivation
1451	BPRM BROTH BASE (Bacteroides Phage Recovery Medium) ISO 10705:4

Cat Nº	*Bordetella*
	Detection/Isolation
1107	BORDET-GENGOU AGAR BASE

Cat Nº	*Brucella*
	Cultivation
1012	BRUCELLA AGAR
1223	BRUCELLA BROTH
1374	BRUCELLA MEDIUM BASE
1142	LIVER AGAR
1242	LIVER BROTH
1047	TRYPTOSE AGAR
1322	TRYPTOSE BROTH

Cat Nº	*Burkholderia cepacia*
	Selective isolation
1347	BURKHOLDERIA CEPACIA AGAR BASE

Cat Nº	Campylobacter
	Selective isolation
1129	CAMPYLOBACTER AGAR BASE (BLOOD FREE) (CCDA)
1131	CAMPYLOBACTER AGAR BASE (PRESTON)

Cat Nº	Candida
	Isolation/Identification/Differentiation
1006	BIGGY AGAR
1382	CANDIDA CHROMOGENIC AGAR
	Chlamydospore Production
1164	CORN MEAL AGAR

Cat Nº	*Clostridium*
	Cultivation
1000	ANAEROBIC AGAR
	Cultivation/ Enumeration
1007	REINFORCED CLOSTRIDAL MEDIUM EUROPEAN PHARMACOPOEIA
1087	REINFORCED CLOSTRIDIAL AGAR
1416	DIFFERENTIAL REINFORCED CLOSTRIDIAL BROTH
	Lactate fermenting Clostridium
1247	BRYANT BURKEY BASE BROTH (MODIFIED WITH RESARZURINE)
1104	COLUMBIA AGAR BASE EUROPEAN PHARMACOPOEIA

Cat Nº	*Clostridium difficile*
	Isolation
1447	CLOSTRIDIUM DIFFICILE AGAR BASE

Cat Nº	*Clostridium perfringens*
	Cultivation
1533	THIOGLYCOLLATE MEDIUM USP ISO 7937
	Detection/Isolation/Enumeration
1009	LACTOSE SULFITE BROTH BASE EUROPEAN PHARMACOPOEIA
1029	T.S.C AGAR BASE (TRYPTOSE SULFITE CYCLOSERINE) ISO 7937

Cat Nº	Campylobacter
	Selective isolation
1129	CAMPYLOBACTER AGAR BASE (BLOOD FREE) (CCDA)
1131	CAMPYLOBACTER AGAR BASE (PRESTON)

Cat Nº	Candida
1082	SPS AGAR (SULFITE POLYMIXIN SULFADIAZINE)
1378	SULFITE TRYPTOSE BROTH
1075	TSN AGAR

Cat Nº	Clostridim perfringens in water
	Enumeration/Isolation
1132	CLOSTRIDIUM PERFRINGES AGAR BASE (m-CP)

Cat Nº	Coliforms
	Selective enrichment
1202	MOSSEL EE BROTH EUROPEAN PHARMACOPOEIA
	Selective enrichment
1202	MOSSEL EE BROTH EUROPEAN PHARMACOPOEIA

Cat Nº	*Clostridium perfringens*
	Confirmation
1526	GELATIN LACTOSE MEDIUM
1565	NITRATE MOTILITY BASE MEDIUM

Cat Nº	*Clostridim perfringens* in water
	Enumeration/Isolation
1132	CLOSTRIDIUM PERFRINGES AGAR BASE (m-CP)

Cat Nº	Coliforms
	Selective enrichment
1202	MOSSEL EE BROTH EUROPEAN PHARMACOPOEIA
	Selective isolation
1051	BCP AGAR
1025	DESOXYCHOLATE LACTOSE AGAR
	Detection
1252	A1 MEDIUM
1228	BRILLIANT GREEN BILE BROTH 2% ISO 4831, ISO 4832
1340	E.COLI-COLIFORMS CHROMOGENIC MEDIUM
1118	ENDO AGAR BASE
1210	MAcCONKEY BROTH EUROPEAN PHARMACOPOEIA
	Cultivation
1206	LACTOSE BROTH EUROPEAN PHARMACOPOEIA
	Enumeration
1010	BRILLIANT GREEN BILE AGAR
1365	MINERALS MODIFIED GLUTAMATE BROTH (MMGB) ISO 16649-3
	Detection/Enumeration
1522	EC MEDIUM ISO 7251
1093	VIOLET RED BILE AGAR WITH LACTOSE (VRBL) ISO 4832
1151	TBX CHROMOGENIC AGAR (TRYPTONE BILE X-GLUCURONIDE) ISO 16649-2,3
	Differentiation
1192	ACETATE DIFFERENTIAL AGAR
1212	EWING MALONATE BROTH MODIFIED
1042	KLIGER IRON AGAR
1364	KLIGER IRON AGAR ISO 10273
1200	KOSER CITRATE BROTH
1512	MR-VP MEDIUM
1014	SIMMONS CITRATE AGAR ISO 10273
1227	UREA INDOLE BROTH ISO 10273
1020	DESOXYCHOLATE AGAR
1052	MACCONKEY AGAR EUROPEAN PHARMACOPOEIA
	Isolation/Cultivation/Differentiatium
1039	EOSIN METHYLENE BLUE AGAR (EMB)

Cat Nº	*Coliforms* in water
	Detection
1340	E.COLI-COLIFORMS CHROMOGENIC MEDIUM
1137	ENDO LES AGAR BASE
1310	LAURYL SULFATE BROTH (LAURYL TRYPTOSE BROTH - LTB) ISO 4831
	Enumeration
1365	MINERALS MODIFIED GLUTAMATE BROTH (MMGB) ISO 16649-3
	Selective Isolation/Enumeration
1309	LAURYL SULFATE AGAR (FOR MEMBRANE FILTRATION)
	Detection/Cultivation/Enumeration
1127	FECAL COLIFORMS AGAR BASE (m-FC)
1121	FECAL COLIFORMS BROTH BASE (m-FC)
1013	TRYPTONE BILE SALTS AGAR ISO 9308-1
1076	TTC CHAPMAN AGAR (LACTOSE AGAR TTC WITH TERGITOL 7) ISO 9308-1
1138	TRYPTONE SOY AGAR ISO 9308-1
	Identification
1237	TRYPTOPHAN CULTURE BROTH ISO 9308-1
	Confirmation
1357	PEPTONE WATER with LACTOSE ISO 9308-1

Cat Nº	*E.coli* 0157:H7
	Cultivation/Differentiation
1099	MACCONKEY AGAR WITH SORBITOL

Cat Nº	*Enterobacter sakazakii*
	Selective enrichment
1445	LAURYL SULPHATE TRYPTOSE BROTH MODIFIED (mLST) ISO 22964
	Isolation
1446	ENTEROBACTER SAZAKAZII ISOLATION CHROMOGENIC AGAR (ESIA) ISO 22964

Cat Nº	Enterobacteria
	Selective isolation
1344	LACTOSE AGAR WITH BROMOTHYMOL BLUE & CRYSTAL VIOLET (DRIGALSKI)
	Detection/Cultivation
1118	ENDO AGAR BASE
1206	LACTOSE BROTH EUROPEAN PHARMACOPOEIA
1355	NUTRIENT AGAR ENRICHED WITH SODIUM CHLORIDE ISO 21528
	Cultivation/Enumeration
1092	VIOLET RED BILE AGAR WITH GLUCOSE (VRBG) ISO 21528 EUROPEAN PHARMACOPOEIA

Cat Nº	Enterobacteria
	Cultivation/Enumeration
1144	VIOLET RED BILE AGAR WITH LACTOSE & GLUCOSE (VRBLG) EUROPEAN PHARMACOPOEIA
1362	EE BROTH ISO 21528-1
	Isolation
1098	MACCONKEY AGAR W/O CRYSTAL VIOLET AND W/O SODIUM CHLORIDE
1045	DCLS AGAR (DESOXYCHOLATE, CITRATE, LACTOSE, SUCROSE)
1067	DESOXYCHOLATE CITRATE AGAR EUROPEAN PHARMACOPOEIA
1080	XLD AGAR (XYLOSE LYSINE DESOXYCHOLATE AGAR) EUROPEAN PHARMACOPOEIA
1274	XLD AGAR (XYLOSE LYSINE DESOXYCHOLAATE AGAR) ISO 6579
1159	XLT4 AGAR BASE
	Isolation/Differentiation
1030	HEKTOEN ENTERIC AGAR
	Isolation/Cultivation/Differentiation
1039	EOSIN METHYLENE BLUE AGAR (EMB)
1050	LEVINE AGAR (EMB)
1052	MACCONKEY AGAR EUROPEAN PHARMACOPOEIA
	Differentiation
1192	ACETATE DIFFERENTIAL AGAR
1212	EWING MALONATE BROTH MODIFIED
1504	INDOLE NITRATE MEDIUM (TRYPTICASEIN NITRATE MEDIUM)
1037	MACCONKEY AGAR wo CRYSTAL VIOLET
1509	MANNITOL NITRATE MOTILITY MEDIUM
1510	MIO MEDIUM (MOTILITY - INDOLE - ORNITHINE)
1112	MOELLER KCN BROTH BASE
1023	PHENOL RED DEXTROSE AGAR
1235	PHENOL RED DEXTROSE BROTH
1239	PHENOL RED SUCROSE BROTH
1040	PHENYLALANINE AGAR
1046	TRIPLE SUGAR IRON AGAR
1110	UREA AGAR BASE (CHRISTENSEN) ISO 6579
1226	UREA BROTH
1042	KLIGER IRON AGAR
1044	LYSINE IRON AGAR
1115	PHENOL RED BROTH BASE
1014	SIMMONS CITRATE AGAR ISO 10273
	Differentiation/Enumeration
1320	BCP GLUCOSE AGAR (ENTEROBACTERIACEAE CONFIRMATORY AGAR) ISO 21528:2

Cat Nº	Enterobacteria
	Identification
1208	LYSINE DECARBOXYLASE BROTH
1364	KLIGER IRON AGAR ISO 10273
1514	SIM MEDIUM
1458	IRON AGAR

Cat Nº	*Enterococcus*
	Selective enrichment
1422	AZIDE DEXTROSE BROTH
1204	ENTEROCOCCUS SELECTIVE BROTH (ENTEROCOCCOSEL BROTH)
1070	ENTEROCOCCUS SELECTIVE AGAR (ENTEROCOCCOSEL AGAR)
	Detection/Isolation
1027	KAA CONFIRMATORY AGAR
1209	KAA PRESUMPTIVE BROTH
1346	KF STREPTOCOCCAL BROTH
1230	EVA BROTH (ETHYL VIOLET AZIDE, LITSKY)
	Enumeration
1238	ROTHE BROTH (GLUCOSE BROTH WITH AZIDE)
	Selective isolation/Enumeration
1034	KF STREPTOCOCCAL AGAR
	Isolation/Identification

1031	BILE ESCULIN AGAR ISO 10273
1359	BILE ESCULIN AZIDE BROTH
	Differentiation
1035	MACCONKEY AGAR Nº 2
1037	MACCONKEY AGAR w/o CRYSTAL VIOLET
	Confirmation
1018	ENTEROCOCCUS CONFIRMATORY AGAR

Cat Nº	*Enterococcus* resistant to vancomycin
	Differentiation
1410	VANCOMYCIN SCREEN AGAR

Cat Nº	*Enterococcus* in water
	Detection/Enumeration
1412	m-EI CHROMOGENIC AGAR BASE
1109	SLANETZ-BARTLEY MEDIUM ISO 7899-2
1435	SLANETZ-BARTLEY MEDIUM wo TTC ISO 7899-2
	Isolation/Identification
1005	BILE ESCULIN AZIDE AGAR ISO 7899-2

Cat Nº	*Flexibacter maritimus*
	Cultivation
1264	FMM Broth

Cat Nº	Yeast & Molds
	Cultivation
1261	POTATO DEXTROSE BROTH
1024	SABOURAUD DEXTROSE AGAR EUROPEAN PHARMACOPOEIA
1088	SABOURAUD DEXTROSE AGAR + CYCLOHEXIMIDE
1205	SABOURAUD DEXTROSE BROTH EUROPEAN PHARMACOPOEIA
1506	SABOURAUD FLUID MEDIUM USP
1054	SABOURAUD MALTOSE AGAR
1213	SABURAUD MALTOSE BROTH
1312	YEAST EXTRACT AGAR FOR MOLDS
1194	YEAST MOLD AGAR
	Cultivation/Enumeration
1316	WORT AGAR
1444	WORT BROTH
1527	O.G.A MEDIUM (OXYTETRACYCLINE GLUCOSE AGAR)
1160	ROSE BENGAL AGAR + CHLORAMPHENICOL + DICHLORAN (DRBC AGAR) ISO 21527-1
	Cultivation/Isolation
1081	ROSE BENGAL AGAR + CLORANPHENICOL
1090	SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL
1134	SABOURAUD DEXTROSE AGAR + CHLORAMPHENICOL EUROPEAN PHARMACOPOEIA
1089	SABOURAUD DEXTROSE AGAR+CLORAMPHENICOL+ CYCLOHEXIMIDE
	Cultivation/Isolation/Enumeration
1038	MALT EXTRACT AGAR
1301	CHLORAMPHENICOL AGAR

Cat Nº	Yeast & Molds
	Cultivation/Isolation/Enumeration
1094	GLUCOSE CHLORAMPHENICOL AGAR
1245	MALT EXTRACT BROTH
1258	GLUCOSE CHLORAMPHENICOL BROTH
	Identification/Cultivation/Enumeration
1022	POTATO DEXTROSE AGAR EUROPEAN PHARMACOPOEIA

Cat Nº	Yeast & Molds in Water
	Enumeration
1049	YEAST EXTRACT AGAR ISO 6222

Cat Nº	Yeast & Molds - Dermatophytes
	Cultivation
1166	SABOURAUD DEXTROSE AGAR 2%
	Isolation
1097	YEAST EXTRACT SOY AGAR

Cat Nº	Osmophilic Yeast
	Isolation/Cultivation
1057	OSMOPHILIC AGAR

Cat Nº	Yeast & Molds - Pathogenic fungi
	Isolation
1072	MYCOBIOTIC AGAR (Fungal Selective Agar)

Cat Nº	Yeast & Molds - Xerophilic fungi
	Enumeration/isolation
1161	DICHLORAN GLYCEROL AGAR

Cat Nº	Lactic Acid Bacteria
	Cultivation
1539	ELLIKER MEDIUM
1043	MRS AGAR
1433	MRS Agar low pH ISO 15214
1215	MRS BROTH

1096	ROGOSA SL AGAR
1234	ROGOSA SL BROTH
1413	SAN FRANCISCO MEDIUM, MODIFIED
	Cultivation/Enumeration
1318	M17 AGAR
	Isolation/Detection
1307	ORANGE SERUM AGAR

Cat Nº	*Legionella*
	Cultivation
1311	LEGIONELLA CYE AGAR BASE ISO 11731-2

Cat Nº	*Listeria*
	Selective enrichment
1120	LISTERIA ENRICHMENT BROTH BASE FRASER ISO 11290-2
1182	LISTERIA FRASER BROTH BASE ISO 11290-1
1183	LISTERIA HALF FRASER BROTH BASE ISO 11290-1
1279	UVM-I LISTERIA SELECTIVE ENRICHMENT BROTH, MODIFIED
1280	UVM-II LISTERIA SELECTIVE ENRICHMENT BROTH, MODIFIED
1345	LISTERIA CHROMOGENIC AGAR BASE ISO 11290-1

Cat Nº	*Listeria*
	Detection
1133	LISTERIA AGAR BASE OXFORD ISO 11290-1
1141	LISTERIA AGAR BASE PALCAM ISO 11290-2
	Detection/Enumeration
1345	LISTERIA CHROMOGENIC AGAR BASE ISO 11290-1
	Confirmation
1328	BLOOD AGAR BASE Nº 2 ISO 7932
1398	TSYE AGAR (TRYPTONE SOY YEAST EXTRACT AGAR) ISO 11290-1
1339	TSYEB BROTH (TRYPTONE SOY YEAST EXTRACT BROTH) ISO 11290-1

Cat Nº	Marine Heterotrophic Bacteria
	Isolation/enumeration
1059	MARINE AGAR
1217	MARINE BROTH

Cat Nº	*Mycobacteria*
	Cultivation
1116	LOWENSTEIN-JENSEN MEDIUM BASE

Cat Nº	*Mycoplasma*
	Enrichment
1262	PPLO BROTH BASE w/o CRYSTAL VIOLET
	Isolation/Cultivation
1140	PPLO AGAR BASE w/o CRYSTAL VIOLET

Cat Nº	*Neisseria* & *Haemophilus*
	Isolation/Cultivation
1106	GC AGAR BASE
1058	MUELLER HINTON AGAR

Cat Nº	Nitrophilous fungi & Bacteria
	Cultivation
1015	CZAPEK DOX AGAR MODIFIED
1250	CZAPEK-DOX MODIFIED BROTH

Cat Nº	Non fermenting bacilli
	Identification/Differentiation
1500	OF BASAL MEDIUM (HUGH AND LEIFSON)
1065	SELLERS AGAR

Cat Nº	Proteolytic microorganisms
	Detection
1069	CALCIUM CASEINATE AGAR

Cat Nº	Proteolytic microorganisms
	Enumeration
1300	NUTRIENT GELATIN

Cat Nº	*Pseudomonas*
	Selective isolation/Identification
1102	CETRIMIDE AGAR BASE EUROPEAN PHARMACOPOEIA
	Isolation/Enumeration
1356	PSEUDOMONAS AGAR BASE ISO 13720
	Identification/Enumeration
1207	ASPARAGINE BROTH
	Confirmation
1211	ACETAMIDE BROTH
	Differentiation
1391	ACETAMIDE AGAR
	Identification
1531	KING A MEDIUM (PSEUDOMONAS P AGAR) USP
1532	KING B MEDIUM (PSEUDOMONAS F AGAR) USP

Cat Nº	*Pseudomonas in water*
	Cultivation
1156	NUTRIENT AGAR UNE-EN 12780, EN ISO 16266
	Identification/Enumeration
1154	KING B MEDIUM UNE-EN 12780, EN ISO 16266
1153	PSEUDOMONAS CN AGAR BASE UNE-EN 12780, EN ISO 16266
	Confirmation
1155	ACETAMIDE BROTH UNE-EN 12780, EN ISO 16266

Cat Nº	Psychrotrophic Microorganisms
	Enumeration
1053	KING FG AGAR

Cat Nº	*Salmonella*
	Selective enrichment
1221	BRILLIANT GREEN SELENITE BROTH
1219	BRILLIANT GREEN SELENITE BROTH II
1253	BRILLIANT GREEN TETRATHIONATE BILE BROTH EUROPEAN PHARMACOPOEIA
1173	MULLER KAUFFMANN BROTH BASE WITH BRILLIANT GREEN & NOVOBIOCINE (MKTTN) ISO 6579
1130	MULLER KAUFFMAN TETRATIONATE BROTH BASE
1414	RAPAPPORT VASSILIADIS BROTH EUROPEAN PHARMACOPOEIA
1174	RAPPAPORT SOY BROTH (VASSILIADIS) ISO 6579
1240	RAPPAPORT SOY BROTH (VASSILIADIS)

Cat Nº	*Salmonella*
	Selective enrichment
1220	SELENITE CYSTINE BROTH
1222	SODIUM SELENITE BROTH
1114	TETRATHIONATE BROTH BASE USP
	Isolation
1011	BISMUTH SULFITE AGAR (WILSON BLAIR) USP
1078	BRILLIANT GREEN AGAR *European Pharmacopeia*
1143	BRILLIANT GREEN AGAR ISO 6579
1186	SALMONELLA SHIGELLA (SS) AGAR, MODIFIED
1122	SALMONELLA CHROMOGENIC AGAR
1064	SALMONELLA SHIGELLA AGAR (SS AGAR)
1220	SELENITE CYSTINE BROTH
1159	XLT4 AGAR BASE
1045	DCLS AGAR (DESOXYCHOLATE, CITRATE, LACTOSE, SUCROSE)
1067	DESOXYCHOLATE CITRATE AGAR EUROPEAN PHARMACOPOEIA
1025	DESOXYCHOLATE LACTOSE AGAR
1274	XLD AGAR (XYLOSE LYSINE DESOXYCHOLAATE AGAR) ISO 6579
	Isolation/Differentiation
1020	DESOXYCHOLATE AGAR
	Differentiation
1044	LYSINE IRON AGAR
1376	MSRV - MODIFIED SEMISOLD RAPPAPORT VASSILIADIS MEDIUM
	Identification
1176	LYSINE DECARBOXYLASE MEDIUM ISO 6579, ISO 10273
1172	TRIPLE SUGAR IRON AGAR (TSI) ISO 6579
1110	UREA AGAR BASE (CHRISTENSEN) ISO 6579
1226	UREA BROTH
1376	MSRV - MODIFIED SEMISOLD RAPPAPORT VASSILIADIS MEDIUM

Cat Nº	*Staphylococcus*
	Selective enrichment/Enumeration
1232	GIOLITTI-CANTONI BROTH
1287	GIOLITTI-CANTONI BROTH ISO 6888-3
	Isolation
1113	AZIDE BLOOD AGAR BASE
1100	BAIRD PARKER AGAR BASE EUROPEAN PHARMACOPOEIA
1319	BAIRD PARKER AGAR BASE (RPF) ISO-FDIS 6888-2
1017	CHAPMAN STONE AGAR
1152	COLUMBIA CNA AGAR
1032	STAPHYLOCOCCUS AGAR Nº 110
1079	VOGEL-JOHNSON AGAR

Cat Nº	*Staphylococcus*
	Isolation
1032	STAPHYLOCOCCUS AGAR Nº 110
1079	VOGEL-JOHNSON AGAR
	Isolation/Enumeration
1062	MANNITOL SALT AGAR (MSA) (CHAPMAN MEDIUM) EUROPEAN PHARMACOPOEIA
	Differentiation
1037	MACCONKEY AGAR wo CRYSTAL VIOLET
	Identification
1028	DNase TEST AGAR
	Confirmation
1331	BRAIN HEART INFUSION BROTH ISO 6888-1

Cat Nº	Methicillin Resistant Staphylococcus aureus
	Detection
1423	MRSA AGAR CHROMOGENIC
	Detection/Differentiation
1498	MRSA AGAR, MODIFIED CHROMOGENIC

Cat Nº	*Streptococcus*
	Cultivation/Isolation
1113	AZIDE BLOOD AGAR BASE
1152	COLUMBIA CNA AGAR
1106	GC AGAR BASE
1236	TODD HEWITT BROTH
	Identification
1128	BLOOD AGAR BASE + NALIDIXIC ACID

Cat Nº	*Streptococcus thermophilus*
	Cultivation
1254	ESTY BROTH
1555	ESTY MEDIUM

Cat Nº	*Vibrio*
	Isolation
1407	ALKALINE PEPTONE WATER
1074	TCBS AGAR

Cat Nº	*Yersinia*
	Selective enrichment
1361	IRGASAN TICARCHILLIN AND POTASSIUM CHLORATE BROTH (ITC BROTH) ISO 10273
	Isolation
1126	YERSINIA SELECTIVE AGAR BASE ISO 10273

Cat Nº	*Yersinia*
	Isolation/Identification
1031	BILE ESCULIN AGAR ISO 10273
	Cultivation
1060	NUTRIENT AGAR ISO 6579 ISO 10273
	Identification/Differentiation
1364	KLIGER IRON AGAR ISO 10273
1014	SIMMONS CITRATE AGAR ISO 10273
1227	UREA INDOLE BROTH ISO 10273
	Confirmation
1176	LYSINE DECARBOXYLASE MEDIUM ISO 6579, ISO 10273

Cat Nº	OTHER APPLICATIONS
	Media for general Use
1048	BRAIN HEART INFUSION AGAR (BHI AGAR)
1400	BRAIN HEART INFUSION BROTH (BHI BROTH)
1401	BUFFERED PEPTONE WATER EUROPEAN PHARMACOPEIA
1402	BUFFERED PEPTONE WATER ISO 6579, ISO 22964
1406	BUFFERED SALINE PEPTONE WATER
1104	COLUMBIA AGAR BASE EUROPEAN PHARMACOPEIA
1229	COLUMBIA BROTH
1021	DEXTROSE AGAR
1203	DEXTROSE BROTH (GLUCOSE BROTH)
1036	EUGON AGAR
1323	HEART INFUSION BROTH
1244	LETHEEN BROTH MODIFIED
1399	LPT DILUTION BROTH
1449	NEUTRALIZING AGAR
1314	NUTRIENT AGAR (DEV REGULATIONS)
1060	NUTRIENT AGAR ISO 6579, ISO 10273
1156	NUTRIENT AGAR UNE-EN 12780, EN ISO 16266
1216	NUTRIENT BROTH
1251	NUTRIENT BROTH Nº2
1403	PEPTONE WATER (TRYPTONE WATER)
1405	SALINE PEPTONE WATER ISO 6887
1177	STANDARD NUTRIENT AGAR I
1286	STANDARD NUTRIENT BROTH I
1003	TRYPTICASEIN DEXTROSE MEDIUM
1041	TRYPTICASEIN GLUCOSE EXTRACT AGAR
1068	TRYPTICASEIN SOY AGAR (TSA) EUROPEAN PHARMACOPOEIA
1224	TRYPTICASEIN SOY BROTH (TSB) EUROPEAN PHARMACOPOEIA
1243	TRYPTOSE PHOSPHATE BROTH
1198	TSA AGAR Nº MODIFIED
1380	VEGETAL PEPTONE BROTH (TSB VEGETABLE)

Cat Nº	OTHER APPLICATIONS
	Media for general Use
1268	YEAST EXTRACT SOY BROTH (TSB NON-ANIMAL SOURCE)
	Antibiotic Assay
1520	ANTIBIOTIC MEDIUM Nº 1 (SEED AGAR) USP EUROPEAN PHARMACOPEIA
1002	ANTIBIOTIC MEDIUM Nº 2 (BASE AGAR) USP
1534	ANTIBIOTIC MEDIUM Nº 3 USP
1524	ANTIBIOTIC MEDIUM Nº 5 (STREPTOMYCIN TEST AGAR) USP
1004	ANTIBIOTIC MEDIUM Nº 8 (BASE AGAR WITH LOW pH) USP

1528	ANTIBIOTIC MEDIUM Nº 11 (NEOMYCIN TEST AGAR) USP
1525	ANTIBIOTIC MEDIUM Nº 12
	Hemolytic Activity
1108	BLOOD AGAR BASE
1328	BLOOD AGAR BASE Nº 2 ISO 7932
1104	COLUMBIA AGAR BASE EUROPEAN PHARMACOPOEIA
1058	MUELLER HINTON AGAR
1055	MUELLER HINTON AGAR II
1068	TRYPTICASEIN SOY AGAR (TSA) EUROPEAN PHARMACOPOEIA
1561	TRYPTICASEIN SOY AGAR (TSA) Nº2
1189	TRYPTICASEIN SOY BLOOD AGAR BASE
	Maintenance of strains
1048	BRAIN HEART INFUSION AGAR (BHI AGAR)
1502	C.T.A. MEDIUM
1068	TRYPTICASEIN SOY AGAR (TSA) EUROPEAN PHARMACOPOEIA
1314	NUTRIENT AGAR (DEV REGULATIONS)
1060	NUTRIENT AGAR ISO 6579, ISO 10273
1156	NUTRIENT AGAR UNE-EN 12780, EN ISO 16266
	Plate count media
1111	LETHEEN AGAR MODIFIED
1071	R2A AGAR EUROPEAN PHARMACOPOEIA
1056	STANDARD METHODS AGAR (P.C.A) Acc. to APHA, ISO 4833
1068	TRYPTICASEIN SOY AGAR (TSA) EUROPEAN PHARMACOPOEIA
	Beer Fermentation Process
1438	NOCIVE BREWERS BACTERIA AGAR BASE, MODIFIED
1440	NOCIVE BREWERS BACTERIA BROTH BASE, MODIFIED
1061	RAKA-RAY AGAR BASE
1562	UNIVERSAL BEER MEDIUM
1026	WL DIFFERENTIAL AGAR
1086	WL NUTRIENT AGAR
Cat Nº	**OTHER APPLICATIONS**
	Sensitivity Test Media
1058	MUELLER HINTON AGAR
1055	MUELLER HINTON AGAR II
1214	MUELLER HINTON BROTH
1503	WILKINS CHALGREN MEDIUM
1568	WILKINS CHALGREN MEDIUM II
	Sterility Test Media
1241	THIOGLYCOLLATE BROTH NIH, USP
1508	THIOGLYCOLLATE FLUID MEDIUM EUROPEAN PHARMACOPOEIA
1533	THIOGLYCOLLATE MEDIUM USP, ISO 7937
1516	THIOGLYCOLLATE MEDIUM WITHOUT INDICATOR USP
	Transport Media
1535	AMIES TRANSPORT MEDIUM WITH CHARCOAL
1530	AMIES TRANSPORT MEDIUM WITHOUT CHARCOAL
1529	CARY BLAIR MEDIUM
1518	STUART TRANSPORT MEDIUM
	Urinary Germs test
1016	CLED AGAR (CYSTINE LACTOSE ELECTROLYTE DEFICIENT)
1303	CLED AGAR WITH ANDRADE´S INDICATOR
1424	URINARY TRACT INFECTIONS CHROMOGENIC (UTIC)

Culture Media for **Industrial Microbiology**

Laboratorios CONDA, one of the world leaders in the design and manufacturing of high quality culture media, currently offers more than 400 different products, among which you will find chromogenic media, ISO-formulated media and custom-made media for many different industrial applications. From hygiene control, through food and beverage poisoning prevention, to microbiologial examination of cosmetic and pharmaceutical products, CONDA supplies a wide variety of different media for each field so that customers can find the most suitable product for their needs.

CONDA complies with the ISO 9001 standard, and our products comply with various international standards such as ISO, the European Pharmacopoeia, FDA, APHA, USP and AOAC standards.

ANALYSIS CHART

INDUSTRIES AND RELATED BACTERIA

- MEAT & FISH INDUSTRY
- WATER & BEVERAGES
- DAIRY PRODUCTS
- BAKERY
- PROCESSED FOODS
- BEER INDUSTRY
- WASTE WATER
- COSMETIC INDUSTRY
- PHARMACEUTICAL INDUSTRY
- MICROBIOLOGY DEHYDRATED CULTURE MEDIA GUIDE

	Acid tolerant pathogens	Aerobes mesophiles	Anaerobes count	Antibiotic assay	*Bacillus cereus*	Beer industry process	*Campylobacter*	*Clostridium perfringens*	*Clostridium* sulfite reducers	*Clostridium tyrobutyricum*	*Coliforms*	Cosmetic testing	*Enterobacter sakazakii*	*Enterobacteria*	*Enterococci*	*Escherichia coli*	*Lactobacillus*	*Legionella*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Salmonella/Shigella*	*Staphylococcus aureus*	Sterility testing	*Streptococcus thermophilus*	*Vibrio*	Yeast and molds	*Yersinia*
MEAT & FISH INDUSTRY		●			●		●	●	●		●			●		●	●		●	●	●	●			●	●	●
WATER & BEVERAGES	●	●						●	●		●			●	●	●		●	●	●						●	
DAIRY PRODUCTS		●					●		●	●	●		●	●	●	●	●		●		●	●		●		●	●
BAKERY		●			●				●		●			●		●			●		●	●				●	
PROCESSED FOODS		●									●			●		●			●		●	●				●	●
BEER INDUSTRY						●								●		●			●							●	
WASTE WATER		●	●								●			●	●	●				●					●		
COSMETIC INDUSTRY			●								●	●		●		●				●		●				●	
PHARMA - INDUSTRY		●	●	●										●		●				●	●	●	●			●	

MEAT & FISH INDUSTRY

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	*Aerobes mesophiles*	*Bacillus cereus*	*Campylobacter*	*Clostridium perfringens*	*Clostridium* sulfite reducers	Coliforms	*Enterobacteria*	*Escherichia coli*	*Lactobacillus*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Salmonella/Shigella*	*Staphylococcus aureus*	*Vibrio*	Yeast and molds	*Yersinia*
Acetate Differential Agar	1192		Differentiation									●				●				
Alkaline Peptone Water	1407		Enrichment Medium															●		
Anaerobic Agar	1000		Cultivation					●	●											
Bacillus Cereus Selective Agar Base	1124	6021/ 5152	Enumeration / Isolation			●														
Baird Parker Agar Base	1319	5129	Selective Isolation	ISO 6888-1													●			
Baird Parker Agar Base (RPF)	1319	6024	Selective Isolation	ISO 6888-2													●			
BCP Glucose Agar	1320		Differentiation /Enumeration	ISO 21528:2							●									
Bismuth Sulfite Agar (Wilson Blair)	1011		Selective Isolation	USP												●				
Blood Agar Base No. 2	1328		Confirmation	ISO 7932		●														
Blood Free Campylobacter Agar Base	1129	6053	Selective Isolation				●													
Brain Heart Infusion Broth	1331		Confirmation	ISO 6888-1													●			
Brilliant Green Agar	1078		Selective Isolation	Eur. Pharma USP												●				
Brilliant Green Selenite Broth	1221		Selective Enrichment													●				
Brilliant Green Selenite Broth II	1219		Selective Enrichment													●				
Brilliant Green Tetrathionate Bile Broth	1253		Selective Enrichment	Eur. Pharma												●				
Buffered Peptone Water	1402		Diluent	ISO 6579												●				
Carbohydrates Utilization Broth	1342		Confirmation	ISO 11290-2										●						
Chapman Stone Agar	1017		Selective Isolation/ Differentiation														●			
DCLS Agar	1045		Selective Isolation													●				
Desoxycholate Citrate Agar	1067		Selective Isolation	Eur. Pharma												●				
Desoxycholate Lactose Agar	1025		Isolation/Enumeration							●										
Dextrose Agar	1021		Total Count		●															
Differential Reinforced Clostridial Broth	1416		Enumeration					●	●											
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection							●		●								
EE Broth	1362		Enumeration	ISO 21528-1							●									
Endo Agar Base	1118		Confirmation / Differentiation							●										
Ewing Malonate Broth Modified	1212		Differentiation							●						●				
Fraser Broth Base	1182	6001	Enrichment Medium	ISO 11290-1										●						
Gelatin Lactose Medium	1526		Confirmation					●												
Giolitti - Cantoni Broth	1287	5208	Detection	ISO 6888/5944													●			
Hektoen Enteric Agar	1030		Isolation/Differentiation								●									
Irgasan Ticarcillin & Potassium Chlorate	1361	6051	Enrichment Medium	ISO 10273																●
Kligler Iron Agar	1364		Presumptive Isolation	ISO 10273																●
Koser Citrate Broth	1200		Differentiation									●								
Lactose Broth	1206		Cultivation	Eur. Pharma						●						●				

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	*Aerobes mesophiles*	*Bacillus cereus*	*Campylobacter*	*Clostridium perfringens*	*Clostridium sulfite reducers*	*Coliforms*	*Enterobacteria*	*Escherichia coli*	*Lactobacillus*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Salmonella/Shigella*	*Staphylococcus aureus*	*Vibrio*	*Yeast and molds*	*Yersinia*
Lactose Sulfite Broth Base	1009		Selective Detection/ Enumeration	Eur. Pharma				●												
Levine Agar (EMB)	1050		Isolation/Differentiation								●									
Listeria Agar Base Oxford	1133	6003	Selective Detection	ISO 11290-1										●						
Listeria Enrichment Broth Base Fraser	1120	6001/ 6002	Enrichment Medium	ISO 11290-1										●						
Listeria Agar Base Palcam	1141	6004	Selective/Differential Medium	ISO 11290-2										●						
Listeria Chromogenic Agar Base	1345	6031/ 6040	Selective Detection / Enumeration	ISO 11290-2										●						
Lysine Decarboxylase Broth	1208		Identification								●					●				
Lysine Decarboxylase Broth	1176		Biochemical Confirmation	ISO 6579												●				
Lysine Iron Agar	1044		Differentiation							●						●				
Macconkey Agar	1052		Isolation/Identification	Eur. Pharma							●									
Malt Extract Agar	1038		Cultivation																●	
Malt Extract Broth	1245		Isolation/ Enumeration																●	
Mannitol Nitrate Motility Medium	1509		Differentiation								●									
Mannitol Salt Agar (MSA)	1062		Isolation	Eur. Pharma													●			
Mio Medium	1510		Identification								●									
Moeller KCN Broth Base	1112		Differentiation								●									
Mossel EE Broth	1202		Selective Enrichment	Eur. Pharma							●					●				
MRS Agar	1043		Cultivation										●							
MRS Broth	1215		Cultivation										●							
MRSV Medium	1376		Detection of Motility													●				
MR-VP Medium	1512		Differentiation								●									
Muller Kauffmann Broth Base with Brilliant Green & Novobiocine (MKTTN)	1173		Selective Enrichment	ISO 6579												●				
Muller Kauffmann Tetrathionate Broth Base	1130		Selective Enrichment													●				
Nitrate Motility Base Medium	1565		Confirmation					●												
Nutrient Agar	1060		Cultivation	ISO 6579 ISO 10273												●				
Nutrient Agar with Sodium Chloride	1355		Confirmation	ISO 21528-1							●									
OGA Medium (Oxytetracycline Glucose Agar)	1527	6018	Enumeration / Selection																●	
Peptone Sorbitol & Biliar Salts Broth (PSB Broth)	1298		Diluent	ISO 10273																●
Potato Dextrose Agar	1022		Identification/Cultivation /Enumeration	Eur. Pharma															●	
Potato Dextrose Broth	1261		Cultivation																●	
Pseudomonas Agar Base	1356	6036	Isolation	ISO 13720											●					
Rappaport Soy Broth (Vassiliadis)	1174		Selective Enrichment	ISO 6579												●				

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	*Aerobes mesophiles*	*Bacillus cereus*	*Campylobacter*	*Clostridium perfringens*	*Clostridium* sulfite reducers	Coliforms	*Enterobacteria*	*Escherichia coli*	*Lactobacillus*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Salmonella/Shigella*	*Staphylococcus aureus*	*Vibrio*	Yeast and molds	*Yersinia*
Reinforced Clostridial Medium	1007		Cultivation/Enumeration	Eur. Pharma				●	●											
Rogosa SL Agar	1096		Selective Cultivation										●							
Rogosa SL Broth	1234		Selective Cultivation										●							
Rose Bengal Agar + Chloramphenicol	1081		Cultivation/Selective Isolation																●	
Salmonella Chromogenic Agar	1122		Isolation													●				
Salmonella Shigella Agar (SS)	1064		Selective Isolation													●				
Salmonella Shigella Agar with Sodium Desoxycholate & Calcium Chloride	1360		Selective Isolation/ Differentiation	ISO 10273																●
Salmonella Shigella Modified Agar	1186		Selective Isolation													●				
San Francisco Medium	1413		Cultivation										●							
SBF Broth	1384		Enrichment Medium													●				
Selenite Cystine Broth	1220		Selective Enrichment													●				
Sim Medium	1514	5205	Identification/Differentiation								●	●				●				
Sodium Selenite Broth	1222		Selective Isolation													●				
SPS Agar	1082		Isolation					●												
Standard Methods Agar (PCA)	1056		Total Count	ISO 4833	●															
Staphylococcus Agar No. 110	1032		Isolation														●			
TBX Chromogenic Agar	1151		Selective Detection/ Enumeration	ISO 16649-2								●								
TCBS Agar	1074		Isolation															●		
Tetrathionate Broth Base (Muller Kauffmann)	1114		Selective Enrichment	USP												●				
Thioglycollate USP Medium	1533		Cultivation	USP ISO 7937				●												
Triple Sugar Iron Agar (TSI)	1172		Confirmation	ISO 6579												●				
Tryptone Soy Yeast Extract Agar (TSYEA)	1398		Cultivation	ISO 11290-1										●						
Tryptone Soy Yeast Extract Broth (TSYEB)	1339		Confirmation	ISO 11290-1										●						
Tryptose Sulfite Broth	1378		Selective Medium					●												
TSC Agar Base	1029	6020/ 5152	Detection / Enumeration	ISO 7937				●												
TSN Agar	1075		Selective Isolation					●												
Urea Agar Base (Christensen)	1110		Differentiation	ISO 6579							●									
Urea Indol Broth	1227	5205	Identification	ISO 10273							●									
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation /Enumeration	Eur Pharma, ISO 21528							●									
Violet Red Bile Agar with Lactose (VRBL)	1093		Selective Detection / Enumeration	ISO 4832						●										
XLD Agar	1274		Selective Isolation	ISO 6579												●				
XLT4 Agar Base	1159	6062	Isolation													●				
Yersinia Selective Agar Base	1126	6033	Selective Medium	ISO 10273																●

WATER & BEVERAGES

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Acid tolerant pathogens	Aerobes mesophiles	*Clostridium perfringens*	*Clostridium* sulfite reducers	Coliforms	*Enterobacteria*	Enterococci	*Escherichia coli*	*Legionella*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	Yeast and molds
A1 Medium	1252		Detection						●							
Acetamide Agar	1391		Confirmation												●	
Acetamide Broth	1155		Confirmation	UNE-EN 12780 EN ISO 16266											●	
Anaerobic Agar	1000		Cultivation				●	●								
Asparagine Broth	1207		Presumptive Identification / Enumeration												●	
Azide Dextrose Broth	1422		Selective Enrichment								●					
BCP Agar	1051		Isolation						●							
Bile Esculin Azide Agar	1005		Selective Isolation / Presumptive Identification	ISO 7899-2							●					
Brilliant Green Bile Agar	1010		Enumeration						●							
Brilliant Green Bile Broth 2%	1228		Detection	ISO 4831, ISO 4832					●							
Carbohydrates Utilization Broth	1342		Confirmation	ISO 11290-1										●		
Cetrimide Agar Base	1102		Selective Isolation / Identification	Eur. Pharma											●	
Clostridium Perfringens Agar Base (m-CP)	1132		Enumeration / Isolation				●									
Desoxycholate Lactose Agar	1025		Isolation / Enumeration						●							
Dextrose Agar	1021		Total Count			●										
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection						●			●				
EC Medium	1522		Enumeration / Differentiation	ISO 7521					●							
Endo Agar Base	1118		Confirmation / Differentiation						●							
Endo Les Agar Base	1137		Detection / Enumeration						●							
Enterococcus Confirmatory Agar	1018		Confirmation								●					
Eosin Methylene Blue Agar (EMB)	1039		Isolation / Differentiation						●							
EVA Broth (Ethyl Violet Azide Broth)	1230		Confirmation								●					
Fecal Coliforms Agar Base (m-FC)	1127	6023	Detection / Enumeration						●							
Fecal Coliforms Broth Base	1121	6023	Detection / Enumeration						●							
Gelatin Lactose Medium	1526		Confirmation				●									
King B Medium	1154		Identification / Enumeration	UNE-EN 12780 ISO 16266											●	
Koser Citrate Broth	1200		Differentiation									●				
Lactose Broth	1206		Cultivation	Eur. Pharma					●							
Lactose Sulfite Broth Base	1009		Selective Detection/Enumeration	Eur. Pharma			●									
Lauryl Sulfate Agar	1309		Selective Isolation/Enumeration						●							
Lauryl Sulfate Broth	1310		Detection	ISO 4831					●							
Legionella CYE Agar Base	1311	6022/ 6025	Selective Cultivation	ISO 11731									●			
Listeria Agar Base Oxford	1133	6003	Selective Detection	ISO 11290-1										●		
Listeria Enrichment Broth Base Fraser	1120	6001/ 6002	Enrichment Medium	ISO 11290-1										●		
Listeria Agar Base Palcam	1141	6004	Selective / Differential Medium	ISO 11290-2										●		

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Acid tolerant pathogens	Aerobes mesophiles	*Clostridium perfringens*	*Clostridium* sulfite reducers	Coliforms	*Enterobacteria*	*Enterococci*	*Escherichia coli*	*Legionella*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	Yeast and molds
Listeria Chromogenic Agar Base	1345	6031/ 6040	Selective Detection / Enumeration	ISO 11290-2										●		
Lysine Decarboxylase Broth	1208		Identification							●						
Macconkey Agar No. 2	1035		Identification								●					
Macconkey Agar w/o Crystal Violet	1037		Cultivation / Differentiation								●					
Macconkey Broth	1210		Detection	Eur. Pharma					●							
m-EI Chromogenic Agar Base	1412		Detection / Enumeration								●					
Minerals Modified Glutamate Broth	1365		Enumeration						●							
MR-VP Medium	1512		Differentiation									●				
Nitrate Motility Base Medium	1565		Confirmation				●									
Nutrient Agar	1156		Identification / Enumeration	UNE-EN 12780											●	
Orange Serum Agar	1307		Isolation / Detection		●											
Peptone Water with Lactose	1357		Confirmation	ISO 9308-1					●							
Pseudomonas CN Agar Base	1153		Identification / Enumeration	UNE-EN 12780 ISO 16266											●	
R2A Agar	1071		Total Count	Eur. Pharma		●										
Sim Medium	1514	5205	Identification/Differentiation									●				
Simmons Citrate Agar	1014		Differentiation	ISO 10273								●				
Slanetz-Bartley Medium	1109		Detection / Enumeration	ISO 7899-2							●					
Triple Sugar Iron Agar (TSI)	1046		Identification/Differentiation	Eur. Pharma								●				
Trypticasein Glucose Extract Agar	1041		Enumeration			●										
Tryptone Bile Salts Agar	1013		Detection / Enumeration	ISO 9308-1					●			●				
Tryptone Soy Agar	1138		Detection / Enumeration	ISO 9308-1					●			●				
Tryptone Soy Yeast Extract Agar (TSYEA)	1398		Cultivation	ISO 11290-1										●		
Tryptone Soy Yeast Extract Broth (TSYEB)	1339		Confirmation	ISO 11290-1										●		
Tryptophan Culture Broth	1237		Detection	ISO 9308-1					●			●				
Tryptose Sulfite Broth	1378		Selective Medium				●									
TTC Chapman Agar	1076	6030	Enumeration	ISO 9308-1					●							
Urea Indol Broth	1227	5205	Identification	ISO 10273								●				
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation / Enumeration	Eur. Pharma						●		●				
Wort Agar	1316		Cultivation / Enumeration													●
Wort Broth	1444		Enumeration / Count													●
XLD Agar (Xylose Lysine Desoxycholate)	1080		Isolation	Eur. Pharma ISO 21528						●						
XLT4 Agar Base	1159	6062	Isolation							●						
Yeast Extract Agar	1049		Enumeration	ISO 6222		●										●

DAIRY PRODUCTS

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Campylobacter	Clostridium sulfite reducers	Clostridium tyrobutyricum	Coliforms	Enterobacter sakazakii	Enterobacteria	Enterococci	Escherichia coli	Lactobacillus	Listeria monocytogenes	Salmonella/Shigella	Staphylococcus aureus	Streptococcus thermophilus	Yeast and molds	Yersinia
Acetate Differential Agar	1192		Differentiation										●			●				
Anaerobic Agar	1000		Cultivation				●													
Baird Parker Agar Base	1319	5129	Selective Isolation	ISO 6888-1													●			
Baird Parker Agar Base (RPF)	1319	6024	Selective Isolation	ISO 6888-2													●			
Bile Esculin Agar	1031		Isolation/Identification	ISO 10273								●								
Bismuth Sulfite Agar (Wilson Blair)	1011		Selective Isolation	USP												●				
Blood Free Campylobacter Agar Base	1129	6053	Selective Isolation			●														
Brilliant Green Selenite Broth	1221		Selective Enrichment													●				
Brilliant Green Tetrathionate Bile Broth	1253		Selective Enrichment	Eur. Pharma												●				
Bryant & Burkey Base Broth (Modified with Resarzurine)	1247		Detection					●												
Buffered Peptone Water	1402		Diluent	ISO 6579												●				
Buti Medium for Milk	1184		Detection					●								●				
Carbohydrates Utilization Broth	1342		Confirmation	ISO 11290-2											●					
Chapman Stone Agar	1017		Selective Isolation / Differentiation														●			
Chloramphenicol Agar	1301		Selective Isolation/Enumeration	ISO 7954															●	
DCLS Agar	1045		Selective Isolation													●				
Desoxycholate Agar	1020		Isolation / Enumeration						●											
Desoxycholate Citrate Agar	1067		Selective Isolation	Eur. Pharma												●				
Desoxycholate Lactose Agar	1025		Isolation/ Enumeration						●											
Dextrose Agar	1021		Total Count		●															
Differential Reinforced Clostridial Broth	1416		Enumeration				●													
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection						●				●							
EE Broth	1362		Enumeration	ISO 21528-1							●									
Elliker Medium	1539		Cultivation											●						
Endo Agar Base	1118		Confirmation/Differentiation						●											
Enterobacter Sakazakii Isolation Agar Chromogenic (ESIA)	1446		Isolation	ISO 22964						●										
ESTY Broth	1254		Selective Cultivation															●		
ESTY Medium	1555		Selective Enumeration															●		
Ewing Malonate Broth Modified	1212		Differentiation						●							●				
Fraser Broth Base	1182	6001	Enrichment Medium	ISO 11290-1											●					
Giolitti - Cantoni Broth	1287	5208	Detection	ISO 6888/5944													●			
Glucose Chloramphenicol Agar	1094		Selective Isolation / Enumeration																●	
Glucose Chloramphenicol Broth	1258		Selective Isolation / Enumeration																●	
Hektoen Enteric Agar	1030		Isolation/ Differentiation								●									

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Campylobacter	Clostridium sulfite reducers	Clostridium tyrobutyricum	Coliforms	Enterobacter sakazakii	Enterobacteria	Enterococci	Escherichia coli	Lactobacillus	Listeria monocytogenes	Salmonella/Shigella	Staphylococcus aureus	Streptococcus thermophilus	Yeast and molds	Yersinia
Irgasan Ticarcillin & Potassium Chlorate	1361	6051	Enrichment Medium	ISO 10273																●
KAA Confirmatory Agar	1027		Isolation/Confirmation									●								
KAA Presumptive Broth	1209		Presumptive Detection									●								
Kligler Iron Agar	1364		Presumptive Isolation	ISO 10273																●
Koser Citrate Broth	1200		Differentiation										●							
Lactose Broth	1206		Cultivation	Eur. Pharma					●							●				
Lauryl Sulfate Tryptose Broth Modified	1445		Selective Enrichment	ISO 22964						●										
Levine Agar (EMB)	1050		Isolation / Differentiation								●									
Listeria Agar Base Oxford	1133	6003	Selective Detection	ISO 11290-1											●					
Listeria Enrichment Broth Base Fraser	1120	6001/ 6002	Enrichment Medium	ISO 11290-1											●					
Listeria Agar Base Palcam	1141	6004	Selective/Differential Medium	ISO 11290-2											●					
Listeria Chromogenic Agar Base	1345	6031/ 6040	Selective Detection / Enumeration	ISO 11290-2											●					
Lysine Decarboxylase Broth	1208		Identification								●					●				
Lysine Decarboxylase Broth	1176		Biochemical Confirmation	ISO 6579												●				
M 17 Agar	1318		Cultivation/Enumeration														●			
Macconkey Agar	1052		Isolation / Identification	Eur. Pharma							●									
Macconkey Agar No. 2	1035		Identification									●								
Macconkey Broth	1210		Detection	Eur. Pharma					●											
Malt Extract Agar	1038		Cultivation																●	
Malt Extract Broth	1245		Isolation / Enumeration																●	
Mannitol Nitrate Motility Medium	1509		Differentiation								●		●							
Mannitol Salt Agar (MSA)	1062		Isolation	Eur. Pharma													●			
Mio Medium	1510		Identification								●		●			●				
Mossel EE Broth	1202		Selective Enrichment	Eur. Pharma					●											
MRS Agar	1043		Cultivation											●						
MRS Broth	1215		Cultivation											●						
MRSV Medium	1376		Detection													●				
MR-VP Medium	1512		Differentiation								●		●							
Muller Kauffmann Broth Base with Brilliant Green & Novobiocine (MKTTN)	1173		Selective Enrichment	ISO 6579												●				
Muller Kauffmann Tetrathionate Broth Base	1130		Selective Enrichment													●				
Nutrient Agar	1060		Cultivation	ISO 6579 ISO 10273												●				
Nutrient Agar with Sodium Chloride	1355		Confirmation	ISO 21528-1							●									
OGA Medium (Oxytetracycline Glucose Agar)	1527	6018	Enumeration / Selection																●	
Osmophilic Agar	1057		Cultivation																●	

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Campylobacter	Clostridium sulfite reducers	Clostridium tyrobutyricum	Coliforms	Enterobacter sakazakii	Enterobacteria	Enterococci	Escherichia coli	Lactobacillus	Listeria monocytogenes	Salmonella/Shigella	Staphylococcus aureus	Streptococcus thermophilus	Yeast and molds	Yersinia
Peptone Sorbitol & Biliar Salts Broth (PSB Broth)	1298		Diluent	ISO 10273																●
Potato Dextrose Agar	1022		Identification/Cultivation / Enumeration	Eur. Pharma															●	
Potato Dextrose Broth	1261		Cultivation																●	
Rappaport Soy Broth (Vassiliadis)	1174		Selective Enrichment	ISO 6579												●				
Reinforced Clostridial Medium	1007		Cultivation / Enumeration	Eur. Pharma			●													
Rose Bengal Agar + Chloramphenicol	1081		Cultivation/Selective Isolation																●	
Rose Bengal Agar + Dichloran + Chloramphenicol (DRBC Agar)	1160		Selective Cultivation	ISO 21527-1															●	
Rothe Broth (Glucose Broth with Azide)	1238		Enumeration									●								
Salmonella Chromogenic Agar	1122		Isolation													●				
Salmonella Shigella Agar with Sodium Desoxycholate & Calcium Chloride	1360		Selective Isolation/ Differentiation	ISO 10273																●
San Francisco Medium	1413		Cultivation											●						
SBF Broth	1384		Enrichment Medium													●				
Selenite Cystine Broth	1220		Selective Enrichment													●				
Sim Medium	1514	5205	Identification/Differentiation								●		●			●				
Sodium Selenite Broth	1222		Selective Isolation													●				
Standard Methods Agar (PCA)	1056		Total Count	ISO 4833	●															
Standard Methods Agar with Powdered Milk	1033		Total Count		●															
Staphylococcus Agar No. 110	1032		Isolation														●			
TBX Chromogenic Agar	1151		Selective Detection / Enumeration	ISO 16649-2									●							
Tetrathionate Broth Base (Mueller Kauffmann)	1114		Selective Enrichment	USP												●				
Triple Sugar Iron Agar (TSI)	1172		Biochemical Confirmation	ISO 6579												●				
Tryptone Soy Yeast Extract Agar (TSYEA)	1398		Cultivation	ISO 11290-1											●					
Tryptone Soy Yeast Extract Broth (TSYEB)	1339		Confirmation	ISO 11290-1											●					
Urea Indol Broth	1227	5205	Identification	ISO 10273							●		●			●				
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation/Enumeration	Eur. Pharma ISO 21528							●									
Violet Red Bile Agar with Lactose (VRBL)	1093		Selective Detection/ Enumeration	ISO 4832					●											
Wort Agar	1316		Cultivation/Enumeration																●	
Wort Broth	1444		Enumeration / Count																●	
XLD Agar	1274		Selective Isolation	ISO 6579												●				
XLT4 Agar Base	1159	6062	Isolation													●				
Yeast Extract Agar (for Molds)	1312		Cultivation																●	
Yersinia Selective Agar Base	1126	6033	Selective Medium	ISO 10273																●

BAKERY

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	*Bacillus cereus*	*Clostridium* sulfite reducers	Coliforms	*Enterobacteria*	*Escherichia coli*	*Listeria monocytogenes*	*Salmonella/Shigella*	*Staphylococcus aureus*	Yeast and molds
Anaerobic Agar	1000		Cultivation				●							
Bacillus Cereus Selective Agar Base	1124	6021/ 5152	Enumeration / Isolation			●								
Baird Parker Agar Base	1319	5129	Selective Isolation	ISO 6888-1									●	
Baird Parker Agar Base (RPF)	1319	6024	Selective Isolation	ISO 6888-2									●	
BCP Agar	1051		Isolation					●						
BCP Glucose Agar	1320		Differentiation / Enumeration	ISO 21528-2					●	●		●		
Bismuth Sulfite Agar (Wilson Blair)	1011		Selective Isolation	USP								●		
Brilliant Green Agar	1078		Selective Isolation	Eur. Pharma USP								●		
Brilliant Green Bile Broth 2%	1228		Detection	ISO 4831, ISO 4832				●						
Brilliant Green Selenite Broth	1221		Selective Enrichment									●		
Brilliant Green Tetrathionate Bile Broth	1253		Selective Enrichment	Eur. Pharma								●		
Carbohydrates Utilization Broth	1342		Confirmation	ISO 11290-2							●			
Chapman Stone Agar	1017		Selective Isolation / Differentiation										●	
Dextrose Agar	1021		Total Count		●									
Differential Reinforced Clostridial Broth	1416		Enumeration				●							
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection					●		●				
EE Broth	1362		Enumeration	ISO 21528-1					●					
Fraser Broth Base	1182	6001	Enrichment Medium	ISO 11290-1							●			
Giolitti - Cantoni Broth	1287	5208	Detection	ISO 6888/5944									●	
Hektoen Enteric Agar	1030		Isolation / Differentiation						●			●		
Lactose Broth	1206		Cultivation	Eur. Pharma				●				●		
Lauryl Sulfate Agar	1309		Selective Isolation / Enumeration					●						
Levine Agar (EMB)	1050		Isolation / Differentiation						●					
Listeria Agar Base Oxford	1133	6003	Selective Detection	ISO 11290-1							●			
Listeria Enrichment Broth Base Fraser	1120	6001/ 6002	Enrichment Medium	ISO 11290-1							●			
Listeria Agar Base Palcam	1141	6004	Selective/Differential Medium	ISO 11290-2							●			
Listeria Chromogenic Agar Base	1345	6031/ 6040	Selective Detection / Enumeration	ISO 11290-2							●			
Lysine Decarboxylase Broth	1208		Identification						●			●		
Lysine Decarboxylase Broth	1176		Biochemical Confirmation	ISO 6579								●		
Macconkey Agar	1052		Isolation / Identification	Eur. Pharma					●					
Malt Extract Agar	1038		Cultivation											●
Malt Extract Broth	1245		Isolation / Enumeration											●
Mannitol Nitrate Motility Medium	1509		Differentiation						●	●				
Mannitol Salt Agar (MSA)	1062		Isolation	Eur. Pharma									●	

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	*Bacillus cereus*	*Clostridium* sulfite reducers	Coliforms	*Enterobacteria*	*Escherichia coli*	*Listeria monocytogenes*	*Salmonella/Shigella*	*Staphylococcus aureus*	Yeast and molds
Mio Medium	1510		Identification						●	●		●		
Moeller KCN Broth Base	1112		Differentiation						●	●		●		
Mossel EE Broth	1202		Selective Enrichment	Eur. Pharma				●						
MRSV Medium	1376		Detection									●		
MR-VP Medium	1512		Differentiation						●	●				
Muller Kauffmann Broth Base with Brilliant Green & Novobiocine (MKTTN)	1173		Selective Enrichment	ISO 6579								●		
Muller Kauffmann Tetrathionate Broth Base	1130		Selective Enrichment									●		
Nutrient Agar	1060		Cultivation	ISO 6579 ISO 10273								●		
Nutrient Agar with Sodium Chloride	1355		Confirmation	ISO 21528-1					●					
OGA Medium (Oxytetracycline Glucose Agar)	1527	6018	Enumeration / Selection											●
Osmophilic Agar	1057		Cultivation											●
Potato Dextrose Agar	1022		Identification/Cultivation/Enumeration	Eur. Pharma										●
Potato Dextrose Broth	1261		Cultivation											●
Rappaport Soy Broth (Vassiliadis)	1174		Selective Enrichment	ISO 6579								●		
Reinforced Clostridial Medium	1007		Cultivation/Enumeration	Eur. Pharma			●							
Rose Bengal Agar + Chloramphenicol	1081		Cultivation/Selective Isolation											●
Rose Bengal Agar + Dichloran + Chloramphenicol (DRBC Agar)	1160		Selective Cultivation	ISO 21527-1										●
Salmonella Chromogenic Agar	1122		Isolation									●		
SBF Broth	1384		Enrichment Medium									●		
Selenite Cystine Broth	1220		Selective Enrichment									●		
Sim Medium	1514	5205	Identification/Differentiation						●	●		●		
Sodium Selenite Broth	1222		Selective Isolation									●		
Staphylococcus Agar No. 110	1032		Isolation										●	
TBX Chromogenic Agar	1151		Selective Detection / Enumeration	ISO 16649-2						●				
Tetrathionate Broth Base (Muller Kauffmann)	1114		Selective Enrichment	USP								●		
Triple Sugar Iron Agar (TSI)	1172		Biochemical Confirmation	ISO 6579								●		
Tryptone Soy Yeast Extract Agar (TSYEA)	1398		Cultivation	ISO 11290-1							●			
Tryptone Soy Yeast Extract Broth (TSYEB)	1339		Confirmation	ISO 11290-1							●			
Urea Agar Base (Christensen)	1110		Differentiation	ISO 6579					●					
Urea Indol Broth	1227	5205	Identification	ISO 10273					●	●		●		
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation / Enumeration	Eur. Pharma ISO 21528					●					
Violet Red Bile Agar with Lactose (VRBL)	1093		Selective Detection / Enumeration	ISO 4832				●						
XLD Agar	1274		Selective Isolation	ISO 6579								●		
XLT4 Agar Base	1159	6062	Isolation									●		

PROCESSED FOODS

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	*Aerobes mesophiles*	*Coliforms*	*Enterobacteria*	*Escherichia coli*	*Listeria monocytogenes*	*Salmonella/Shigella*	*Staphylococcus aureus*	*Yeast and molds*	*Yersinia*
Baird Parker Agar Base	1319	5129	Selective Isolation	ISO 6888-1							●		
Baird Parker Agar Base (RPF)	1319	6024	Selective Isolation	ISO 6888-2							●		
BCP Agar	1051		Isolation			●							
BCP Glucose Agar	1320		Differentiation/Enumeration	ISO 21528-2			●	●		●			
Bismuth Sulfite Agar (Wilson Blair)	1011		Selective Isolation	USP						●			
Brilliant Green Agar	1078		Selective Isolation	Eur. Pharma USP						●			
Brilliant Green Bile Broth 2%	1228		Detection	ISO 4831 ISO 4832		●							
Brilliant Green Selenite Broth	1221		Selective Enrichment							●			
Brilliant Green Tetrathionate Bile Broth	1253		Selective Enrichment	Eur. Pharma						●			
Carbohydrates Utilization Broth	1342		Confirmation	ISO 11290-2					●				
Chapman Stone Agar	1017		Selective Isolation / Differentiation								●		
DCLS Agar	1045		Selective Isolation							●			
Desoxycholate Citrate Agar	1067		Selective Isolation	Eur. Pharma						●			
Desoxycholate Lactose Agar	1025		Isolation / Enumeration			●							
Dextrose Agar	1021		Total Count		●								
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection			●		●					
EE Broth	1362		Enumeration	ISO 21528-1			●						
Endo Agar Base	1118		Confirmation / Differentiation			●							
Fraser Broth Base	1182	6001	Enrichment Medium	ISO 11290-1					●				
Giolitti - Cantoni Broth	1287	5208	Detection	ISO 6888 / 5944							●		
Hektoen Enteric Agar	1030		Isolation / Differentiation				●			●			
Irgasan Ticarcillin & Potassium Chlorate	1361	6051	Enrichment Medium	ISO 10273									●
Kligler Iron Agar	1364		Presumptive Isolation	ISO 10273									●
Koser Citrate Broth	1200		Differentiation					●					
Lactose Broth	1206		Cultivation	Eur. Pharma		●				●			
Lauryl Sulfate Agar	1309		Selective Isolation / Enumeration			●							
Levine Agar (EMB)	1050		Isolation / Differentiation				●						
Listeria Agar Base Oxford	1133	6003	Selective Detection	ISO 11290-1					●				
Listeria Enrichment Broth Base Fraser	1120	6001/ 6002	Enrichment Medium	ISO 11290-1					●				
Listeria Agar Base Palcam	1141	6004	Selective / Differential Medium	ISO 11290-2					●				
Listeria Chromogenic Agar Base	1345	6031/ 6040	Selective Detection / Enumeration	ISO 11290-2					●				
Lysine Decarboxylase Broth	1176		Biochemical Confirmation	ISO 6579						●			
Macconkey Agar	1052		Isolation / Identification	Eur. Pharma			●						
Malt Extract Agar	1038		Cultivation									●	
Malt Extract Broth	1245		Isolation / Enumeration									●	
Mannitol Nitrate Motility Medium	1509		Differentiation				●	●					
Mannitol Salt Agar (MSA)	1062		Isolation	Eur. Pharma							●		

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Coliforms	Enterobacteria	Escherichia coli	Listeria monocytogenes	Salmonella/Shigella	Staphylococcus aureus	Yeast and molds	Yersinia
Mio Medium	1510		Identification				●	●		●			
Moeller KCN Broth Base	1112		Differentiation				●	●		●			
Mossel EE Broth	1202		Selective Enrichment	Eur. Pharma		●							
MRSV Medium	1376		Detection							●			
MR-VP Medium	1512		Differentiation				●	●					
Muller Kauffmann Broth Base with Brilliant Green & Novobiocine (MKTTN)	1173		Selective Enrichment	ISO 6579						●			
Muller Kauffmann Tetrathionate Broth Base	1130		Selective Enrichment							●			
Nutrient Agar	1060		Cultivation	ISO 6579 ISO 10273						●			
Nutrient Agar with Sodium Chloride	1355		Confirmation	ISO 21528-1			●						
OGA Medium (Oxytetracycline Glucose Agar)	1527	6018	Enumeration / Selection									●	
Osmophilic Agar	1057		Cultivation									●	
Peptone Sorbitol & Biliar Salts Broth (PSB Broth)	1298		Diluent	ISO 10273									●
Potato Dextrose Agar	1022		Identification/Cultivation/Enumeration	Eur. Pharma								●	
Potato Dextrose Broth	1261		Cultivation									●	
Rappaport Soy Broth (Vassiliadis)	1174		Selective Enrichment	ISO 6579						●			
Rose Bengal Agar + Chloramphenicol	1081		Cultivation/Selective Isolation									●	
Rose Bengal Agar + Dichloran + Chloramphenicol (DRBC Agar)	1160		Selective Cultivation	ISO 21527-1								●	
Salmonella Chromogenic Agar	1122		Isolation							●			
Salmonella Shigella Agar with Sodium Desoxycholate & Calcium Chloride	1360		Selective Isolation/ Differentiation	ISO 10273									●
SBF Broth	1384		Enrichment Medium							●			
Selenite Cystine Broth	1220		Selective Enrichment							●			
Sim Medium	1514	5205	Identification/Differentiation				●	●		●			
Sodium Selenite Broth	1222		Selective Isolation							●			
Staphylococcus Agar No. 110	1032		Isolation								●		
TBX Chromogenic Agar	1151		Selective Detection / Enumeration	ISO 16649-2				●					
Tetrathionate Broth Base (Muller Kauffmann)	1114		Selective Enrichment	USP						●			
Triple Sugar Iron Agar (TSI)	1172		Biochemical Confirmation	ISO 6579						●			
Tryptone Soy Yeast Extract Agar (TSYEA)	1398		Cultivation	ISO 11290-1					●				
Tryptone Soy Yeast Extract Broth (TSYEB)	1339		Confirmation	ISO 11290-1					●				
Urea Agar Base (Christensen)	1110		Differentiation	ISO 6579			●						
Urea Indol Broth	1227	5205	Identification	ISO 10273			●	●		●			
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation / Enumeration	Eur. Pharma ISO 21528			●						
Violet Red Bile Agar with Lactose (VRBL)	1093		Selective Detection / Enumeration	ISO 4832		●							
XLD Agar	1274		Selective Isolation	ISO 6579						●			
XLT4 Agar Base	1159	6062	Isolation							●			
Yersinia Selective Agar Base	1126	6033	Selective Medium	ISI10273									●

BEER INDUSTRY

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Beer industry processes	*Enterobacteria*	*Escherichia coli*	*Listeria monocytogenes*	Yeast and molds
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection				●		
Fraser Broth Base	1182	6001	Enrichment Medium	ISO 11290-1				●	
Koser Citrate Broth	1200		Differentiation			●	●		
Levine Agar (EMB)	1050		Isolation / Differentiation			●			
LMDA Agar	1084		Differentiation		●				
Macconkey Agar	1052		Isolation / Identification	Eur. Pharma		●			
Malt Extract Agar	1038		Cultivation						●
Malt Extract Broth	1245		Isolation / Enumeration						●
Mannitol Nitrate Motility Medium	1509		Differentiation			●	●		
Mio Medium	1510		Identification			●	●		
MR-VP Medium	1512		Differentiation			●	●		
NOCIVE BREWERS BACTERIA Agar Base, Modified	1438		Selective Detection		●				
NOCIVE BREWERS BACTERIA Broth Base, Modified	1440		Selective Detection		●				
Potato Dextrose Broth	1261		Cultivation						●
Raka - Ray Agar Base	1061		Selective Isolation		●				
Rose Bengal Agar + Chloramphenicol	1081		Cultivation / Selective Isolation						●
Rose Bengal Agar + Dichloran + Chloramphenicol (DRBC Agar)	1160		Selective Cultivation	ISO 21527-1					●
Sim Medium	1514	5205	Identification/Differentiation			●	●		
Triple Sugar Iron Agar (TSI)	1046		Identification/Differentiation	Eur. Pharma		●	●		
Universal Beer Agar (UBA Medium)	1562		Fermentation Control		●				
Urea Agar Base (Christensen)	1110		Differentiation	ISO 6579		●			
Urea Indol Broth	1227	5205	Identification	ISO 10273		●	●		
WL Differential Agar	1026		Fermentation Control		●				
WL Nutrient Agar	1086		Fermentation Control		●				
Wort Agar	1316		Cultivation / Enumeration						●
Wort Broth	1444		Enumeration						●

WASTE WATER

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Anaerobes count	Coliforms	Enterobacteria	Enterococci	*Escherichia coli*	*Pseudomonas aeruginosa*	*Vibrio*
A1 Medium	1252		Detection				●					
Acetamide Agar	1391		Confirmation								●	
Acetamide Broth	1155		Confirmation	UNE-EN 12780 ISO 16266							●	
Alkaline Peptone Water	1407		Enrichment Medium									●
Anaerobic Agar	1000		Cultivation			●					●	
Asparagine Broth	1207		Presumptive Identification / Enumeration								●	
Azide Dextrose Broth	1422		Selective Enrichment							●		
BCP Agar	1051		Isolation				●					
Brilliant Green Bile Agar	1010		Enumeration				●					
Brilliant Green Bile Broth 2%	1228		Detection	ISO 4831 ISO 4832			●					
Cetrimide Agar Base	1102		Selective Isolation/Identification	Eur. Pharma							●	
Desoxycholate Lactose Agar	1025		Isolation / Enumeration				●					
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection				●			●		
EC Medium	1522		Enumeration / Differentiation	ISO 7251			●					
Endo Agar Base	1118		Confirmation / Differentiation				●					
Endo Les Agar Base	1137		Detection / Enumeration				●					
Enterococcus Confirmatory Agar	1018		Confirmation						●			
EVA Broth (Ethyl Violet Azide Broth)	1230		Confirmation						●			
Fecal Coliforms Agar Base (m-FC)	1127	6023	Detection / Enumeration				●					
Fecal Coliforms Broth Base	1121	6023	Detection / Enumeration							●		
King B Medium	1154		Identification / Enumeration	UNE-EN 12780 ISO 16266							●	
Koser Citrate Broth	1200		Differentiation							●		
Lactose Broth	1206		Cultivation	Eur. Pharma			●					
Lauryl Sulfate Agar	1309		Selective Isolation/Enumeration				●					
Lauryl Sulfate Broth	1310		Detection	ISO 4831			●					
Levine Agar (EMB)	1050		Isolation / Differentiation					●				
Macconkey Agar	1052		Isolation / Identification	Eur. Pharma				●				
Macconkey Agar No. 2	1035		Identification						●			
Macconkey Broth	1210		Detection	Eur. Pharma			●					
Mannitol Nitrate Motility Medium	1509		Differentiation					●		●		
m-EI Chromogenic Agar Base	1412		Detection / Enumeration						●			
Minerals Modified Glutamate Broth	1365		Enumeration				●					
Mio Medium	1510		Identification					●		●		
MR-VP Medium	1512		Differentiation					●		●		
Nutrient Agar	1156		Identification / Enumeration	UNE-EN 12780							●	
Peptone Water with Lactose	1357		Confirmation	ISO 9308-1			●					

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Anaerobes count	Coliforms	Enterobacteria	Enterococci	*Escherichia coli*	*Pseudomonas aeruginosa*	*Vibrio*
Pseudomonas CN Agar Base	1153		Identification / Enumeration	UNE-EN 12780 ISO 16266							●	
Pseudomonas F Agar (King B)	1532		Identification	USP							●	
Pseudomonas P Agar (King A)	1531		Identification	USP							●	
Reinforced Clostridial Medium	1007		Cultivation / Enumeration	Eur. Pharma		●						
Schaedler Agar	1066		Cultivation			●						
Sim Medium	1514	5205	Identification/Differentiation					●		●		
Simmons Citrate Agar	1014		Differentiation	ISO 10273				●		●		
TCBS Agar	1074		Selective Isolation									●
Triple Sugar Iron Agar (TSI)	1046		Identification/Differentiation	Eur. Pharma				●		●		
Trypticasein Glucose Extract Agar	1041		Total Count		●							
Tryptone Bile Salts Agar	1013		Detection / Enumeration	ISO 9308-1			●			●		
Tryptone Soy Agar	1138		Detection / Enumeration	ISO 9308-1			●			●		
Tryptophan Culture Broth	1237		Detection	ISO 9308-1			●			●		
TTC Chapman Agar	1076	6030	Enumeration	ISO 9308-1			●					
Urea Agar Base (Christensen)	1110		Differentiation	ISO 6579				●				
Urea Indol Broth	1227	5205	Identification	ISO 10273				●		●		
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation / Enumeration	Eur. Pharma ISO 21528				●				
Wilkins Chalgren Medium	1503		Isolation / Cultivation			●						

COSMETIC INDUSTRY

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Anaerobes count	Coliforms	Cosmetic testing	*Enterobacteria*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	Yeast and molds
Baird Parker Agar Base	1100	5129	Selective Isolation	Eur. Pharma							●	
Cetrimide Agar Base	1102		Selective Isolation/Identification	Eur. Pharma						●		
E. Coli Coliforms Chromogenic Medium	1340		Selective Detection			●			●			
Eosin Methylene Blue Agar (EMB)	1039		Isolation / Differentiation			●		●				
Koser Citrate Broth	1200		Differentiation						●			
Letheen Agar Base Modified	1111		Quaternary Ammonium Compounds				●					
Letheen Broth Base Modified	1244		Microbiological Analysis				●					
LPT Dilution Broth	1399		Homogenization				●					
Macconkey Agar	1052		Isolation / Identification	Eur. Pharma				●				
Malt Extract Agar	1038		Cultivation									●
Malt Extract Broth	1245		Isolation / Enumeration									●
Mannitol Nitrate Motility Medium	1509		Differentiation					●	●			
Mannitol Salt Agar (MSA)	1062		Isolation	Eur. Pharma							●	
MR-VP Medium	1512		Differentiation					●	●			
Neutralizing Agar	1449		Detection / Enumeration				●					
Potato Dextrose Broth	1261		Cultivation									●
Pseudomonas F Agar (King B)	1532		Identification	USP						●		
Pseudomonas P Agar (King A)	1531		Identification	USP						●		
Rose Bengal Agar + Chloramphenicol	1081		Selective Isolation									●
Sabouraud Maltose Broth	1213		Cultivation									●
Staphylococcus Agar No. 110	1032		Isolation								●	
Thioglycollate Broth	1241		Sterility Test	USP	●		●					
Thioglycollate Fluid Medium	1508		Cultivation	Eur. Pharma	●		●					
Thioglycollate Medium w/o Indicator	1516		Cultivation / Isolation	USP	●		●					
Thioglycollate USP Medium	1533		Cultivation	USP ISO 7937	●		●					
Triple Sugar Iron Agar (TSI)	1046		Identification/Differentiation	Eur. Pharma				●	●			
Urea Agar Base (Christensen)	1110		Differentiation	ISO 6579				●				

PHARMACEUTICAL INDUSTRY

Dehydrated culture media and detected bacteria

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Aerobes mesophiles	Anaerobes count	Antibiotic assay	*Enterobacteria*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella/Shigella*	*Staphylococcus aureus*	Sterility testing	Yeast and molds
Antibiotic Medium No. 1 (Seed Agar)	1520		Standard Medium	USP Eur. Pharma			●							
Antibiotic Medium No. 2 (Base Agar)	1002		Standard Medium	USP			●							
Antibiotic Medium No. 3	1534		Standard Medium	USP			●							
Antibiotic Medium No. 5 (Streptomycin Test Agar)	1524		Streptomycin Assay	USP			●							
Antibiotic Medium No. 8 (Base Agar with low pH)	1004		Tetracycline / Other antibiotics	USP			●							
Antibiotic Medium No. 11 (Neomycin Test Agar)	1528		Neomycin / Other antibiotics	USP			●							
Antibiotic Medium No. 12	1525		Amphotericin B				●							
Baird Parker Agar Base	1100	5129	Selective Isolation	Eur. Pharma								●		
Bismuth Sulfite Agar (Wilson Blair)	1011		Selective Isolation	USP							●			
Cetrimide Agar Base	1102		Selective Isolation/Identification	Eur. Pharma						●				
Dextrose Agar	1021		Total Count		●									
Lactose Broth	1206		Cultivation	Eur. Pharma							●			
Levine Agar (EMB)	1050		Isolation / Differentiation					●						
Malt Extract Broth	1245		Isolation / Enumeration										●	●
Mannitol Salt Agar (MSA)	1062		Isolation	Eur. Pharma								●		
Mio Medium	1510		Identification					●	●		●			
Mueller Hinton Agar	1058		Sensitivity Test				●							
Mueller Hinton II Agar	1055		Sensitivity Test				●							
Mueller Hinton Broth	1214		Sensitivity Testing				●							
Potato Dextrose Agar	1022		Identification/Cultivation/Enumeration											●
Potato Dextrose Broth	1261		Cultivation											●
Pseudomonas F Agar (King B)	1532		Identification	USP						●				
Pseudomonas P Agar (King A)	1531		Identification	USP						●				
Sabouraud Dextrose Agar	1024		Cultivation	Eur. Pharma										●
Sabouraud Dextrose Broth	1205		Cultivation	Eur. Pharma										●
Sabouraud Maltose Broth	1213		Cultivation										●	●
Salmonella Chromogenic Agar	1122		Isolation								●			
Selenite Cystine Broth	1220		Selective Enrichment								●			
Thioglycollate Broth	1241		Sterility Test	USP		●							●	
Thioglycollate Fluid Medium	1508		Cultivation	USP Eur. Pharma		●							●	
Thioglycollate Medium without Indicator	1516		Cultivation / Isolation	USP		●							●	
Thioglycollate USP Medium	1533		Cultivation	USP ISO 7937		●							●	
Triple Sugar Iron Agar (TSI)	1046		Identification / Differentiation	Eur. Pharma				●	●		●			
Vogel-Johnson Agar	1079		Isolation									●		
Wilkins Chalgren Medium	1503		Isolation / Cultivation			●								
Wilkins Chalgren II Medium	1568		Sensitivity Test			●								
XLD Agar (Xylose Lysine Desoxycholate)	1080		Isolation	Eur. Pharma							●			

DEHYDRATED CULTURE MEDIA

uses and related industries

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Meat and Fish Industry	Water and Beverages	Dairy Products	Bakery	Processed Foods	Beer Industry	Wastewater	Cosmetic Industry	Pharmaceutical Industry
A1 Medium	1252		Detection of Coliforms			●					●		
Acetamide Agar	1391		Confirmation of *Pseudomonas aeruginosa*			●					●		
Acetamide Broth	1155		Confirmation of *Pseudomonas aeruginosa*	UNE-EN 12780 ISO 16266		●					●		
Acetate Differential Agar	1192		Diferentiation of species of *Shigella*, *E.coli* and non-fermentable Coliforms		●		●						
Alkaline Peptone Water	1407		Enrichment of *Vibrio* species		●						●		
Anaerobic Agar	1000		Cultivation of anaerobes, specially *Clostridium*		●	●	●	●			●		
Antibiotic Medium No. 1 (Seed Agar)	1520		Standard medium used for the preparation of the seed layer in Antibiotic Assays	USP Eur. Pharma									●
Antibiotic Medium No. 2 (Base Agar)	1002		Standard medium used for the preparation of the seed layer in Antibiotic Assays	USP									●
Antibiotic Medium No. 3	1534		Standard medium for use in Antibiotic Assays	USP									●
Antibiotic Medium No. 5 (Streptomycin Test Agar)	1524		Potency assay of Streptomycin	USP									●
Antibiotic Medium No. 8 (Base Agar with Low pH)	1004		Plate assay of tetracycline and other antibiotics	USP									●
Antibiotic Medium No. 11 (Neomycin Test Agar)	1528		Potency assay of neomycin and other antibiotics	USP									●
Antibiotic Medium No. 12	1525		Amphotericin B Testing										●
Asparagine Broth	1207		Presumptive identification and enumeration (MPN) of *Pseudomonas aeruginosa*			●					●		
Azide Dextrose Broth	1422		*Enterococcus* preliminary test and selective enrichment			●					●		
Bacillus Cereus Selective Agar Base	1124	6021/ 5152	Enumeration and isolation of *Bacillus cereus*		●			●					
Baird Parker Agar Base	1100	5129	Selective isolation of coagulase-positive staphylococci	Eur. Pharma								●	●
Baird Parker Agar Base	1319	5129	Selective isolation of coagulase-positive staphylococci	ISO 6888-1	●		●	●	●				
Baird Parker Agar Base (RPF)	1319	6024	Selective isolation of coagulase-positive staphylococci	ISO 6888-2	●		●	●	●				
BCP Agar	1051		Isolation of Coliforms			●		●	●		●		
BCP Glucose Agar	1320		Differentiation and enumeration of Enterobacteriaceae	ISO 21528-2	●			●	●				
Bile Esculin Agar	1031		Isolation and presumptive identification of enterococci	ISO 10273			●						
Bile Esculin Azide Agar	1005		Selective medium for isolation and presumptive identification of *enterococci*	ISO 7899-2		●							
Bismuth Sulfite Agar (Wilson Blair)	1011		Highly selective medium for isolation of *Salmonella spp*, particularly *Salmonella typhi*	USP	●		●	●	●				●
Blood Agar Base No. 2	1328		Confirmation	ISO 7932	●								
Blood Free Campylobacter Agar Base	1129	6053	Selective medium used with supplements, for isolation of *Campylobacter jejuni*, *C.coli* and *C.laridis*		●		●						
Brain Heart Infusion Broth	1331		Confirmation of positive coagulase *Staphylococcus*	ISO 6888-1	●								
Brilliant Green Agar	1078		Highly selective medium for isolation of *Salmonella*	Eur. Pharma USP	●			●	●				
Brilliant Green Bile Agar	1010		Determination of the degree of contamination by Coliforms			●					●		
Brilliant Green Bile Broth 2%	1228		Detection of Coliforms	ISO 4831, ISO 4832		●		●	●		●		
Brilliant Green Selenite Broth	1221		Selective enrichment of *Salmonella species*		●		●	●	●				
Brilliant Green Selenite Broth II	1219		Selective enrichment of *Salmonella species*		●								
Brilliant Green Tetrathionate Bile Broth	1253		Selective enrichment of *Salmonella*	Eur. Pharma	●		●	●	●				
Bryant & Burkey Base Broth (Modified with Resarzurine)	1247		Detection of lactate fermenting clostridial species				●						
Buffered Peptone Water	1402		Diluent for the homogenization of samples	ISO 6579	●		●						
Buti Medium For Milk	1184		Detection of *Clostridium tyrobutyricum*				●						
Carbohydrates Utilization Broth	1342		Confirmation of *Listeria*	ISO 11290-2	●	●	●	●	●				
Cetrimide Agar Base	1102		Selective isolation and identification of *Pseudomonas aeruginosa*	Eur. Pharma		●					●	●	●

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Meat and Fish Industry	Water and Beverages	Dairy Products	Bakery	Processed Foods	Beer Industry	Wastewater	Cosmetic Industry	Pharmaceutical Industry
Chapman Stone Agar	1017		Selective and differential medium for isolation of staphylococci		●		●	●	●				
Chloramphenicol Agar	1301		Selective medium for isolation and enumeration of molds	ISO 7954			●						
Clostridium Perfringens Agar Base (m-CP)	1132		Enumeration and isolation of *Clostridium perfringens*			●							
DCLS Agar	1045		Selective medium for isolation of *Salmonella* and *Shigella*. It is also used to isolate *Vibrio cholerae*		●		●		●				
Desoxycholate Agar	1020		Isolation and enumeration of Coliforms				●						
Desoxycholate Citrate Agar	1067		Highly selective medium for isolation of enteric pathogens, especially *Salmonella* and *Shigella*	Eur. Pharma	●		●		●				
Desoxycholate Lactose Agar	1025		Isolation and enumeration of Coliforms		●	●	●		●		●		
Dextrose Agar	1021		Total counts of microorganisms and for general laboratory purposes		●	●	●	●	●				●
Differential Reinforced Clostridial Broth	1416		Enumeration of all clostridia by the MPN method		●		●	●					
E. Coli Coliforms Chromogenic Medium	1340		Selective medium for simultaneous detection of *E.coli* and other Coliforms		●	●	●	●	●	●	●	●	
EC Medium	1522		Differentiation and enumeration of Coliforms	ISO 7521		●					●		
EE Broth	1362		Enumeration of Enterobacteriaceae	ISO 21528-1	●		●	●	●				
Elliker Medium	1539		Cultivation of streptococci and lactobacilli				●						
Endo Agar Base	1118		Confirmation and differentiation of Coliforms		●	●	●		●		●		
Endo Les Agar Base	1137		Detection and enumeration of Coliforms in water using membrane-filter technique			●					●		
Enterobacter Sakazakii Isolation Agar Chromogenic (ESIA)	1446		Isolation of *Enterobacter Sakazakii*	ISO 22964			●						
Enterococcus Confirmatory Agar	1018		Confirmation of the presence of enterococci			●					●		
Eosin Methylene Blue Agar (EMB)	1039		Isolation and differentiation of Coliforms from other *Enterobacteria*			●						●	
ESTY Broth	1254		Selective medium for cultivation of *Streptococcus thermophilus* in yogurt				●						
ESTY Medium	1555		Selective medium for enumeration of *Streptococcus thermophilus* in yogurt				●						
EVA Broth (Ethyl Violet Azide Broth)	1230		Confirmation of enterococci and as a detector of fecal contamination			●					●		
Ewing Malonate Broth Modified	1212		Differentiation of *Enterobacteria*, especially *Salmonella arizonae*		●		●						
Fecal Coliforms Agar Base (m-FC)	1127	6023	Membrane-filter medium for detection and enumeration of fecal Coliforms			●					●		
Fecal Coliforms Broth Base	1121	6023	Detection and enumeration of fecal Coliforms through the membrane- filter technique			●					●		
Fraser Broth Base	1182	6001	Enrichment medium for detection and isolation of *Listeria*	ISO 11290-1	●		●	●	●	●			
Gelatin Lactose Medium	1526		Confirmation of *Clostridium perfringens*		●	●							
Giolitti - Cantoni Broth	1287	5208	Detection of *Staphylococcus aureus*	ISO 6888 / 5944	●		●	●	●				
Glucose Chloramphenicol Agar	1094		Selective medium for isolation and enumeration of yeast and molds				●						
Glucose Chloramphenicol Broth	1258		Selective medium for isolation and enumeration of yeast and molds				●						
Hektoen Enteric Agar	1030		Isolation and differentiation of Gram-negative enteric bacteria		●		●	●	●				
Irgasan Ticarcillin & Potassium Chlorate	1361	6051	Enrichment medium for *Yersinia enterocolitica*	ISO 10273	●		●		●				
KAA Confirmatory Agar	1027		Isolation and confirmation of enterococci				●						
KAA Presumptive Broth	1209		Presumptive detection of enterococci from foods				●						
King B Medium	1154		Identification and enumeration of *Pseudomonas aeruginosa* by membrane-filtration technique	UNE-EN 12780 ISO 16266		●					●		
Kligler Iron Agar	1364		Presumptive test of *Yersinia enterocolitica*	ISO 10273	●		●		●				
Koser Citrate Broth	1200		Differentiation of *E.coli* from *Enterobacter* on basis of citrate utilization		●	●	●		●	●	●	●	
Lactose Broth	1206		Cultivation of Coliforms and *Salmonella*	Eur. Pharma	●	●	●	●	●		●		●
Lactose Sulfite Broth Base	1009		Selective medium for detection and enumeration of *Clostridium perfringens*	Eur. Pharma	●	●							
Lauryl Sulfate Agar	1309		Selective isolation and enumeration of Coliforms			●		●	●		●		
Lauryl Sulfate Broth	1310		Detection of Coliforms (APHA)	ISO 4831			●						

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Meat and Fish Industry	Water and Beverages	Dairy Products	Bakery	Processed Foods	Beer Industry	Wastewater	Cosmetic Industry	Pharmaceutical Industry
Lauryl Sulfate Tryptose Broth Modified	1445		Selective enrichment medium for *Enterobacter sakazakii*	ISO 22964		●					●		
Legionella Cye Agar Base	1311	6022/ 6025	Selective medium for cultivation of *Legionella*	ISO 11731-2		●							
Letheen Agar Base Modified	1111		Determination of bacterial activity of quaternary ammonium compounds									●	
Letheen Broth Base Modified	1244		Microbiological analysis									●	
Levine Agar (EMB)	1050		Isolation and differentiation of *Enterobacteria*		●		●	●	●	●	●		●
Listeria Agar Base Oxford	1133	6003	Selective medium for detection of *Listeria monocytogenes*	ISO 11290-1	●	●	●	●	●				
Listeria Enrichment Broth Base Fraser	1120	6001/ 6002	Enrichment medium for detection and isolation of *Listeria*	ISO 11290-1	●	●	●	●	●				
Listeria Agar Base Palcam	1141	6004	Selective and differential medium for detection of *Listeria monocytogenes*	ISO 11290-2	●	●	●	●	●				
Listeria Chromogenic Agar Base	1345	6031/ 6040	Selective medium for detection and enumeration of *Listeria monocytogenes*	ISO 11290-2	●	●	●	●	●				
LMDA Agar	1084		Differentiation of a high variety of bacteria							●			
LPT Dilution Broth	1399		Solvent for homogenization of samples									●	
Lysine Decarboxylase Broth	1208		Identification of microorganisms, especially *enteric* bacilli, based on the decarboxylation of lysine		●	●	●	●					
Lysine Decarboxylase Broth	1176		Biochemical confirmation of *Salmonella*	ISO 6579	●		●	●	●				
Lysine Iron Agar	1044		Studies of decarboxylation of lysine for rapid differentiation of *Salmonella arizonae*		●								
M17 Agar	1318		Cultivation and enumeration of lactic streptococci				●						
Macconkey Agar	1052		Isolation and identification of *Enterobacteriaceae*	Eur. Pharma	●		●	●	●	●	●	●	
Macconkey Agar No. 2	1035		Identification of enterococci in the presence of coliforms and non lactose-fermenters			●	●				●		
Macconkey Agar without Crystal Violet	1037		Cultivation of staphylococci and enterococci and differentiation of enteric microorganisms			●							
Macconkey Broth	1210		For detection of Coliforms	Eur. Pharma		●	●				●		
Malt Extract Agar	1038		Cultivation of fungi and yeasts		●		●	●	●	●		●	
Malt Extract Broth	1245		Isolation and enumeration of yeast and molds, as well as for sterility tests		●		●	●	●	●		●	●
Mannitol Nitrate Motility Medium	1509		Rapid differentiation of Enterobacteria		●		●	●	●	●	●	●	
Mannitol Salt Agar (MSA)	1062		Isolation of pathogenic staphylococci	Eur. Pharma	●		●	●	●			●	●
m-EI Chromogenic Agar Base	1412		Detection and enumeration of *Enterococcus* through membrane filtration technique			●					●		
Minerals Modified Glutamate Broth	1365		Enumeration of Coliforms			●					●		
Mio Medium	1510		Identification of *Enterobacteriaceae*		●		●	●	●	●	●		●
Moeller KCN Broth Base	1112		Differentiation of enteric bacilli		●			●	●				
Mossel EE Broth	1202		Selective enrichment of *Enterobacteriaceae*, specially *Salmonella* and Coliforms	Eur. Pharma	●		●	●	●				
MRS Agar	1043		Cultivation of lactobacilli in general		●		●						
MRS Broth	1215		Cultivation of lactobacilli in general		●		●						
MRSV Medium	1376		A semi-solid medium for detection of motile *Salmonella* species		●		●	●	●				
MR-VP Medium	1512		Differentiation of *Escherichia- Enterobacter* group (Methyl Red and Voges-Proskauer reactions)		●	●	●	●	●	●	●	●	
Mueller Hinton Agar	1058		Sensitivity Test										●
Mueller Hinton II Agar	1055		Sensitivity Test										●
Mueller Hinton Broth	1214		Sensitivity testing to antibiotics in liquid media										●
Muller Kauffmann Broth Base with Brilliant Green & Novobiocine (MKTTN)	1173		Liquid medium for selective enrichment of *Salmonella*	ISO 6579	●		●	●	●				
Muller Kauffmann Tetrathionate Broth Base	1130		Selective enrichment of *Salmonella*		●		●	●	●				
NOVICE BREWERS BACTERIA Agar Base, Modified	1438		Selective medium used for detection of contaminating/spoilage micro-organisms							●			
NOVICE BREWERS BACTERIA Broth Base, Modified	1440		Selective medium used for detection of contaminating/spoilage micro-organisms							●			
Neutralizing Agar	1449		Neutralization of antimicrobial chemicals									●	

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Meat and Fish Industry	Water and Beverages	Dairy Products	Bakery	Processed Foods	Beer Industry	Wastewater	Cosmetic Industry	Pharmaceutical Industry
Nitrate Motility Base Medium	1565		Confirmation of *Clostridium perfringens*		●	●							
Nutrient Agar	1060		Cultivation of non-fastidious microorganisms	ISO 6579 ISO 10273	●		●	●	●				
Nutrient Agar	1156		Identification and enumeration of *Pseudomonas aeruginosa* by membrane-filtration technique	UNE-EN 12780		●					●		
Nutrient Agar with Sodium Chloride	1355		Confirmation of Enterobacteriaceae	ISO 21528-1	●		●	●	●				
OGA Medium (Oxytetracycline Glucose Agar)	1527	6018	Enumeration and selection of yeast and molds		●		●	●	●				
Orange Serum Agar	1307		Isolation and detection of different acid tolerant pathogens in citrus products			●							
Osmophilic Agar	1057		Cultivation of osmophilic yeasts				●	●	●				
Peptone Water with Lactose	1357		Confirmation of membrane results for total Coliforms	ISO 9308-1		●					●		
Peptone Sorbitol & Biliar Salts Broth (PSB Broth)	1298		Diluent	ISO 10273	●		●		●				
Potato Dextrose Agar	1022		Identification, cultivation and enumeration of yeasts and molds	Eur. Pharma	●		●	●	●				●
Potato Dextrose Broth	1261		Cultivation of yeasts and molds		●		●	●	●	●		●	●
Pseudomonas Agar Base	1356	6036	Isolation of *Pseudomonas*	ISO 13720	●								
Pseudomonas CN Agar Base	1153		Identification and enumeration of *Pseudomonas aeruginosa* by membrane-filtration technique	UNE-EN 12780 ISO 16266		●					●		
Pseudomonas F Agar (King B)	1532		Identification of *Pseudomonas aeruginosa* based on production of fluorescein	USP							●	●	●
Pseudomonas P Agar (King A)	1531		Identification of *Pseudomonas aeruginosa* based on production of pyocyanin	USP							●	●	●
Raka - Ray Agar Base	1061		Selective medium to isolate lactic-acid bacteria							●			
Rappaport Soy Broth (Vassiliadis)	1174		Liquid medium for selective enrichment of *Salmonella*	ISO 6579	●		●	●	●				
Reinforced Clostridial Medium	1007		Cultivation and enumeration of *Clostridium* and other anaerobes	Eur. Pharma	●		●	●			●		
Rogosa SL Agar	1096		Selective medium for cultivation of lactobacilli		●								
Rogosa SL Broth	1234		Selective medium for cultivation of lactobacilli		●								
Rose Bengal Agar + Chloramphenicol	1081		Cultivation and selective isolation of yeast and molds		●		●	●	●	●		●	
Rose Bengal Agar + Dichloran + Chloramphenicol (DRBC Agar)	1160		Selective medium for cultivation of yeast and molds	ISO 21527-1			●	●	●	●			
Rothe Broth (Glucose Broth with Azide)	1238		Quantitative determination of enterococci				●						
R2A Agar	1071		Total aerobe count	Eur. Pharma		●							
Sabouraud Dextrose Agar	1024		Cultivation of yeast and molds	Eur. Pharma									●
Sabouraud Dextrose Broth	1205		Cultivation of yeast and molds	Eur. Pharma									●
Sabouraud Maltose Broth	1213		Cultivation of yeast, molds and acidophilic bacteria, as well as for sterility tests									●	●
Salmonella Chromogenic Agar	1122		Isolation of *Salmonella*		●		●	●	●				●
Salmonella Shigella Agar (SS)	1064		Selective medium for isolation of *Salmonella* and *Shigella*		●								
Salmonella Shigella Agar with Sodium Desoxycholate & Calcium Chloride	1360		Selective medium for isolation and differentiation of *Yersinia enterocolitica*	ISO 10273	●		●		●				
Salmonella Shigella Modified Agar	1186		Selective medium for isolation of *Salmonella* and *Shigella*		●								
San Francisco Medium	1413		Cultivation of *Lactobacillus*		●		●						
SBF Broth	1384		Enrichment medium for *Salmonella*		●		●	●	●				
Schaedler Agar	1066		Cultivation of anaerobic microorganisms from contaminated specimens								●		
Selenite Cystine Broth	1220		Selective enrichment of *Salmonella* and some strains of *Shigella*		●		●	●	●				●
Sim Medium	1514	5205	Identification and differentiation of Enterobacteriaceae		●	●	●	●	●	●	●		
Simmons Citrate Agar	1014		Determination of citrate utilization by Enterobacteriaceae	ISO 10273		●					●		
Slanetz-Bartley Medium	1109		Detection and enumeration of intestinal *Enterococci* by membrane-filtration technique	ISO 7899-2		●							
Sodium Selenite Broth	1222		Selective isolation of *Salmonella*		●		●	●	●				
SPS Agar	1082		Isolation of *Clostridium perfringens*		●								
Standard Methods Agar (PCA)	1056		Total microbial plate count	ISO 4833	●		●						

	Cat. No.	Suppl. Cat. No.	Uses	Ref. Method	Meat and Fish Industry	Water and Beverages	Dairy Products	Bakery	Processsed Foods	Beer Industry	Wastewater	Cosmetic Industry	Pharmaceutical Industry
Standard Methods Agar with Powdered Milk	1033		Bacterial plate count of microorganisms (APHA* Formula)				●						
Staphylococcus Agar No. 110	1032		Isolation of staphylococci		●		●	●	●			●	
TBX Chromogenic Agar	1151		Selective medium for detection and enumeration of *E.coli*	ISO 16649-2	●		●	●	●				
TCBS Agar	1074		Selective isolation of *Vibrio spp*		●						●		
Tetrathionate Broth Base (Muller Kauffmann)	1114		Selective enrichment of *Salmonella* species	USP	●		●	●	●				
Thioglycollate Broth	1241		Sterility assays of biological and pharmaceutical products	USP Eur. Pharma								●	●
Thioglycollate Fluid Medium	1508		Cultivation of aerobic and anaerobic microorganisms in sterility tests	USP								●	●
Thioglycollate Medium without Indicator	1516		Cultivation and isolation of obligate and facultative aerobic, anaerobic and microaerophilic bacteria for sterility tests	USP								●	●
Thioglycollate USP Medium	1533		Cultivation of aerobic and anaerobic microorganisms in sterility tests	USP ISO 7937	●							●	●
Triple Sugar Iron Agar (TSI)	1046		Identification and differentiation of *Enterobacteria*	Eur. Pharma		●				●	●	●	●
Triple Sugar Iron Agar (TSI)	1172		Biochemical confirmation of *Salmonella*	ISO 6579	●		●	●	●				
Trypticasein Glucose Extract Agar	1041		Plate count enumeration of bacteria			●					●		
Tryptone Bile Salts Agar	1013		Detection and enumeration of *E. coli* and other Coliforms	ISO 9308-1		●					●		
Tryptone Soy Agar	1138		Detection and enumeration of *E.coli* and other Coliforms	ISO 9308-1		●					●		
Tryptone Soy Yeast Extract Agar (TSYEA)	1398		Cultivation of *Listeria spp*	ISO 11290-1	●	●	●	●	■				
Tryptone Soy Yeast Extract Broth (TSYEB)	1339		Confirmation of *Listeria monocytogenes*	ISO 11290-1	●	●	●	●	●				
Tryptophan Culture Broth	1237		Detection of *E. coli* and other Coliforms by indole production	ISO 9308-1		●					●		
Tryptose Sulfite Broth	1378		Selective medium for *Clostridium perfringens*		●	●							
TSC Agar Base	1029	6020/ 5152	Detection and enumeration of *Clostridium perfringens*	ISO 7937	●								
TSN Agar	1075		Selective isolation of *Clostridium perfringens*		●								
TTC Chapman Agar	1076	6030	Enumeration of Coliforms by membrane - filtration technique	ISO 9308-1		●					●		
Universal Beer Agar (UBA Medium)	1562		Control of industrial fermentation processes							●			
Urea Agar Base (Christensen)	1110		Differentiation of *Enterobacteria* on the basis of urease production	ISO 6579	●			●	●	●	●	●	
Urea Indol Broth	1227	5205	Identification of *Enterobacteria* on the basis of urease and indole production and the transdeamination of tryptophan (TDA)	ISO 10273	●	●	●	●	●	●	●		
Violet Red Bile Agar with Glucose (VRBG)	1092		Cultivation and enumeration of *Enterobacteria*	Eur. Pharma ISO 21528	●	●	●	●	●		●		
Violet Red Bile Agar with Lactose (VRBL)	1093		Selective medium for detection and enumeration of Coliforms	ISO 4832	●		●	●	●				
Vogel-Johnson Agar	1079		Isolation of *Staphylococcus aureus*										●
Wilkins Chalgren Medium	1503		Susceptibility testing as well as for isolation and culture of anaerobic bacteria in general								●		●
Wilkins Chalgren II Medium	1568		General development and microbial sensitivity tests of anaerobic agents										●
WL Differential Agar	1026		Control of industrial fermentation processes							●			
WL Nutrient Agar	1086		Determination of microbial flora							●			
Wort Agar	1316		Cultivation and enumeration of yeasts			●	●			●			
Wort Broth	1444		Enumeration and count of yeasts			●	●			●			
XLD Agar	1274		Solid selective medium for isolation of *Salmonella*	ISO 6579	●		●	●	●				
XLD Agar (Xylose Lysine Desoxycholate)	1080		Isolation of enteropathogenic bacteria, especially from the genus of *Shigella* and *Salmonella*	Eur. Pharma		●							●
XLT4 Agar Base	1159	6062	Isolation of pathogenic *Enterobacteria*, especially *Salmonella*		●	●	●	●	●				
Yeast Extract Agar	1049		Nutrient medium for enumeration of a wide spectrum of bacteria, yeast and molds	ISO 6222		●							
Yeast Extract Agar (for molds)	1312		Cultivation of yeast and molds				●						
Yersinia Selective Agar Base	1126	6033	Selective Medium for *Yersinia enterocolitica*	ISO 10273	●		●		●				

Take your **Notes**

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