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Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Compendium of Microbiological Criteria for Food

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1. INTRODUCTION

1.1 This document

Food Standards Australia New Zealand (FSANZ) has produced this compendium to provide best practice guidance for food regulators and the food industry. It contains basic information on microorganisms (pathogens and indicators) significant to food safety, and microbiological criteria for food safety management. Criteria are included for ready-to-eat foods, as well as several specific food commodities. General information on environmental monitoring in a food production setting is also provided.

Commodity chapters have been developed by experts from FSANZ and food regulatory agencies, in consultation with relevant food industry sectors. FSANZ will update this document periodically as further information is developed.

1.2 Through-chain food safety management

The microbiological safety of food is best ensured by implementing food hygiene controls at each stage of food handling throughout the food chain. In Australia food safety requirements are set out in Chapters 3 and 4 of the *Australia New Zealand Food Standards Code* (the Code). In New Zealand, the *Food Act 2014* and *Animal Products Act 1999* and associated regulations specify food handling controls.

Microbiological testing can be a useful tool to support through-chain control measures. It may occur at different points in the food system from primary production, through production/processing and retail. Microbiological criteria may be established to examine ingredients, in-process products, end products, or environmental samples.

1.3 Microbiological criteria

Microbiological criteria are established to support decision making about a food or process based on microbiological testing. Criteria can be developed and applied for different purposes across the food supply chain, with different consequences if the limits are not met.

Internationally, the Codex Alimentarius Commission (Codex) and the International Commission on Microbiological Specifications for Foods (ICMSF) have provided the lead on contemporary food safety management approaches and applying microbiological criteria. An important principle is that microbiological criteria are established at specified points in the food chain for a particular purpose. Generally, this is to establish the safety of the food at that stage, or to verify that the food safety control system is working as intended.

There are three main types of microbiological criteria used by food regulatory agencies: food safety criteria, process hygiene criteria and guideline criteria. These are explained below and illustrated in Figure 1.

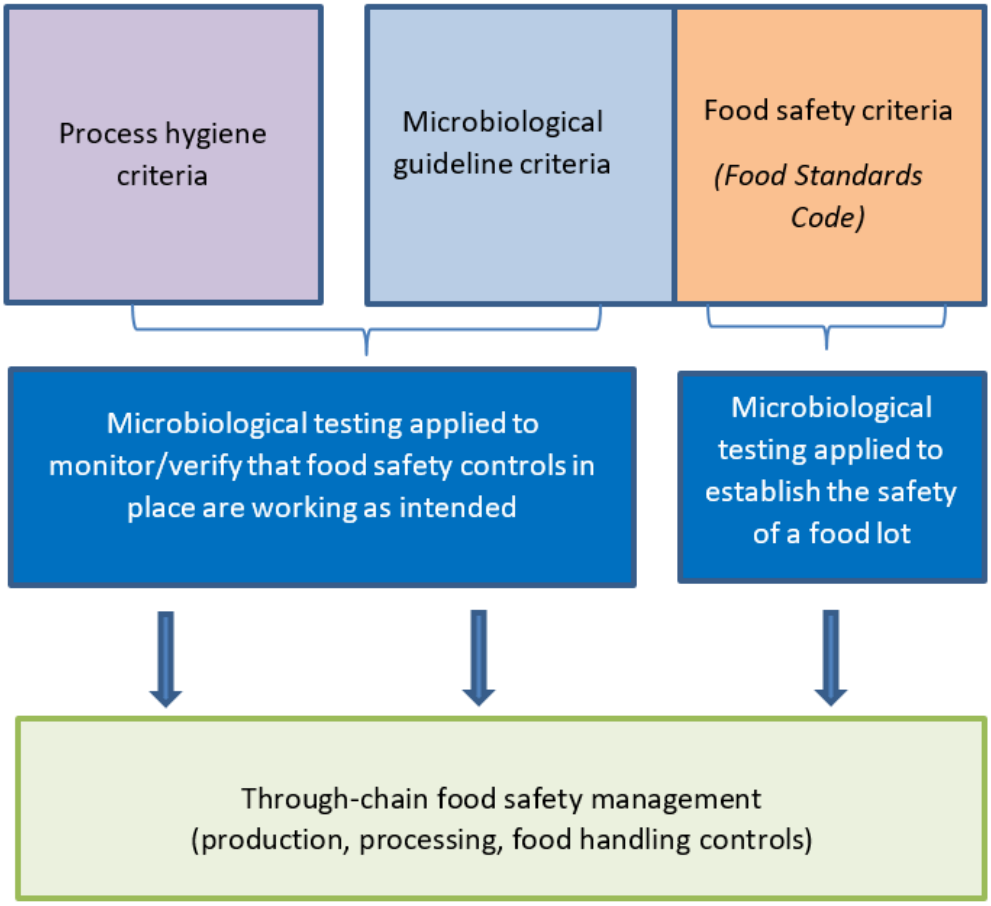
- **Food safety criteria**: end-point microbiological criteria that are applied to determine the safety of a food lot or batch. These criteria are regulatory requirements in the Code, in Standard 1.6.1 and associated Schedule 27. They can be applied by relevant authorities to sample and test the safety of a food lot available for sale (e.g. at any point following final product manufacture).

- **Process hygiene criteria**: microbiological criteria that are applied to verify hygiene measures or process controls are working as intended. These criteria are usually based on indicator organisms and are applied at a specified point (or multiple points) in the

manufacturing process. Process hygiene criteria for infant formula, dairy products and raw chicken are included in this compendium.

- **Microbiological guideline criteria** are also used by regulatory agencies to check that food for sale (i.e. final product) is safe and suitable and that a food business's food handling controls and hygienic practices are adequate. Guideline criteria indicate whether the microbiological status of a food product is within the normal/acceptable range, so can signal conformance with food safety controls. These criteria may include food safety criteria as defined above. Microbiological guideline criteria for ready-to-eat (RTE) foods, infant formula and dairy products are included in this compendium.

Figure 1. Types of microbiological criteria



Food businesses may also establish process hygiene criteria and microbiological guidelines for their operations. They may also set **microbiological specifications** for raw materials, ingredients or finished products. These are criteria applied as part of a purchase arrangement to determine acceptability of ingredients or foods, to ensure product safety or quality.

1.4 Proactive management using process hygiene criteria

Adopting a proactive approach to microbiological testing provides additional assurance that a food produced will be safe and suitable. By applying process hygiene criteria at various stages of the food production process, testing can confirm whether controls at each step have been effective. A loss of control can be detected before a food safety limit is exceeded.

This approach is especially useful in identifying contamination sources in complex, multi-stage production processes. It helps identify, at the earliest opportunity, situations that need investigation and/or corrective action. Non-conformance with a process hygiene criterion should result in actions to adjust the process, as appropriate, and ensure ongoing control of production.

A frequent schedule of testing according to process hygiene criteria can supplement more extensive testing against microbiological guideline criteria, to provide more regular and reliable assurance.

Mandatory food safety criteria for some foods have also been established in the Code, in Standard 1.6.1 and its associated Schedule 27. Where applicable, these criteria have been included in this compendium. However, applying testing programs to detect loss of control before a food safety limit is exceeded is a predictive and proactive approach that will help ensure production of safe and suitable food.

1.5 Glossary of terms

An explanation of common terms used in microbiological testing for food safety management is provided below.

Aseptic technique – A method of collecting a sample to ensure that microbiological contamination does not occur during sampling. This means ensuring that the sample does not contact anything that is not sterile.

Commercial laboratory – external testing should be done by a laboratory that holds relevant accreditation from the National Association of Testing Authorities, Australia (NATA). Such laboratories will comply with relevant international and Australian standards providing assurance of consistently reliable testing data to industry.

Composite sample – The consolidation of a number of samples from the same lot/batch to produce a single sample (or test portion) for *qualitative* (absence or presence) microbiological testing only. This can reduce the cost of testing. Compositing of samples must not reduce the sensitivity of an analytical method at very low levels of contamination. Generally the maximum number of 25g samples composited is fifteen, so the result is reported as “Not detected”, “ND” or “Absent in 375g”. Where five samples of 25g are composited and tested (e.g. as in the alternative sampling plan for *L. monocytogenes* in dairy microbiological criteria), results would be reported as “ND/125g”. Compositing fewer samples may be necessary depending on the laboratory’s capacity to handle large volumes of diluents.
Note: Compositing of samples is not appropriate for quantitative tests e.g. *E. coli*.

Corrective actions – actions taken to bring a process back under control following a deviation outside of the set limits or criteria. These should include actions that prevent a possible recurrence of the same failure.

Lot (or batch) – The Food Standards Code defines a *lot* as:

> *lot* means an amount of a food that the manufacturer or producer identifies as having been prepared, or from which foods have been packaged or otherwise separated for sale, under essentially the same conditions, for example:
>
> (a) from a particular preparation or packing unit; and
>
> (b) during a particular time ordinarily not exceeding 24 hours.

Microbiological guideline criteria – microbiological criteria used to verify that overall food handling controls and hygienic practices within the business are adequate. These criteria may include food safety criteria, which are regulatory requirements applied to determine the safety of a food and are listed in Schedule 27 of the Code.

Pre-requisite programs – essential over-arching food safety practices and conditions that are documented within a manufacturer's food safety program.

Process hygiene criteria – microbiological criteria used to verify hygiene measures or control of process at a specified point in the process.

Qualitative tests – tests that establish the *presence* or *absence* of an organism in a quantity of food, e.g. *Listeria monocytogenes* not detected in 125g.

Quantitative tests – tests that determine the *number* of organisms in a sample
e.g. 150 *E. coli*/g.

Representative samples – A sample drawn from a lot/batch should reflect as accurately as possible the properties of the entire batch from which it is taken. It may be an individual sealed or wrapped food item, or a sub-sample from a larger unit (e.g.100g from a 20-kg block of cheese). Sterilised equipment such as spatulas, triers, pipettes, bottles and bags will be needed for taking sub-samples for microbiological analysis.

Root cause analysis – investigation to identify the cause of a failure of a process to meet set limits or criteria. This analysis may identify corrective actions.

Sample integrity – The microbiological integrity of the unit, which is crucial to obtaining meaningful results. Contamination of samples during sampling will result in unnecessary and costly investigations or product wastage. Sub-samples must be aseptically collected and placed in sterile containers. All samples for microbiological analysis should be securely transported under temperature-controlled conditions (where appropriate) to the testing laboratory as soon as possible after sampling. Sample security should be considered and addressed.

Sampling plans – Testing of foods usually involve either 2-class or 3-class sampling plans.

- *2-class sampling plans* are performed when the microorganism of concern is not permitted in the food and are described using the terms n, c and m. A two-class plan is used for testing the presence or absence of organisms such as *Salmonella* spp. or *L. monocytogenes*.

  For example: n=5, c=0, m=not detected
  n = number sample units to be drawn randomly from a lot/batch
  c = maximum allowable number of sample units yielding a positive result (presence/absence testing) or exceeding the microbiological limit m. For pathogens, c is usually set to zero.
  m = microbiological limit, separates a good result from an unacceptable result.

- 3-class sampling plans are usually adopted if an acceptable level of microorganisms is permitted in a unit-volume and involves quantitative testing. These plans separate *good* results, from *marginally acceptable results*, and *unacceptable* results using the terms m and M.

  For example: n = 5, c = 1, m = 1, M = 10
  m = microbiological limit which separates good results from marginal results.

c = the maximum number of samples which may exceed the limit given for m
M = microbiological limit above which results are unacceptable or defective

The term m reflects the upper limit under good manufacturing practice, while M marks the limit beyond which the level of contamination is considered hazardous, unacceptable or indicative of an ineffective control (depending on the reason for testing).

- Alternative sampling plans may be appropriate (e.g. see Dairy chapter).

Testing method – method of analysis used for any microbiological testing; should be the most recent Australian Standard (e.g. AS 5013 series) or ISO method, or other validated method that provides equivalent sensitivity, reproducibility and reliability.

1.6 Abbreviations

B. cereus	*Bacillus cereus*
cfu	colony forming units
CPS	*Staphylococcus aureus* and other coagulase positive staphylococci
E. coli	*Escherichia coli*
g	gram
HACCP	hazard analysis critical control point
L. monocytogenes	*Listeria monocytogenes*
ml	millilitre
NATA	National Association of Testing Authorities
ND	not detected
PRP	pre-requisite program
PHC	process hygiene criteria
RTE	ready to eat
S. aureus	*Staphylococcus aureus*
SPC	standard plate count
spp.	species

2. READY-TO-EAT FOODS

Ready-to-eat (RTE) foods are intended to be consumed by the final consumer without any further process that may eliminate or reduce pathogenic microorganisms that could be present. They may be commodity based (e.g. dairy or meat products), but commonly include a combination of ingredients from more than one commodity group. 'Ready-to-eat food' is defined in Standard 3.2.2 of the Code.

The safety and suitability of RTE foods is ensured through adherence to food handling controls and good hygiene practices that prevent or minimise contamination by and growth of pathogenic microorganisms.

2.1 Purpose and scope

Food samples may be taken for a variety of surveillance and monitoring purposes. When microbiological testing of food samples is done, it is important that relevant tests and suitable limits are applied so results are interpreted correctly and consistently.

The purpose of this section is to:

- provide information on which microbiological tests apply to RTE foods, based on their characteristics and processing factors
- outline guideline microbiological criteria for assessment, including limits for interpreting results
- provide an indication of follow-up actions to be taken in response to findings.

The reference limits provided allow an assessment of a single or multiple samples. They are not intended to be sampling plans for the acceptance/ rejection of food lots, but used for evaluating food handling controls.

The guideline criteria provided are not intended to be used for food products that have food safety criteria in the Code. Criteria for specific commodity products (e.g. dairy products) are also provided in other sections of this compendium.

2.2 Microbiological tests

As RTE foods include a wide range of products, the decision on which microbiological test to apply will depend on:

- the type of ingredients used
- whether ingredients are cooked or raw
- the cooking or other processing involved in manufacture
- the level of handling after cooking or processing
- whether the food requires temperature control for safety (i.e. whether food characteristics, such as pH and water activity, allow pathogens to grow)
- presence and type of packaging
- shelf life.

Appendix 1 and 2 provide information on pathogens and indicator microorganisms/tests significant to food safety. A summary of bacterial pathogen–food associations for microorganisms routinely tested and applicable to RTE foods is provided below.

Laboratory methods are not specified in this section. The method used will depend on the reason for testing and factors such as speed, sensitivity, whether identification or quantification is required, as well as cost. For regulatory testing against food safety criteria in

Schedule 27 of the Code, Standard 1.6.1 specifies reference methods to be used. For other testing, validated methods should be used.

2.3 Bacterial pathogen–food associations

The main bacterial pathogen–food associations for ready-to-eat foods are listed below.

Pathogen	Associated foods	Why
Bacillus cereus	Cooked foods such as: • rice dishes including sushi • potato and pasta dishes • meat, vegetable and fish dishes (stews, curries etc.)	Spores are widespread in the environment and may be present on raw ingredients. The spores survive and are activated by cooking. When food is then cooled too slowly or displayed out of temperature control for extended periods, warm conditions allow for vegetative cells to grow to high levels and produce toxins.
Campylobacter spp.	Main food vehicles: • undercooked/improperly handled poultry • raw meat • unpasteurised milk • contaminated water	*Campylobacter* spp. can colonise the intestinal tract of food-producing animals, such as chickens, cattle, sheep and pigs. Inadequate processing (e.g. undercooked poultry, unpasteurised milk) and cross contamination of RTE foods or food contact surfaces with raw meat and poultry can result in sufficient numbers being present in food to cause illness.
Clostridium botulinum	Main foods: • vacuum-packed foods, including sous vide foods • home-canned and -bottled foods • fermented, salted and smoked meat and seafood • honey (infant botulism)	Spores are widespread in the environment and may be present on raw ingredients. The spores survive and are activated by cooking. In anaerobic conditions, such as in canned food, vegetative cells can grow to high levels and produce toxins, even at low temperatures.
Clostridium perfringens	Cooked foods such as: • meats, particularly rolled and large joints • meat containing products such as stews, gravies, curries and pies • vegetable dishes (curries, soups etc.)	Spores are widespread in the environment and are a part of normal intestinal flora of animals. The spores survive and are activated by cooking. Slow cooling/reheating, particularly of large volumes of food, provides warm, anaerobic conditions that allow for vegetative cells to grow to high levels that cause illness when ingested.
Listeria monocytogenes	RTE foods that can support the growth of *L. monocytogenes* and have an extended refrigerated shelf life. Foods that have been associated with outbreaks include soft cheeses, delicatessen meats, cooked chicken, smoked seafood, salads and rockmelon	*L. monocytogenes* is widespread in the environment and able to persist in food processing environments. RTE foods can become contaminated post processing through contamination from food contact surfaces. *L. monocytogenes* is able to grow at refrigeration temperatures and can reach high levels in food that supports its growth.

Pathogen	Associated foods	Why
Salmonella spp.	A wide range of foods have been implicated in outbreaks of foodborne salmonellosis: • animal products such as eggs (particularly raw or lightly cooked egg dishes), poultry, raw meat, milk and dairy products • fresh produce (such as leafy greens, seed sprouts, melons) • low moisture foods such as spices, peanut butter, chocolate and flour	*Salmonella* is widely dispersed in the environment. A primary reservoir is the intestinal tracts of vertebrates, including livestock, wildlife, domestic pets, and humans. Contaminated raw foods that are eaten without further processing (such as cooking), cross contamination during food handling and poor hygiene and temperature control practices are factors contributing to foodborne salmonellosis.
Shiga toxin-producing *Escherichia coli* (STEC)	Foods include: • inadequately cooked ground beef (hamburger patties) • uncooked fermented comminuted meat (e.g. salami) • raw or inadequately pasteurised dairy products (milk and cheese) • fresh produce such as leafy greens and sprouted seeds	Ruminants, in particular cattle and sheep, are the major animal reservoir of STEC. Infected animals shed the bacteria in their faeces, resulting in contamination of the environment. Primary products (such as meat, milk and fresh produce) can be either contaminated directly by faecal material or indirectly via contaminated water or soil. STEC infection is associated with contaminated foods that are eaten without further processing or have been inadequately processed.
Staphylococcus aureus and other coagulase-positive staphylococci (CPS)	A variety of foods, particularly those high in protein and requiring extensive handling during preparation. These can include: • meat and meat products • poultry and egg products • milk and dairy products • cream or custard filled bakery products • sandwich fillings	Food handlers are the main source of food contamination via direct contact (staphylococci can normally be present in people's nasal passages, throat and skin). Contamination of food can occur via hands or respiratory secretions. Time and temperature abuse of contaminated food can result in growth of *S. aureus* and production of enterotoxin in the food.
Vibrio parahaemolyticus	Foods predominantly associated with foodborne illness caused by *V. parahaemolyticus* are fish, shellfish and crustaceans (particularly raw molluscs and crustacea)	*V. parahaemolyticus* occurs in coastal and estuarine waters and is a natural contaminant of seafood. Initial levels will depend on environmental factors at harvest. Illness is associated with eating raw or lightly cooked seafood, or cooked seafood that has been cross contaminated. Inadequate refrigeration of seafood contaminated with *V. parahaemolyticus* allows growth to levels that cause illness.

2.4 Interpretation of results

The tables below provide guidance on interpreting results for the microbiological examination of RTE foods for pathogenic microorganisms (Table 2.1) and for indicator microorganisms (Table 2.2 and 2.3). The limits apply to foods sampled in the retail chain (i.e. food for sale at retail, food service wholesale and distribution) up to and including end of shelf life.

There are four categories of microbiological assessment defined based on the detection or level of microorganism found:

- *Satisfactory*: results are within expected microbiological levels (lower range) and present no food safety concern. No action required.

- *Marginal*: results are within expected microbiological levels but are at the upper range. Some action may be required to ensure food handling controls continue to be effective.

- *Unsatisfactory*: results are outside expected microbiological levels and indicate poor food handling practices. Further actions are required to re-establish effective food handling controls.

- *Potentially hazardous***:** results exceed expected microbiological levels to a level that presents an immediate food safety concern. Further action is required to:
    - prevent affected product still available from being distributed or sold
    - determine the likely source/cause of the problem and ensure corrective actions are implemented.

Interpretation of results should also be based on knowledge of the food product and the production process. Care must be taken when interpreting results obtained in the absence of this information.

Standard plate count (SPC)

SPC (also termed aerobic colony count, total viable count or aerobic mesophilic count) provides a general assessment of quality. The reference Australian Standard (AS 5013 series) and International Organization for Standardization (ISO) methods are described as horizontal methods for enumeration of microorganisms, providing a colony count on a solid medium after aerobic incubation at 30°C.

Interpreting results for SPC (see Table 2.3) should take into account the processing and handling the food has received, the type of packaging and the stage of shelf life:

- Processing and handling – the microbial level initially present will depend on the type and duration of processing. For example, heat processes such as cooking will result in low counts ($<10^3$ cfu/g (colony forming units per gram)), canned products should be commercially sterile, and raw RTE foods will have much higher counts due to the natural flora present. Handling after processing such as slicing, portioning, packaging, etc. may increase the microbial load, noting this should be minimised by good hygienic practices.
- Packaging – the type of packaging can influence the rate of microbial growth. For example, vacuum packaging or modified atmosphere packaging will inhibit the growth of aerobic organisms.
- Shelf life – foods sampled towards the end of shelf life will have a higher count than at the point of production. It would be expected that this may be at the higher end of the ‘marginal’ range.

Table 2.1 Interpreting results for pathogen testing in RTE food

Hazard	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
Bacillus cereus and other pathogenic *Bacillus* spp.	$>10^5$	Potentially hazardous	Inadequate time and temperature control during cooling and subsequent storage allowing spores to germinate and multiply. The use of poor quality highly contaminated raw ingredients, such as plant-based powders and spices, may also be a contributing factor. Inadequate acidification of foods using pH to control growth (e.g. acidified rice for sushi).	• Product disposition action to assess safety and determine if disposal or product recall is needed. Reprocessing of product is not an option due to potential for toxin formation. • Investigate and review temperature and time profiles used for the cooling and storage of cooked foods. • Identify high-risk raw ingredients and consider limits for *B. cereus.* • Investigate pH and acidification process (as applicable).
	10^3 – $\leq 10^5$	Unsatisfactory	As above.	• Investigate and review temperature and time profiles used for the cooling and storage of cooked foods. • Identify high-risk raw ingredients and consider limits for *B. cereus.*
	10^2 – $<10^3$	Marginal	Process controls not fully achieved or possible raw material contamination.	• Proactive investigation to ensure adequate temperature and time profiles used for cooling and storage of cooked foods are being used. • Assess quality of high-risk raw ingredients.
	$<10^2$	Satisfactory		

Campylobacter spp.	Detected in 25g	Potentially hazardous	Inadequate processing of raw products (especially poultry and raw milk) or cross contamination of raw materials and prepared foods. The use of inadequately treated water can also be a factor.	• Product disposition action to assess safety and determine if disposal or product recall is needed.
				• An investigation should assess:
				  ○ the adequacy of processing used (e.g. adequate cooking, pasteurisation)
				  ○ the adequacy of measures implemented to prevent cross contamination
				  ○ the possibility of untreated water being used.

	Not detected in 25g	Satisfactory		Ensure sample has not been frozen, as results may not be accurate (*Campylobacter* levels are reduced by freezing).

Hazard	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
Clostridium perfringens	$>10^5$	Potentially hazardous	Inadequate time and temperature control during cooling, storage, processing or reheating. Slow or inadequate cooling, reheating or cooking of large production volumes a possible factor.	• Product disposition action to assess safety and determine if disposal or product recall is needed. Reprocessing of product is not an option due to potential for toxin formation. • Investigate and review temperature and time profiles used for cooling and storage of cooked foods (i.e. times taken to reach required internal temperatures). • Assess capacity of business and equipment used to effectively process the volume of food handled.
	10^3 – $\leq 10^5$	Unsatisfactory	As above.	• Investigate and review temperature and time profiles used for cooking, cooling, storage and reheating of cooked foods (i.e. times taken to reach required internal temperatures). • Assess capacity of business and equipment used to effectively process the volume of food handled.
	10^2 – $<10^3$	Marginal	Process controls not fully achieved.	• Proactive investigation to ensure adequate temperature and time profiles used for cooling, processing, reheating and storage of cooked foods are being implemented.
	$<10^2$	Satisfactory		

Listeria monocytogenes
 	
1. RTE food in which growth of *L. monocytogenes* can occur*
	Detected in 25g	Potentially hazardous	Post-processing or post-harvest contamination or inadequate process control.
			
			Higher levels in product in the marketplace may be due to poor temperature control during storage and /or distribution or inappropriate length of shelf life.
				• Product disposition action to assess safety and determine if disposal or product recall is needed. Vulnerability of likely consumers to be considered.
				• An investigation should assess:
				  ○ the raw materials used
				  ○ adequacy of cleaning and sanitising of premises and equipment, particularly preferred harbourage sites
				  ○ adequacy of premises construction and maintenance
				  ○ the effectiveness of processing controls
				  ○ the adequacy of process flow.
				• Increased sampling, including environmental sampling.

	Not detected in 25g	Satisfactory		

*Schedule 27 of the Code specifies microbiological criteria for RTE food based on whether growth of *L. monocytogenes* can occur or not occur.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
Listeria monocytogenes 2. RTE food in which growth of *L. monocytogenes* will not occur*	>10^2	Potentially hazardous	Post-processing or post-harvest contamination or inadequate process control.	• Product disposition action to assess safety and determine if disposal or product recall is needed. Vulnerability of likely consumers should be considered. • An investigation should be done, as above.
	Detected but ≤10^2	Satisfactory if a listericidal process has not been applied. Marginal if a listericidal process has been applied.	Indicates better process control required.	• While regulatory limits are met, the presence of *L. monocytogenes* should be investigated if the food has received a listericidal process. For foods that have not, ongoing trend analysis should be used to monitor levels. • Product disposition action may be needed to assess safety and determine if disposal or recall is required. Vulnerability of likely consumers should be considered.
	Absent in 25g	Satisfactory		

Salmonella spp.	Detected in 25g	Potentially hazardous	Inadequate processing of raw products, cross contamination or contaminated raw materials. Poor time and temperature control is a contributing factor for multiplication.	• Product disposition action to assess safety and determine if disposal or product recall is needed.
				• An investigation should assess:
				  ○ raw material suitability
				  ○ the adequacy of processing used (e.g. adequate cooking, pH, water activity)
				  ○ the adequacy of measures implemented to prevent cross contamination
				  ○ the effectiveness of cleaning and sanitising equipment (e.g. blenders, vitamisers, other processing equipment)
				  ○ the adequacy of time and temperature controls used.
				• Health and hygiene practices may also need investigation if an infected food handler is suspected.
				• Confirmation of identity, serotyping, phage typing where cases of foodborne illness suspected.

	Not detected in 25g	Satisfactory		

*****Schedule 27 of the Code specifies microbiological criteria for RTE food based on whether growth of *L. monocytogenes* can occur or not occur.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
Shiga toxin-producing *Escherichia coli* (STEC)	Detected in 25g	Potentially hazardous	Inadequate processing of raw products or cross contamination of raw materials and prepared foods. Poor time and temperature control is a contributing factor for multiplication.	• Product disposition action to assess safety and determine if disposal or product recall is needed. • An investigation should assess: ○ raw material suitability ○ the adequacy of processing used (e.g. adequate cooking, pH, water activity) ○ the adequacy of measures used to prevent cross contamination ○ the adequacy of time and temperature controls used. • Additional sampling of foods and environmental samples may be needed. • Confirmation of toxigenic strains and serotyping where cases of foodborne illness are suspected.
	Not detected in 25g	Satisfactory		
Staphylococcus aureus and other coagulase-positive staphylococci	$>10^4$	Potentially hazardous	Inadequate temperature control and poor hygienic practices.	• Product disposition action to assess safety and determine if disposal or product recall is needed. Reprocessing of product is not an option due to potential for toxin formation. • Food handling practices should be investigated to: ○ ensure food handlers are taking all practicable measures to prevent unnecessary contact with RTE food ○ ensure good levels of personal hygiene ○ review temperature and time controls. • Testing for enterotoxin should be considered where cases of foodborne illness are suspected.
	10^3 – $\leq 10^4$	Unsatisfactory	As above.	• Food handling practices should be investigated as above. • The level of *S. aureus* determined at the time of analysis may not be the highest level that occurred in the food. If cases of foodborne illness are suspected, testing for enterotoxin should be considered.
	10^2 – $<10^3$	Marginal	Hygiene and handling controls not fully achieved.	• Proactive investigation to ensure hygiene practices and temperature controls are effectively implemented.
	$<10^2$	Satisfactory		

Hazard	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
Vibrio parahaemolyticus	$>10^4$	Potentially hazardous	Poor temperature control (rapid chilling and storage at < 5°C), inadequate processing, cross contamination or high contamination levels in harvested seafood.	• Product disposition action to assess safety and determine if disposal or product recall is needed. May need confirmation to determine whether the genetic markers of virulence are present and the *V. parahaemolyticus* are able to cause disease. • An investigation should assess: ○ the source of raw product and potential for high levels of contamination (e.g. harvest water temperature and water salinity) ○ the adequacy of the time and temperature controls (chilling and storage) implemented post- harvest ○ the adequacy of the processing used (e.g. adequate cooking) ○ likelihood of cross contamination • Confirmation of identity and typing may be required where cases of foodborne illness are suspected.
	$10^2 – 10^4$	Unsatisfactory	As above.	• An investigation should be done, as above.
	$<3 – 10^2$	Marginal	Indication that temperature control or food handling controls are not fully achieved. It may be expected that naturally contaminated raw seafood may have low levels present (<100 cfu/g).	• Proactive investigation to ensure temperature and food handling controls are effectively implemented.
	<3	Satisfactory		

RTE = ready-to-eat, cfu/g = colony forming units per gram.

Table 2.1 does not include an exhaustive list of pathogens and for some foods/circumstances, testing of other microorganisms may be appropriate. The microbiological testing applied should be appropriate to the type of food being examined and the handling it has received.

Table 2.2 Interpreting test results for indicator organisms in RTE foods

Indicator	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
Enterobacteriaceae***** (includes coliforms)	$>10^4$	Unsatisfactory	For processed foods indicates that contamination has occurred post processing (cross contamination from food contact surfaces, raw products or food handlers) or there has been inadequate processing. Poor temperature time control may also be a contributing factor.	• Review: ○ processing controls used (such as cooking temperatures) ○ cleaning and sanitising practices for premises and equipment ○ food handler hygiene ○ time and temperature control. Additional food or environmental samples may be required for investigation.
	10^2 –10^4	Marginal	Some cross contamination or inadequate processing indicated.	Proactive investigation to ensure processing and hygiene controls are being implemented. Results may need to be compared with other food samples from the production environment for interpretation.
	$<10^2$	Satisfactory		
*Escherichia coli****** *(E. coli)*	$>10^2$	Unsatisfactory	For raw and processed foods indicates potential for there to have been contamination of faecal origin from poor hygienic practices (cross contamination from food contact surfaces, raw foods or food handlers) or there has been inadequate processing. For RTE foods that have not been processed (e.g. fresh produce), contamination from the primary production environment should be considered.	• Review: ○ processing controls used (such as cooking temperatures) ○ cleaning and sanitising practices for premises and equipment ○ food handler hygiene ○ time and temperature control ○ primary production controls (e.g. harvest practices, water quality, fertilizers, other inputs as appropriate). Additional food or environmental samples may be required for investigation and testing for enteric pathogens considered if appropriate.

RTE = ready-to-eat, cfu/g = colony forming units per gram.
*Process hygiene criteria and associated actions for Enterobacteriaceae and *E. coli* in specific food products are provided in commodity chapters.

Indicator	Result (cfu/g)	Interpretation	Likely cause	Recommended actions
	3 – <10^2	Marginal	While low levels may occasionally be found in RTE food, widespread detection in several foods or areas of the food production environment suggests poor hygienic practices.	Proactive investigation to ensure processing and hygiene controls are being implemented.
	<3	Satisfactory		

Listeria spp. (other than *L. monocytogenes*)	>10^2	Unsatisfactory	Detection of *Listeria* spp. at this level signifies that conditions may also be favourable for *L. monocytogenes* to be present. This may be due to poor food handling controls or cross contamination.
			
			Higher levels may also suggest poor temperature control or inappropriate length of shelf life.
				• Investigate:
				  ○ the raw materials used
				  ○ adequacy of cleaning and sanitising of premises and equipment
				  ○ adequacy of construction and maintenance of premises
				  ○ the effectiveness of processing controls.
				• Additional sampling, including environmental sampling should be considered (including specific testing for *L. monocytogenes*).

	≤10^2	Marginal	Indicates that food handling controls or cross contamination may become a problem.	Proactive investigation to ensure production, processing and hygiene controls are being implemented as intended. Consider additional sampling of the environment and food products.
	Not detected in 25g	Satisfactory		

RTE = ready-to-eat, cfu/g = colony forming units per gram

Table 2.3 Interpreting results for standard plate counts in RTE foods

Food category		Examples	Result (cfu/g)		
			Satisfactory	Marginal	Unsatisfactory
Category 1	Applies to foods fully cooked for immediate sale or consumption.	• Hot takeaway food such as pizza, fish and chips, etc. (a la carte)	$< 10^3$	$10^3 – <10^5$	$\geq 10^5$
Category 2a	Applies to foods in which all components of the foods have been cooked but there is minimal handling or storage before sale or consumption.	• Whole cooked bakery products such as pies, sausage rolls, quiches • Whole cooked chicken	$< 10^4$	$10^4 – <10^6$	$\geq 10^6$
Category 2b	Applies to foods in which all components of the foods have been cooked but there is minimal handling and the food is packaged for extended refrigerated shelf life.	• Packaged cook/chill meals (e.g. curries, pastas, soups) • Vacuum-packed, MAP meals or foods (e.g. packaged sliced meats)	$<10^4$	$10^4 – <10^7$	$\geq 10^7$
Category 3	Applies to foods in which all components of the foods have been cooked and there is some handling and/or refrigerated storage before sale or consumption.	• Fully cooked bakery products (pies, quiches, cooked deserts etc.) that are chilled/portioned/further handled • Unpackaged sliced meats • Cooked shellfish (molluscs, crustaceans)	$<10^5$	$10^5 – <10^7$	$\geq 10^7$
Category 4	Applies to foods that contain some components that have not been cooked.	• Dips such as hummus, tzatziki etc. • Bakery products containing fresh cream or uncooked fillings (e.g. cold set custard) • Sandwiches* • Sushi rolls	$<10^6$	$10^6 – <10^7$	$\geq 10^7$
Category 5	Foods in Category 5 either have an inherently high plate count because of the normal microbial flora present or as a result of the processing received. Includes fermented, preserved and dried food products and fresh fruit and vegetables.	• Fermented foods including fermented and cured meats, fermented vegetables (e.g. sauerkraut, olives), ripened cheeses, yoghurts, cultured butter, etc. • Preserved foods (pickled, marinated or salted fish or vegetables) • Dried foods (fruits, nuts, seeds, herbs, spices, dried fish/meat) • Whole fresh fruits and vegetables and foods containing these e.g. salads, sandwiches containing salad or vegetable ingredients	N/A	N/A	N/A

RTE = ready-to-eat, cfu/g = colony forming units per gram. *For sandwiches that contain salad or vegetable ingredients, higher counts may be attributed to the microbial flora associated with those ingredients.

3. POWDERED INFANT FORMULA PRODUCTS

Safe production of powdered infant formula products[1] depends on maintaining a high level of hygiene control to prevent entry and establishment of pathogens such as *Salmonella* and *Cronobacter* [2] spp. in processing areas. Guidance on the hygienic manufacture of powdered infant formulae and on the subsequent hygienic preparation, handling and use of reconstituted formula products is provided in the Codex *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children* (CAC/RCP 66 – 2008).

3.1 Microbiological criteria

Information on process control and microbiological criteria for powdered infant formula and powdered follow-on formula are provided in the Tables 3.1 and 3.2 below.

Food safety criteria

Standard 1.6.1 of the Food Standards Code specifies microbiological food safety criteria for *Salmonella* and *Cronobacter* spp. in powdered infant formula and powdered follow-on formula.

Non-regulatory criteria

To meet the criteria specified for *Salmonella* and *Cronobacter* spp., infant formula manufacturers should use microbiological sampling and testing as part of monitoring and verification of their food safety control system. This may include testing ingredients, the processing environment, in-process samples and the final product.

Codex's code of practice recommends that manufacturers take steps to ensure the microbiological quality of dry-mix ingredients meets the requirements for finished products.

Testing for Enterobacteriaceae/coliforms and standard plate counts (SPC) is useful to verify that the hygiene measures in place are working as intended. This provides assurance that the potential for pathogens to be in the processing environment and to cross-contaminate infant formula products is being controlled.

SPC provides a useful indication of the hygienic status of wet processing steps. A trend in counts above the recommended limits may indicate a build-up of bacteria in equipment such as evaporators, or contamination due to leaks in plate-heat exchangers (Codex, 2008). These limits shouldn't be applied to powdered infant formula products that contain lactic acid-producing microorganisms.

Testing for *Bacillus cereus* is recommended because this organism can survive pasteurisation and drying processes and has the capacity to grow when infant formula powders are reconstituted. Monitoring is also important because seasonal conditions can cause spikes in *B. cereus* levels in powders.

The reference methods for microbiological testing should be the most recent Australian Standard (AS 5013 series) or ISO methods, or other validated methods that provide equivalent sensitivity, reproducibility and reliability.

[1] Infant formula products is defined in Standard 1.1.2 of the Code
[2] Referred to as *Enterobacter sakazakii* prior to 2008.

Table 3.1 Process control for the production of powdered infant formula and follow-on formula

Stage of process		Pre-operational	Pasteurisation	Evaporation and spray drying	Dry blending	Packing (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation Maintenance of dry conditions	Pasteurisation	Time/temperature PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)
How do we know if it was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records	• Pre-operational checks acceptable • Production records show time temperature • PRP verification records	• Pre-operational checks • PRP verification records • Post-pasteurisation inputs – controlled by raw material/approved supplier	• Pre-operational checks acceptable • Production records • PRP verification records
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC SPC n= 5 c=2 m=500/g M= 5000/g AND Enterobacteriaceae* n=10 c=2 m=0/10g	In-process samples meet PHC SPC n= 5 c=2 m=500/g M= 5000/g AND Enterobacteriaceae n=10 c=2 m=0/10g	**Product meets microbiological guideline criteria**

PHC = process hygiene criteria; PRP = pre-requisite program; SPC = standard plate count. n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded. For manufacturers that purchase dry ingredients only, then blend and pack product, the pasteurisation and drying steps will not be applicable.

* Codex proposed a 2-class sampling plan for Enterobacteriaceae given low levels that occur when stringent hygiene conditions are maintained. This criterion assumes that:

- the product is sufficiently homogenous so that high level contaminations will fail (more than two samples would exceed 'm')
- in practice, positives would not normally be found if strict hygiene measures are in place. If occasional positives are found, the manufacturer would take appropriate actions. See Codex CAC/RCP 66 – 2008.

Table 3.2 Microbiological guideline criteria for powdered infant formula products

Product types	Test	Sampling plan		Alternative sampling plan for small batches	Frequency
Powdered infant formula products	*Salmonella* spp./25g	n = 60 c = 0 not detected in 25g	OR	4 composites of 15 samples (limit: ND/1500g)	Every 10 batches
	B. cereus/g	n = 5 c = 1 m = 100 M = 1000		1 sample (limit: 100/g)	Every 10 batches
	Cronobacter spp./10g	n = 30 c = 0 not detected in 10g		2 composites of 15 samples (limit: ND/300g)	Every 10 batches
	Coliforms/*g OR* Enterobacteriaceae/*g**	n = 5 c = 2 m = <3 M = 10 n = 10 c = 2 m = 0/10g		1 sample (limit: 3/g) 1 sample (limit: 0/10g)	Every 10 batches
	SPC/g **	n = 5 c = 2 m = 1,000 M = 10,000		1 sample (limit: 1,000/g)	Every 10 batches
Follow-on formula	*Salmonella* spp./25g	n = 60 c = 0 not detected in 25g		4 composites of 15 samples (limit: ND/1500g)	Every 10 batches
	B. cereus/g	n = 5 c = 1 m = 100 M = 1000		1 sample (limit: 100/g)	Every 10 batches
	Coliforms/*g OR* Enterobacteriaceae/*g*	n = 5 c = 2 m = <3 M = 10 n = 10 c = 2 m = 0/10g		1 sample (limit: 3/g) 1 sample (limit: 0/10g)	Every 10 batches
	SPC/g **	n = 5 c = 2 m = 1,000 M = 10,000		1 sample (limit: 1,000/g)	Every 10 batches

Regulatory requirements are highlighted in orange; ND = not detected; SPC = standard plate count.
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded
* Codex proposed a 2-class sampling plan for Enterobacteriaceae given low levels that occur when stringent hygiene conditions are maintained. This criterion assumes that:

- the product is sufficiently homogenous so that high level contaminations will fail (more than two samples would exceed 'm')
- in practice, positives would not normally be found if strict hygiene measures are in place. If occasional positives are found, the manufacturer would take appropriate actions. See Codex CAC/RCP 66 – 2008.

** Not applicable for infant formula products with lactic acid cultures.

3.2 Corrective actions

Failure to consistently meet the above criteria may indicate a trend toward potential loss of process control. Appropriate actions include:

- evaluation of product safety through increased sampling of final product for *Cronobacter* and *Salmonella* before release of the product
- evaluation of environmental and process hygiene controls—before production is resumed—to confirm they are suitable and are able to maintain hygiene control continuously.

3.3 References

Codex (2008) *Code of hygienic practice for powdered infant formulae for infants and young children* (CAC/RCP 66 – 2008), http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

FAO/WHO (2004) *Enterobacter sakazakii* and other microorganisms in powdered infant formula: Meeting Report, Microbiological Risk Assessment Series No. 6 http://www.fao.org/3/a-y5502e.pdf

FAO/WHO (2006) *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting Report, Microbiological Risk Assessment Series No. 10, http://www.fao.org/3/a-a0707e.pdf

ICMSF (International Commission on Microbiological Specifications for Foods) (2011) *Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance*. Springer, New York.

4. MEAT PRODUCTS

4.1 Raw chicken meat

The main microbiological hazards associated with raw poultry meat are contamination with *Salmonella* and *Campylobacter* (FSANZ, 2005; FSANZ 2010; Walker et al, 2019). An effective food safety management system includes control points throughout production and processing to control these hazards.

Microbiological testing is one indicator of effective process control in production areas and during processing of poultry meat. It should not be used as a sole measure of compliance or in isolation from other measures; rather as an indicator of an effective food safety control system operating within a business.

Government regulatory bodies may also utilise information collected from verification points by the business (including microbiological testing of carcases) to support assessments of processing establishments. Information collected by businesses may assist regulators to verify the overall performance of the business's food safety system.

These microbiological targets should be used within the context of through-chain controls to[3]:

- support and to verify effective application of process controls
- provide feedback to food business operators on microbiological levels which should be achieved when applying best practices
- assist in identifying situations (products and processes) requiring investigative action and/or control action.

Process hygiene criteria

Campylobacter

A microbiological target for *Campylobacter* of <10,000 cfu per whole chicken carcase[4] at the end of processing (after final chill and just prior to dispatch) assists in verifying that the whole process is under control.

If processors meet the designated target this verifies that their process is maintaining suitable control. Corrective actions to be taken when the criteria are not met should include review of process controls, including:

- for birds prior to entering the slaughter facility
- following evisceration and prior to birds entering the washing process
- for the carcase decontamination process
- for chilling of poultry meat carcases.

Further detail is provided in Table 4.1 below.

Noting that 5000 *Campylobacter* organisms per carcase could be considered sufficient to cause a risk of cross contamination to ready-to-eat foods in the kitchen environment, it is recommended that a technique to count down to a lower level is used. This can be readily

[3] This guidance should be read in conjunction with the Australian Standard AS 4465-2006 *Construction of Premises and Hygienic Production of Poultry Meat for Human Consumption* and Appendix A to provide additional and specific guidance with regard to *Salmonella* and *Campylobacter* across the industry.

[4] Please note that based on available data these targets are only for chicken meat at this stage.

achieved in the processing plant and can demonstrate good process control (see Methods of analysis).

Salmonella

A microbiological target for *Salmonella* has not been proposed; however, if present, serotypes should be identified. Specific *Salmonella* serotypes of public health or industry significance (i.e. *Salmonella* Typhimurium or *Salmonella* Enteriditis) must be notified immediately where required to the relevant authority to ensure appropriate controls are applied. The controls through the process which reduce counts of *Campylobacter* are the same which can control *Salmonella*.

Identity of *Salmonella* types on carcases is important as an assessment of control measures throughout the food chain and so requires an examination of risk and control of hazards as stated in the Primary Production and Processing Standard for Poultry Meat (Standard 4.2.2). There are many ways that a company can demonstrate risk assessment and may include targeted microbiological surveillance and may necessitate investigation and corrective actions further back up the food chain, such as, but not limited to:

- breeder farms
- hatcheries
- feed production
- transport
- broiler farms
- livehaul equipment and transport
- processing plant equipment cleaning and maintenance.

Verification points

The performance of through-chain system controls within poultry processing cannot be fully verified through the isolated application of microbiological end-point testing. In order to do so, information should be gathered that relates to processing performance at designated verification points through the entire chain, including live bird receipt, evisceration, carcase decontamination and chilling. Achieving performance targets at each of these verification points, may provide evidence to demonstrate effective operation of the process controls in place. Ideally, information collected should form part of the periodic validation for each business's food safety management system as part of their compliance arrangement in consultation with the regulatory authority.

In order to effectively monitor verification points (Attachment 1), businesses should assess their individual circumstances and develop an appropriate monitoring regime including sampling size and monitoring frequency that accounts for a number of factors within the business. These factors may include (but not be limited to) the size of the business, the quantum of productive output, how the product is presented to the end user and the risks associated with the scale of activities being conducted. This should be done in consultation with the enforcement agency. Additionally, all processors should maintain records to demonstrate process control (including details of appropriate corrective actions if out of specification).

Regular monitoring of all verification points enables businesses to make timely assessments of food safety system performance, which may be then further verified by an associated microbiological test. A microbiological testing programme needs to be developed by each processor to regularly demonstrate that process control is achieving suitable management of microbiological contamination. This should be completed at a frequency which builds confidence and demonstrates that the management of process control is sufficient to minimise the risks. As noted above, microbiological testing is not a sole determinant of an

effective food safety management system. Therefore, in developing a monitoring plan, each food business should consider what appropriate corrective actions in each instance may be, and when they are to be taken if monitoring activities indicate that applied process controls may not be operating effectively.

Method of analysis

Three methods of increasing sensitivity are provided. At a minimum, the method to be used should be the most recent Australian Standard (AS 5013 series) or ISO method, or other validated method that provides equivalent sensitivity, reproducibility and reliability.

1. AS.5013.6:2015 Food microbiology. Method 6: Examination for specific organisms – *Campylobacter* will achieve a limit of detection (LOD) of 5000 *Campylobacter* organisms per carcass when rinsing whole carcasses with 500ml of rinsate and plating out one ml of rinsate.

2. The New Zealand Ministry of Primary Industries has developed a technique which is used in the poultry industry whereby the carcases are rinsed with 400ml of rinsate and 2ml of rinsate are plated on to 6 plates. This gives an LOD of 200 *Campylobacter* organisms (Lee *et al.* 2014). The modified *Campylobacter* method below is a further adaptation of this technique which can be used for carcasses or portions. Other validated methods are also appropriate such as a miniaturized most probable number method as published by Chenu *et al*. (2013).

3. Modified *Campylobacter* method to lower the LOD to 100 cfu per carcase:

- Each carcase is rinsed with 200ml of sterile buffered peptone water for 2 minutes. 2ml of rinse fluid is inoculated over eight (8) *Campylobacter* Blood Free Agar plates (250µl per plate).
- A 100µl aliquot of rinse is plated onto a ninth plate for higher concentrations of organisms. The plates are placed in sealed containers with atmosphere generating sachets (CampyGen™, Oxoid) and incubated at 42 ± 0.5°C for 48 ± 2 hours.
- After incubation, up to five representative colonies are selected from across the eight plates. The selected colonies are confirmed as *Campylobacter* by oxidase activity and latex agglutination test, *Campylobacter* Dryspot *Campylobacter* Test™ (Oxoid).

Note: A 200mL rinse was used to achieve greater sensitivity and 8 x 250µL inoculums used for greater precision.
The number of *Campylobacter* cfu per sample is calculated by adding up the number of confirmed colonies counted on the 8 plates:
(plate 1 + plate 2 + plate 3 + plate 4 + plate 5 + plate 6 + plate 7 + plate 8) x 200ml/2ml = number of *Campylobacter* organisms per poultry sample.

For duplicate plates of higher dilutions:
cfu per sample = *(number colonies confirmed as *Campylobacter*/n) x count characteristic *Campylobacter* morphology colonies (plate 1 + plate 2)/2 x 200ml/0.1ml x 1/dilution = number of *Campylobacter* organisms/poultry carcass sample.

Where:
n = number or characteristic colonies examined (usually 5 unless there are less than 5 characteristic colonies altogether).
* Usually five/five if the first colony of five is confirmed as positive. It will be reported as a proportion of five, if the remaining colonies are required to be confirmed e.g. three/five.

Table 4.1 Raw chicken system assessment – Control point checklist

Verification Point	1 – Control Point: Live Bird Receipt	2 – Control Point: Evisceration	3 - Control Point: Carcase Decontamination	4 – Control Point: Storage, Further Processing and Distribution
Performance target	8 - 12 hours off feed	No unacceptable carcases enter the carcase wash.	>5 ppm FAC pH 5 – 7 ORP >650mV Note : Upper limits need to ensure compliance with S18-7 in Food Standards Code.	Poultry meat carcases chilled to <7°C within 6 hours of stunning, ≤5°C within 12 hours and maintained at ≤5°C.
Outcome	Only birds that are fit for human consumption are to be processed, no feed in the crop, minimal gut spillage. This control point is designated to identify and control hazards prior to entering the slaughter facility. It applies to all stock prior to processing and can be applied to all processing establishments regardless of size. Farms are required to remove feed (but not water) from flocks prior to pick-up and transport, and provide evidence (i.e. a declaration) to the processor that feed withdrawal and any veterinary withholding periods have been met and birds have been examined to ensure suitability for slaughter for human consumption.	Each carcase is subject to inspection and appropriate disposition. This control point is designated to identify and control hazards associated with evisceration. It applies to all processing establishments performing manual or mechanical evisceration. The purpose of this control point is to establish a system that identifies contamination or any other condition that makes a carcase otherwise unacceptable and manages it at the earliest possible opportunity. This reduces pressure on the control points further along the processing chain and minimises contaminants entering the washing process.	Each carcase is subject to an overall reduction in microbial load through the wash/chill process. This control point is designated to control hazards associated with microbiological contamination of carcases. It applies to all processing establishments washing carcases after manual or mechanical evisceration using either a spin wash/chill system or other immersion or washing process. The purpose of this control point is to establish a system that achieves a consistent validated reduction in overall microbial load on carcases to improve food safety in regards to pathogens of concern (i.e. *Campylobacter* spp. and *Salmonella* spp.) in addition to improving product quality. The microbiological verification targets are indicative of the effective	Each carcase is subject to a chilling process that supports an overall reduction in microbial load through processing. This control point is designated to control hazards associated with microbiological growth on poultry carcases. The purpose of this control point is to establish a system that achieves a consistent validated reduction of temperature to meet the requirements of AS4465 and minimise microbiological growth to maintain minimal pathogenic loads on carcases and preserve product quality.

Verification Point	1 – Control Point: Live Bird Receipt	2 – Control Point: Evisceration	3 - Control Point: Carcase Decontamination	4 – Control Point: Storage, Further Processing and Distribution
	Significant evidence exists that demonstrates that poultry presented for slaughter with less than 8 hours feed withdrawal are subject to greater levels of contamination by spilled ingesta. Similarly, flocks that are off feed greater than approximately 12 hours are susceptible to bile production and can suffer from increased rates of intestinal tearing during evisceration due to weakening of the gastrointestinal tract due to sloughing of cells. Therefore, the target between 8-12 hours, should be considered in order to meet the outcome without compromising further processing.		operation of the carcase decontamination process.	
When	Prior to birds being presented for slaughter at abattoir.	At point of final inspection, prior to birds entering the washing process (i.e. the end of the evisceration) during processing operations.	Wash water is measured at the point of overflow from the washing system (or a designated point within decontamination system) at defined intervals during processing.	Temperature of carcase and monitored at the end of processing (after final chill and just prior to dispatch).
What	All birds must be kept off feed for a sufficient period of between 8 - 12 hours prior to slaughter to ensure crops are empty. Birds are to remain on water until pickup commences (minimum of 2 hours before pick-up).	All carcases must be subject to a visual inspection from a suitably trained person, prior to entering the wash. Unacceptable birds must be removed from the processing line and managed separately.	The operation of the carcase decontamination system (e.g. spin wash/chiller, inside/outside washer) must conform to a set of validated operating conditions that demonstrates a reduction in microbiological contamination to ensure effectiveness. Target operational requirements are >5 ppm free available	Carcases must be chilled to a surface temperature of 7°C or less within 6 hours of stunning and further reduced to a core temperature <5°C within 12 hours of stunning. Edible offal must be chilled to <5°C within 1 hour.

Verification Point	1 – Control Point: Live Bird Receipt	2 – Control Point: Evisceration	3 - Control Point: Carcase Decontamination	4 – Control Point: Storage, Further Processing and Distribution
	Ante-mortem inspection of poultry prior to slaughter by a suitably qualified person.		chlorine (F.A.C.) concentration and pH between 5 and 7. Alternative operational limits (e.g. for peracetic acid or chlorine dioxide) may be applied if sufficient validation information is provided.	Frozen product reduced to -15°C or less within 96 hours of stunning. Core temperatures of product maintained at <5°C through distribution.
How	Ensure birds sourced from approved suppliers/accredited farms. Identification and removal of unhealthy or diseased poultry. Presentation of grower declaration (i.e. evidence that birds are free from chemical residues, time off feed parameters have been met etc.). Demonstration by the company that withholding periods for in-feed medication /treatments are met. Physical verification by crop check.	Ensure complete evisceration to minimise carcase contamination (operation of plant and performance of personnel as appropriate). At every processing break, and at least once per shift, evisceration machinery is disengaged from the line and all intestines and faeces are removed from the operating parts of the machine. Identification and removal of unacceptable carcases from the process. Appropriate management system for unacceptable carcases (e.g. re-work process). Verification through monitoring of evisceration efficiency at final inspection point (e.g. 100-bird assessment).	Collect and analyse samples of wash water from overflow point (or at most contaminated point during processing) and record pH, free available chlorine (F.A.C.) concentration and/or ORP to ensure that minimal performance targets are met and demonstrate effective decontamination of carcases. Ensure continuous overflow and replenishment of active chemical to maintain effective and sanitary operational conditions. Verification through microbiological testing of final product: Target levels of <10,000 cfu/carcase *Campylobacter* spp.	Monitor temperature of chiller water during carcase/offal chilling. Monitor time and temperature of carcases at exit of chiller and during post-chill processing via calibrated deep muscle probe thermometer. Monitor operational air temperature of chillers/freezers. Monitor temperature of chilled/frozen product during storage, at dispatch and through the distribution chain (e.g. data logger).

Verification Point	1 – Control Point: Live Bird Receipt	2 – Control Point: Evisceration	3 - Control Point: Carcase Decontamination	4 – Control Point: Storage, Further Processing and Distribution
Why	Ensure that only suitable birds are presented for processing. Minimise contamination of carcases during processing due to spilled intestinal contents or bile staining (intestinal tearing). Appropriate animal welfare outcomes.	Minimise the contamination of carcases and ensure the effective operation of evisceration machinery. Ensure that condemned and inedible carcases aren't entering the washing process. Minimise contamination of wash water and improve reduction of microbial load. Enable timely corrective actions to be implemented in order to minimise the amount of product affected.	Achieve consistent sufficient reduction of microbiological hazards through-chain and minimise microbiological contamination of carcases. Enable sufficient process control for microbiological hazards to ensure that the wash/chill system operates effectively and does not increase microbiological loads on carcases. Enable timely responses to be implemented in the event of non-conformance to ensure adequate process control is maintained and minimal product is affected.	Control hazards associated with the growth of microbiological organisms associated with final product carcases to maintain acceptability of carcases and shelf life. Enable sufficient process control during further processing and handling of product. Enable timely responses to be implemented in the event of non-conformance to minimise the amount of product affected.
Corrective action	Withhold from slaughter (e.g. feed detected in crop/<8hrs feed withdrawal, chemical residue suspected). Removal and humane slaughter of injured/diseased birds (disposal). Removal of dead birds (disposal). Isolation of suspect/diseased flocks. Notify relevant authority when required.	Adjust equipment to allow for correct operation appropriate to carcase size. Identify and re-train relevant operational staff. Isolate and re-work affected product within appropriate timeframes. Adjust/service/repair equipment to manufacturer's specification. Halt killing process and clean and sanitise evisceration equipment prior to restarting	Ensure correct operation of spin chiller (e.g. overflow) and adjust sanitiser and/or pH levels to return to within operational parameters. Adjust automatic dosing equipment. Identify and re-train relevant operational staff. Notify relevant authority when required.	Adjust operational temperature of chilling system (e.g. add ice to spin chiller). Seal cold storage rooms to maintain temperature and monitor via data logging. Halt further stunning/killing process and hold in-process carcasses in chilling system. Identify alternative storage arrangements. Isolate/freeze/test-and-hold potentially affected product.

Verification Point	1 – Control Point: Live Bird Receipt	2 – Control Point: Evisceration	3 - Control Point: Carcase Decontamination	4 – Control Point: Storage, Further Processing and Distribution
		Notify relevant authority when required.		Condemn affected product where temperature exceeds 5°C for a period of time that may compromise the wholesomeness of the product, and the product isn't compromised to the point where remedial processing wouldn't ensure the product is still acceptable for human consumption. Notify relevant authority when required.

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5. DAIRY PRODUCTS

5.1 Introduction

The main microbiological hazards associated with dairy products include *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, pathogenic *Escherichia coli* and *Cronobacter* spp. (FSANZ 2006; ICMSF 2005). An effective food safety management system includes control measures throughout primary production, transport and processing to control these hazards and should be documented in every dairy business's food safety program.

Implementing control measures at various stages of production will eliminate or reduce microbiological hazards to acceptable levels. Controls may include specific treatments during processing such as heating or acidification, or broader pre-requisite program activities such as cleaning and sanitation, good manufacturing practices and pest control.

Manufacturers conduct routine microbiological sampling and testing as one means of verifying that controls have worked as intended. This testing may involve ingredients, the processing environment, in-process samples and final product. Test results are compared to pre-determined criteria to assess the effectiveness of the control.

Different types of microbiological criteria are useful for different purposes. They include food safety criteria, microbiological guideline criteria and process hygiene criteria (see Glossary and Figure 1). A business may establish both process hygiene criteria and microbiological guideline criteria to verify that food safety controls have been effective. Microbiological testing can indicate when a control has failed, but has statistical limitations – especially when dealing with large batches of non-homogenous product – making it unreliable as the sole means of indicating that controls have been effective. Therefore, it is best to consider microbiological testing as an important support of other verification activities, which in combination will provide increased confidence in the effectiveness of food safety management.

5.2 Dairy microbiological testing

Testing process

A typical process for microbiological testing to verify food safety controls of dairy products is as follows (see Glossary for explanation of underlined terms):

1. Representative samples are taken from a lot (or batch).
2. Sampling technique needs to be aseptic to ensure sample integrity is maintained.
3. The number of samples taken depends on the sampling plan. This is usually five samples (but can be more) for 2-class and 3-class sampling plans, or one sample for minimum testing.
4. The type of test/s to be conducted are determined based on the purpose of testing and the criteria (guidance on appropriate process hygiene criteria or microbiological guideline criteria for verification are provided in product category tables in this document).
5. For qualitative tests (*Salmonella* spp. and *L. monocytogenes*) samples can be composite samples.
6. For quantitative tests (*E. coli*, coliforms, standard plate count (SPC), *S. aureus*), samples need to be tested individually.
7. Samples are transported either to an in-house laboratory or commercial laboratory. If a commercial laboratory is used it should be accredited by the National Association of Testing Authorities (NATA).

8. Samples are tested according to validated testing methods.
9. Results are received and compared to either the process hygiene criteria or the microbiological guideline criteria (listed in product tables).
10. Where results fall outside set criteria, corrective actions (including product disposition) and a root cause analysis are undertaken (see Corrective actions section).
11. The frequency of testing will depend on the product category (or categories) being manufactured (see product tables). In some circumstances a more intensive sampling regime may be warranted.

Sampling plans and testing frequency

Sampling plans
Testing of foods usually involves either 2-class or 3-class sampling plans. A 2-class sampling plan is generally used to test the presence or absence of a microorganism (e.g. *Salmonella*). A 3-class, quantitative sampling plan is used to test if a permitted microorganism is at an acceptable level. See the Glossary section for further details.

Alternative sampling plans are a provision for manufacturers producing small batches to reduce testing costs. In this case, a single sample may be appropriate to represent a batch for quantitative testing, as an alternative to the five samples required by a 3-class sampling plan. Where only a single sample is tested (n = 1), no sample (c = 0) should exceed the value of m provided in the microbiological criteria tables.

> For example, the limit for *E. coli* in cheese is n = 5, c = 1, m = 10, M = 100. If a manufacturer chooses to test only one sample, the limit becomes 10 *E. coli*/gram. (Under this sampling regime, if a sample result is 10–100/g, then the batch may be re-sampled and tested with 5 samples to determine if it complies with requirements, or whether corrective action is needed (depending on the reason for testing).

For qualitative tests (testing presence or absence), five samples still need to be collected but can be composited for analysis.

*Manufacturers of product for export will still need to meet the sampling requirements of the Code e.g. when cheese is being tested for *E. coli*, n = 5 samples.

Testing frequency
The testing frequencies provided in this chapter are based on the risk profile of the product and are a compromise between confidence that all batches are acceptable, and the impost of resourcing and costs associated with frequent testing. Where there is an extended period between every 10 or 20 batches manufactured, ensuring that testing occurs at least once every two months would be an acceptable alternative.

A more frequent sampling regime may be appropriate under some circumstances, or as required by a state regulator (e.g. a poor compliance record, or when a clearance program (see Clearance program section) is initiated following a pathogen detection). This regime involves more frequent testing to provide increased assurance that the corrective actions have been effectively implemented. New dairy manufacturing businesses may also be expected to undertake more frequent product testing to validate procedures.

A major risk with less frequent testing is that a business may be unable to demonstrate exactly when an issue originated, bringing into question the acceptability of all product manufactured since the last acceptable test was completed.

5.3 Microorganisms important in the dairy industry

Indicator organisms

Indicator organisms are often used as process hygiene criteria as they are a reliable and cost-effective way of verifying that hygiene measures or process controls are working as intended. The most commonly used indicator organisms in the dairy industry are coliforms, Enterobacteriaceae, standard plate counts (SPC) and *E. coli*. Further detail on each is provided in Appendix 2, and in Dairy Food Safety Victoria's technical information note *Indicator organisms in the dairy industry*.

Indicator organisms are eliminated by effective pasteurisation and their detection in finished dairy products may indicate failure of controls designed to prevent post-process contamination. Exceeding a target level for indicator organisms should prompt an investigation into the cause, followed by corrective action to rectify the issue. The presence of low levels of indicator organisms in some products may be inevitable due to the nature of the manufacturing process. Trending of results over time can provide a benchmark for such products and is a good way of identifying when controls are becoming less effective.

Pathogens

While pathogens such as *L. monocytogenes*, *Salmonella* spp. and *S. aureus* (and other coagulase-positive staphylococci or CPS) are significantly reduced by pasteurisation, monitoring for their presence is useful for verifying that controls specifically targeted to these pathogens are effective. *L. monocytogenes* is commonly found in moist dairy processing environments. Testing for this pathogen can verify that environmental controls that prevent its transfer from the environment to food are effective.

Salmonella spp. can survive for long periods in dry environments, colonise dairy processing equipment and be present in some post-pasteurisation additions or inclusions. Regular testing for *Salmonella* can verify that it is controlled.

S. aureus and other CPS are commonly found on the skin of food handlers and have a higher tolerance for low water activity foods (down to a_w 0.83). Testing for *S. aureus* is advisable in products that are manually handled, have had a slow or inadequate acidification (e.g. some types of cheese) or have experienced temperature abuse.

Further information on pathogens is provided in Appendix 1.

Controlling pathogen growth in dairy products

Different dairy products have intrinsic characteristics (e.g. low a_w, salt, acidification), or are subjected to specific processes (e.g. heat treatments) that restrict microbial growth. Manufacturers use process verification activities such as testing for moisture or salt content, pH/acidity or phosphatase to check that determined criteria for each parameter are being met. Microbiological testing provides additional support to verify that these processes have been effective.

5.4 Microbiological criteria for dairy product categories

In general, the main risk factors for microbial hazards in dairy products are inadequate heat treatment or post-pasteurisation contamination during further processing. Pasteurisation (see Standard 4.2.4 clause 15) or equivalent treatment (e.g. see ANZDAC 2007 for heat treatments) is the major control of microbiological hazards. As a critical control point, it is closely monitored and verified. Food safety risks for dairy products therefore predominately result from post-pasteurisation contamination. Process and hygiene controls, supported by the documented pre-requisite programs in the food safety program, serve to mitigate food safety risks due to post-pasteurisation contamination in dairy products. Microbiological verification, along with other verification activities, demonstrates that these controls are working effectively.

Process hygiene criteria are applied at specified points during manufacturing. They identify if controls at these points are not working as intended and provide a means of ongoing assessment of hygiene programs. A simple production process may have few opportunities for post-pasteurisation contamination, and applying process hygiene criteria for end product testing may effectively demonstrate that the process was well controlled. However, a more complex, multi-stage process may present many more opportunities for contamination, so testing at several points during the process is useful to confirm controls at each stage are effective and to assist in identifying potential contamination points.

Microbiological food safety control measures will vary for different types of dairy products, due to their different production methods and final physicochemical composition.

A basic representation of the stages of production of major dairy product categories, highlighting the control measures and the process hygiene and microbiological guideline criteria appropriate for verification of microbiological controls are provided in the tables below.

The product tables provide sampling plans, including testing frequency, appropriate for microbiological verification of food safety controls for major dairy product categories. They are generic and may be adapted and used to assist your business to develop sampling plans and set acceptable limits when developing microbiological testing procedures. The guidelines are comprehensive and include a full range of microbiological criteria applicable to ready-to-eat dairy products.

Guidance on sampling of milk and milk products is provided in ISO 707:2008 (IDF 50:2008) and the AS 5013 series.

There may be some cases where a manufacturer can justify a different sampling plan or suggest an alternative means of verification in place of microbiological testing. For example, a certificate of analysis stating that *Salmonella* spp. are not present in post-pasteurisation ingredients may substitute for testing for *Salmonella* in your microbiological testing procedures. An alternative testing frequency may be suitable if a sustained and consistent testing history, and other science-based evidence, demonstrates that a product/pathogen combination poses a low risk.

For most product categories, alternative options for indicator organisms are provided, and manufacturers can select the most suitable one for their businesses. Exceeding the upper limits in the sampling plans for each indicator organism listed in the tables would signal a potential issue. Trending and monitoring results to better understand the levels typical of a well-controlled process can help the business set, monitor and trend their own appropriate target criteria to verify effective process control. Some microbiological guideline criteria include food safety criteria, which are regulatory requirements (in the Code) that must be met in all food for sale. Food safety criteria are highlighted in orange in the tables below.

These guidelines should be considered in conjunction with your specific state/territory regulatory requirements and export requirements if applicable.

Holding product until test results are received will mitigate the risk of having to remove product from the marketplace or supply chain. However, this process may not be practicable with short shelf-life products.

Where microbiological criteria are not met, the cause of the issue needs to be identified and corrective actions taken to rectify it (see Corrective actions section).

a) Bulk raw milk

Regulatory requirements for primary production of milk are outlined in Standard 4.2.4, Division 2 and summarised in Table 5.1 below. Microbiological testing of the raw milk can verify that these on-farm controls have worked effectively if results meet these criteria.

These raw bulk milk criteria provide information regarding on-farm compliance to Standard 4.2.4, Division 2 and are an indication of raw milk quality. This information is useful for a dairy manufacturer from the perspective of raw milk quality, but is distinct from the microbiological verification program for on-site processing controls. These are addressed in the product tables of this chapter.

Table 5.1 Microbiological criteria for raw bulk milk

Regulatory requirements for primary production of milk	Verification method	Microbiological criteria	Comments
Standard 4.2.4 – states that on farm control measures must:			
(a) include support programs that ensure that premises and equipment are clean and sanitary and that pests are controlled; and	SPC (cfu/ml)	<50,000	- Premium quality <20,000 - Levels greater than 50,000 should result in a review of dairy hygiene practices
(b) ensure that milk is cooled and stored at a temperature that prevents or reduces the growth of microbiological hazards in the milk; and	SPC (cfu/ml)	<50,000	- SPC provides a good index of good hygienic practices and adequate cooling
(c) ensure that milk for human consumption is only sourced from healthy animals.	BMCC (cells/ml)	<400,000	- BMCC greater than 400,000 may indicate clinical mastitis in the herd, requiring identification and treatment of the affected animals - <200,000 is considered good - <150,000 is considered excellent (Dairy Australia, 2018)

BMCC = bulk milk cell count; cfu = colony forming units; SPC = standard plate count

b) Butter and dairy blends

Butter and dairy blends are generally considered low-risk dairy products due to their low water activity and salt content (for salted butters). Unsalted butter may allow the survival and growth of pathogens. The presence of post-pasteurisation inclusions may increase the risk of contamination, hence the increased frequency of testing.

Table 5.2 Process control for the production of butter and dairy blends

Stage of process		Pre-operational	Cream pasteurisation	Holding / culturing (if cultured butter)	Churning	Packing (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation	Time/temperature PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)
How do we know if it was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records (CCP monitoring)	• Pre-operational checks acceptable • Production records show time temperature • PRP verification records	• Pre-operational checks acceptable • Production records show time/temperature • PRP verification records	• Pre-operational checks acceptable • Production records • PRP verification records
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100/g OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100/g OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	**Product meets microbiological guideline criteria**

CCP= critical control point; PHC = process hygiene criteria; PRP = pre-requisite program

Table 5.3 Microbiological guideline criteria for butter and dairy blends

Product types	Test	Sampling plan		Alternative sampling plan for small batches	Frequency
Butter and dairy blends (salted)	CPS/g	n = 5 c = 1 m = 100 M =1000	OR	1 sample (limit: 100/g)	Every 20 batches
	E. coli/g OR Coliforms/*g* OR Enterobacteriaceae/*g*	n = 5 c = 2 m = 3 M = 10 n = 5 c = 2 m = 10 M = 100 n = 5 c = 2 m = 10 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 20 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 20 batches
Unsalted butter and dairy blends, reduced fat and reduced salt spreads	CPS/g	n = 5 c = 1 m = 100 M =1000		1 sample (limit: 100/g)	Every 10 batches
	E. coli/g OR Coliforms/*g* OR Enterobacteriaceae/*g*	n = 5 c = 2 m = 3 M = 10 n = 5 c = 2 m = 10 M = 100 n = 5 c = 2 m = 10 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 10 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches
All butter and dairy blends with post-pasteurisation ingredients and inclusions Testing high-risk ingredients separately for the presence of *Salmonella* spp. may be an appropriate alternative to more frequent testing of finished batches	CPS/g	n = 5 c = 1 m = 100 M =1000		1 sample (limit: 100/g)	Every 10 batches
	E. coli/g OR Coliforms/*g* OR Enterobacteriaceae/*g*	n = 5 c = 2 m = 3 M = 10 n = 5 c = 2 m = 10 M = 100 n = 5 c = 2 m = 10 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 10 batches
	Salmonella spp./25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches

Regulatory requirements are highlighted in orange; CPS = coagulase-positive staphylococci; ND = not detected
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

c) Cheese (heat-treated milk)

The main hazards involved in foodborne illness outbreaks associated with cheese (from heat-treated milk) include *Salmonella* spp., *L. monocytogenes* and *S. aureus*. Outbreaks involving these pathogens have resulted from loss of control at key production steps, use of contaminated starter cultures or contaminated ingredients, post-pasteurisation contamination, or mishandling during transport and/or distribution (FSANZ 2009).

There are many varieties of cheese and ways to classify them. The significant hazard(s) of concern and key verification points will depend on the cheese type and the cheesemaking process used. These should be determined during the HACCP hazard analysis stage for each product. The use of microbiological testing should take into account the particular processing factors and product characteristics for each cheese and complement the monitoring of key controls. The criteria for *L. monocytogenes* will depend on whether the product will support its growth. Cheeses can vary greatly in their physicochemical properties and manufacturers need to establish whether the product will, or will not, support the growth of *L. monocytogenes,* and then apply the appropriate criteria as per the table below. Regardless of the heat treatment used, post-process contamination is a major risk factor for the safety of the final product.

Table 5.4 Process control for the production of cheese (heat-treated milk)

Stage of process		Pre-operational	Pasteurisation	Fermentation (acidification)	Moulding / pressing / draining / salting	Maturation /ripening	Final packaging / storage
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination (and growth) by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation	Rapid acidification PRPs (especially those controlling environmental hygiene)	pH PRPs (especially those controlling environmental hygiene)	Time/temperature/humidity PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)
How do we know if it was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records (CCP monitoring)	• Pre-operational checks acceptable • pH/acidity records • PRP verification records	• Pre-operational checks acceptable • [Salt] records	• Pre-operational checks acceptable • End - physicochemical parameters (e.g. pH/a_w) are met	• Temperature and a_w / pH records
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=1 m=100/ml M=1000 AND CPS n=5 c=2 m=100/ml M=1000/ml	In-process samples meet PHC Coliforms n=5 c=1 m=100/ml M=1000 AND (if relevant) CPS n=5 c=2 m=100/ml M=1000/ml	In-process samples meet PHC Coliforms n=5 c=1 m=100/ml M=1000 AND (if relevant) CPS n=5 c=2 m=100/ml M=1000/ml	**Product meets microbiological guideline criteria**

a_w = water activity; CCP = critical control point; CPS = coagulase-positive staphylococci; PHC = process hygiene criteria; PRP = pre-requisite program
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

Table 5.5 Microbiological guideline criteria for cheese (heat-treated milk)

Product types	Test	Sampling plan		Alternative sampling plan for small batches	Frequency
All cheese (Except categories listed below)	CPS/g*	n = 5 c = 2 m = 100 M = 1000		1 sample (limit: 100/g)	Every 20 batches
	*E. coli/g**	n = 5 c = 1 m = 10 M = 100		1 sample (limit: 10/g)	Every 20 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g (in products that support growth)		5 samples composited (limit: ND/125g)	Every 20 batches
		n = 5 c = 0 m = 100 (in products that will not support growth)		5 samples composited and tested. Enumerate if positive	
Soft and semi-soft cheese (Moisture content greater than 39% and pH greater than 5.0)	CPS/g*	n = 5 c = 2 m = 100 M = 1000		1 sample (limit: 100/g)	Every 10 batches
	E. coli/g *	n = 5 c = 1 m = 10 M = 100		1 sample (limit: 10/g)	Every 10 batches
	Salmonella spp./25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches
Cheese with post-pasteurisation inclusions (excluding starter cultures, fermentation aids and rennet)	CPS/g*	n = 5 c = 2 m = 100 M = 1000	**OR**	1 sample (limit: 100/g)	Every 10 batches
	*E. coli/g**	n = 5 c = 1 m = 10 M = 100		1 sample (limit: 10/g)	Every 10 batches
	Salmonella spp./25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g (in products that support growth)		5 samples composited (limit: ND/125g)	Every 10 batches
		n = 5 c = 0 m = 100 (in products that will not support growth)		5 samples composited and tested. Enumerate if positive	
Shredded, grated and cut cheese (excluding soft and semi-soft cheese)	CPS/g*	n = 5 c = 2 m = 100 M = 1000		1 sample (limit: 100/g)	Every 10 batches
	*E. coli/g**	n = 5 c = 1 m = 10 M = 100		1 sample (limit: 10/g)	Every 10 batches
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g (in products that support growth)		5 samples composited (limit: ND/125g)	Every 10 batches (high risk >39% moisture) Every 20 batches (medium risk <39% moisture)
		n = 5 c = 0 m = 100 (in products that will not support growth)		5 samples composited and tested. Enumerate if positive	

Regulatory requirements are highlighted in orange; CPS = coagulase-positive staphylococci; ND = not detected
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded
* Levels of CPS and *E. coli* (if present) may decline during ageing, therefore testing in process and/or prior to ageing may be more appropriate than final product testing for the purpose of identifying control failures in aged cheeses.
* Where testing for *E. coli* is for the purpose of determining acceptability, this should be conducted on food ready for retail sale.
* Some adjunct cultures may include organisms belonging to the coliform group. Care should be taken when interpreting results from cheeses produced using these types of cultures.

d) Cheese (raw milk)

The production of raw milk cheese has been permitted since 2016 when Standard 4.2.4 was amended. This standard prescribes specific criteria that must be complied with to verify that microbiological hazards are effectively controlled. Verification of these controls is imperative to the production of safe raw milk cheese.

On-farm bulk milk for raw milk cheese

Raw milk used for making raw milk cheese must meet the requirements of Standard 4.2.4 Division 5, which describes specific requirements for temperatures, holding times and microbiological limits for both the raw milk and cheese.

Table 5.6 Process control and criteria for bulk milk for raw cheese

		Milking	Milk cooling and storage
What needs to be controlled?		Animal health Milking hygiene	Pathogen growth Post-milking contamination
How is it controlled?		Herd health management Cleaning and sanitation	Time/temperature
How do we know if it was effective?	**Monitoring records**	Treatment records Animal checks BMCC Cleaning records Environmental monitoring	Time/temperature records • Milk must be cooled to a maximum temperature of 6°C within two hours of milking and must be kept at a temperature not exceeding 5°C. • Milk must not exceed 8°C at any point between collection from the primary production business that produced it and delivery to a dairy processing business Cleaning records
	Microbiological verification	BMCC (cells/ml): n=5, c=1 m=200,000 M=400,000 CPS (cfu/ml): n=5 c=1 m=100 M=1000 *E. coli* (cfu/ml): n=5 c=1 m=10 M=100 SPC (cfu/ml): n=5 c=1 m=25,000 M=50,000 • For results calculated under the moving window concept (as described in the Dairy Food Safety Victoria Raw milk cheese production guidance document *"Farmers – raw milk cheese checklist"*)	

BMCC = bulk milk cell count; cfu = colony forming units; CPS = coagulase positive staphylococci; SPC = standard plate count
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

Process hygiene criteria for raw milk cheese are the same as those described for cheese made from heat-treated milk.

Table 5.7 Microbiological criteria for raw milk cheese

Product type	Test	Sampling plan	Frequency
Raw milk cheese	CPS/g*	n = 5 c =2 m=100 M=1000	Testing frequency should be every batch until it is demonstrated that the requirements can be consistently met, as advised by your state regulator.
	Staphylococcal enterotoxins/*25g*	n=5 c=0 m= not detected in 25g	
	*E. coli/g**	n = 5 c = 1 m = 10 M = 100	
	L. monocytogenes/25g	n = 5 c = 0 not detected in 25g	
	Salmonella spp./*25g*	n = 5 c = 0 not detected in 25g	

Regulatory requirements are highlighted in orange; CPS = coagulase-positive staphylococci
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded
* Levels of CPS and *E.coli* may decline during ageing, therefore testing in process and/or prior to ageing may be more appropriate than final product testing for the purpose of identifying control failures in aged cheeses.

e) Dairy-based dips and desserts

Some dairy-based dips and desserts are acidified to prevent growth of pathogens, while others rely on refrigeration and short shelf life. Growth and toxin production by *S. aureus,* the presence of *L. monocytogenes* in non-acidified products and *Salmonella* spp. in post-pasteurisation additions are potential hazards in these products. Differences in the physicochemical properties and temperature of filling can impact on the hazards associated with this diverse range of products and affect verification requirements. Hot-filled products may not need as extensive testing if records can demonstrate that target temperatures were achieved at filling.

Table 5.8 Process control for the production of dairy-based dips and desserts

Stage of process		Pre-operational	Pasteurisation	Post-pasteurisation inputs & mixing	Final packaging (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation Post-pasteurisation inputs – controlled by raw material/approved supplier	Time/temperature PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)
How do we know if it was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records (CCP monitoring)	• Pre-operational checks acceptable • Production records show time/ temperature • PRP verification records	• Pre-operational checks acceptable • Production records • PRP verification records
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100/g OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	**Product meets microbiological guideline criteria**

CCP = critical control point; PHC = process hygiene criteria; PRP = pre-requisite program
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

Table 5.9 Microbiological guideline criteria for dairy-based dips and desserts

Product types	Test	Sampling plan					Alternative sampling plan for small batches	Frequency
Dairy-based desserts and dips with a pH above 4.5 (e.g. custard, mousse)	CPS/g	n = 5	c = 2	m = 100	M = 1000		1 sample (limit: 100/g)	Every 10 batches
	E. coli/g OR Coliforms*/g* OR Enterobacteriaceae*/g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 10 batches
	L. monocytogenes/25g	n = 5	c = 0	not detected in 25g			5 samples composited (limit: ND/125g)	Every 10 batches
Dairy-based desserts and dips with a pH above 4.5 with high-risk post-pasteurisation inclusions Testing high-risk ingredients separately for the presence of *Salmonella* spp. may be an appropriate alternative to more frequent testing of finished batches	CPS/g	n = 5	c = 2	m = 100	M = 1000		1 sample (limit: 100/g)	Every 10 batches
	E. coli/g OR Coliforms*/g* OR Enterobacteriaceae*/g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g))	Every 10 batches
	L. monocytogenes/25g	n = 5	c = 0	not detected in 25g			5 samples composited (limit: ND/125g)	Every 10 batches
	Salmonella spp./25g	n = 5	c = 0	not detected in 25g		**OR**	5 samples composited (limit: ND/125g)	Every 10 batches
Dairy-based desserts and dips with a pH below 4.5	CPS/g	n = 5	c = 2	m = 100	M = 1000		1 sample (limit: 100/g)	Every 20 batches
	E. coli/g OR Coliforms*/g* OR Enterobacteriaceae*/g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 20 batches
	L. monocytogenes/25g	n = 5	c = 0	m = 100			5 samples composited and tested. Enumerate if positive	Every 20 batches
Dairy-based desserts and dips with a pH below 4.5 with high-risk post-pasteurisation inclusions Testing high-risk ingredients separately for the presence of *Salmonella* spp. may be an appropriate alternative to more frequent testing of finished batches	CPS/g	n = 5	c = 2	m = 100	M = 1000		1 sample (limit: 100/g)	Every 10 batches
	E. coli/g OR Coliforms*/g* OR Enterobacteriaceae*/g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 10 batches
	L. monocytogenes/25g	n = 5	c = 0	m = 100			5 samples composited and tested. Enumerate if positive	Every 10 batches
	Salmonella spp./25g	n = 5	c = 0	not detected in 25g			5 samples composited (limit: ND/125g)	Every 10 batches

Regulatory requirements are highlighted in orange; CPS = coagulase-positive staphylococci; ND = not detected
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

f) Dried milk powders

These powders include whole and skim milk powders and other dairy-derived powders such as whey powder. The main microbiological hazard associated with these products is *Salmonella* spp., which are known to survive for long periods in dry environments and to colonise drying plants and contaminate product. Monitoring for *B. cereus* is recommended, as this toxin-producing organism can survive pasteurisation and drying and may grow when the powder is reconstituted. The low water activity makes this product a low risk for organisms such as *L. monocytogenes* and staphylococci.

Table 5.10 Process control for the production of dried milk powders

Stage of process		Pre-operational	Pasteurisation / post-pasteurisation inputs	Evaporation and spray drying	Packing (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation Post-pasteurisation inputs – controlled by raw material/approved supplier	Time/temperature PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene)
How do we know if it was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records (CCP monitoring)	• Pre-operational checks acceptable • Production records show vacuum / temperature • PRP verification records	• Pre-operational checks acceptable • Production records • PRP verification records
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100/g OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	**Product meets microbiological guideline criteria**

CCP = critical control point; PHC = process hygiene criteria; PRP = pre-requisite program
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

Table 5.11 Microbiological guideline criteria for dried milk powders

Product types	Test	Sampling plan		Alternative sampling plan for small batches	Frequency
Dried milk powder	*Salmonella* spp./25g	n = 5 c = 0 not detected in 25g	OR	5 samples composited (limit: ND/125g)	Every 20 batches
	B. cereus/g	n = 5 c = 1 m = 100 M = 1000		1 sample (limit: 100/g)	Every 20 batches

Regulatory requirements are highlighted in orange; ND = not detected
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

g) Fermented milk products

Standard 2.5.3 states that fermented milk products must have a pH of no more than 4.5. The acidic nature of these product means that most pathogens will not grow in these products. Post-pasteurisation contamination and the addition of inclusions can potentially lead to the presence of some pathogens.

Table 5.12 Process control for the production of fermented milk products

Stage of process		Pre-operational	Pasteurisation	Post-pasteurisation inputs	Ferment	Packing (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination (and growth prior to acidification) by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation Post-pasteurisation inputs – controlled by raw material/approved supplier	PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene) Rapid acidification	PRPs (especially those controlling environmental hygiene & GMP)
How do we know if it was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records (CCP monitoring)	• Pre-operational checks acceptable • Production records show traceable details of inputs • PRP verification records	• Pre-operational checks acceptable • Production records show time temperature & pH • PRP verification records	• Pre-operational checks acceptable • Production records • PRP verification records
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100/g OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100/g OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	**Product meets microbiological guideline criteria**

CCP = critical control point; GMP = good manufacturing process; PHC = process hygiene criteria; PRP = pre-requisite program
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

Table 5.13 Microbiological guideline criteria for fermented milk products

Product types	Test	Sampling plan		Alternative sampling plan for small batches	Frequency
Yoghurt and other fermented milk products (e.g. sour cream)	*E. coli/g* OR Coliforms*/g* OR Enterobacteriaceae*/g*	n = 5 c = 1 m = 3 M = 10 n = 5 c = 1 m = 10 M = 100 n = 5 c = 1 m = 10 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 20 batches
	L. monocytogenes	n = 5 c = 0 m = 100		5 samples composited and tested. Enumerate if positive	Every 20 batches
Yoghurt and other fermented milk products with high-risk post-pasteurisation inclusions Testing high-risk ingredients separately for the presence of *Salmonella* and CPS may be an appropriate alternative to more frequent testing of finished batches	CPS/g	n = 5 c = 2 m = 100 M =1000	**OR**	1 sample (limit: 100/g)	Every 10 batches
	E. coli/g OR Coliforms*/g* OR Enterobacteriaceae*/g*	n = 5 c = 1 m = 3 M = 10 n = 5 c = 1 m = 10 M = 100 n = 5 c = 1 m = 10 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 10 batches
	Salmonella spp./25g	n = 5 c = 0 not detected in 25g		5 samples composited (limit: ND/125g)	Every 10 batches
	L. monocytogenes/25g	n = 5 c = 0 m = 100		5 samples composited and tested. Enumerate if positive	Every 10 batches

Regulatory requirements are highlighted in orange; CPS = coagulase-positive staphylococci; ND = not detected
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

h) Ice cream and other frozen products

Major hazards associated with ice cream include *L. monocytogenes* and *Salmonella* spp. While pathogens may not grow during frozen storage, they can survive, and foodborne outbreaks have been caused by the presence of both these organisms in ice cream. The presence of post-pasteurisation inclusions may increase the risk of contamination by *Salmonella* spp.

Table 5.14 Process control for the production of ice cream and other frozen products

Stage of process		Pre-operational	Pasteurisation / post-pasteurisation inputs	Prepare base and age	Churn	Final packaging (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation	Time/temperature PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene & GMP)	PRPs (especially those controlling environmental hygiene & GMP)
How do we know if it was effective?	**Microbiological verification**	• Cleaning records • Pre-operational checks • Environmental monitoring (see below)	• Verified pasteurisation records (CCP monitoring) • Post-pasteurisation inputs – controlled by raw material/approved supplier	• Pre-operational checks acceptable • Production records show time temperature • PRP verification records	• Pre-operational checks acceptable • PRP verification records	• Pre-operational checks acceptable • Production records • PRP verification records
	Monitoring records	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100 OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	In-process samples meet PHC Coliforms n=5 c=1 m=10/g M=100 OR Enterobacteriaceae n=5 c=1 m=10/g M=100/g	**Product meets microbiological guideline criteria**

CCP = critical control point; GMP = good manufacturing process; PHC = process hygiene criteria; PRP = pre-requisite program
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

Table 5.15 Microbiological guideline criteria for ice cream and other frozen products

Product types	Test	Sampling plan					Alternative sampling plan for small batches	Frequency
Frozen ice cream, frozen ice cream mix, and edible frozen ices	*E. coli*/g *OR* Coliforms/*g OR* Enterobacteriaceae/*g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 20 batches
	L. monocytogenes	n = 5	c = 0	m = 100			5 samples composited and tested. Enumerate if positive	Every 20 batches
Frozen ice cream, frozen ice cream mix, and edible frozen ices with high-risk post-pasteurisation inclusions Testing high-risk ingredients separately for the presence of Salmonella may be an appropriate alternative to more frequent testing of finished batches	*E. coli*/g *OR* Coliforms/*g OR* Enterobacteriaceae/*g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100	**OR**	1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 20 batches
	Salmonella spp./25g	n = 5	c = 0	not detected in 25g			5 samples composited (limit: ND/125g)	Every 20 batches
	L. monocytogenes	n = 5	c = 0	m = 100			5 samples composited and tested. Enumerate if positive	Every 20 batches
Refrigerated ice cream soft serve (e.g. soft serve mix)	*E. coli*/g *OR* Coliforms/*g OR* Enterobacteriaceae/*g*	n = 5 n = 5 n = 5	c = 1 c = 1 c = 1	m = 3 m = 10 m = 10	M = 10 M = 100 M = 100		1 sample (limit: 3/g) 1 sample (limit: 10/g) 1 sample (limit: 10/g)	Every 10 batches
	L. monocytogenes	n = 5	c = 0	not detected in 25g			5 samples composited (limit: ND/125g)	Every 10 batches

Regulatory requirements are highlighted in orange; ND = not detected
n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded

i) Pasteurised liquid milk and cream

Generally, production of liquid milk and cream is a relatively straightforward process with few post-pasteurisation steps. The use of microbiological guideline criteria (end product testing) alone may be adequate to verify post-pasteurisation controls in some cases. However, in-process verification may be appropriate at the stages where product is held, or additional steps occur before packaging.

Table 5.16 Process control for the production of pasteurised liquid milk and cream

Stage of process		Pre-operational	Pasteurisation	Post-pasteurisation steps	Filling and sealing (final product)
What needs to be controlled?		Environment cleanliness	Pathogen presence	Post-pasteurisation contamination (and growth) by pathogens	Post-pasteurisation contamination by pathogens
How is it controlled?		Cleaning and sanitation	Pasteurisation (CCP)	PRPs (especially those controlling environmental hygiene)	PRPs (especially those controlling environmental hygiene) Sanitary packaging materials
How do we know if control was effective?	**Monitoring records**	• Cleaning records • Pre-operational checks • Environmental monitoring	• Verified pasteurisation records (CCP monitoring)	• Production records • PRP verification records • Pre-operational checks acceptable	• Production records • PRP verification records • Pre-operational checks acceptable
	Microbiological verification	Meet environmental monitoring targets		In-process samples meet PHC Coliforms n=5 c=0 m=10/g OR Enterobacteriaceae n=5 c=1 m=10/ml M=100/g	**Product meets microbiological guideline criteria**

CCP = critical control point; PHC = process hygiene criteria; PRP = pre-requisite program.

Table 5.17 Microbiological guideline criteria for pasteurised milk and cream

Product types	Test	Sampling plan		Alternative sampling plan for small batches	Frequency
Pasteurised liquid milk products (Includes flavoured milk and extended shelf life (ESL) products)	*E. coli/ml OR* Coliforms*/ml OR* Enterobacteriaceae*/ml*	n = 5 c = 1 m = 3 M = 10 n = 5 c = 1 m = 10 M = 100 n = 5 c = 1 m = 10 M = 100		1 sample (limit: 3/ml) 1 sample (limit: 10/ml) 1 sample (limit: 10/ml)	Every 10 batches
	L. monocytogenes/25ml	n = 5	c = 0 not detected in 25ml	**OR** 5 samples composited (limit: ND/125ml)	Every 10 batches
Pasteurised liquid cream products	*E. coli/ml OR* Coliforms*/ml OR* Enterobacteriaceae*/ml*	n = 5 c = 1 m = 3 M = 10 n = 5 c = 1 m = 10 M = 100 n = 5 c = 1 m = 10 M = 100		1 sample (limit: 3/ml) 1 sample (limit: 10/ml) 1 sample (limit: 10/ml)	Every 10 batches
	L. monocytogenes/25ml	n = 5	c = 0 not detected in 25ml	5 samples composited (limit: ND/125ml)	Every 10 batches

Regulatory requirements are highlighted in orange; ND = not detected. n = number of sample units; c = the number of sample units allowed to exceed m; m = the acceptable microbiological limit; M = the limit which must not be exceeded. Approved alternative processes to pasteurisation (e.g.HPP) will involve different processing steps; however, the above microbiological criteria would still apply.

j) Powdered infant formula

Guidance and microbiological criteria for powdered infant formula products are provided in Chapter 3.

k) Raw goat milk for consumption

Raw goat milk is permitted for sale in some states. Refer to relevant state or territory (or New Zealand) legislation regarding requirements for this or similar products.

5.5 Corrective actions

Failure to meet either process hygiene criteria or microbiological guideline criteria indicates that a control in the process may not be working as intended. This should initiate a proactive response to ensure that the control is promptly and effectively restored to full effectiveness and that no product for sale is compromised.

An investigation to identify the cause of the failure (root cause analysis) is then necessary, followed by implementation of corrective actions to prevent a recurrence. Where microbiological guideline criteria are not met, initiating a clearance program (see Clearance program section) will provide assurance that the corrective actions have been effective.

Failure to meet microbiological guideline criteria

Table 5.18 below outlines the steps involved in an investigation following failure of microbiological guideline criteria. Your regulator should be notified and can provide assistance in the investigation.

To identify the possible source of the contamination it is important to follow a logical sequence, such as that described below. For example, extensive cleaning should not commence before equipment is inspected, dis-assembled and swabbed, as any cleaning may remove the evidence or possibly adversely affect swab results by leaving chemical residues.

In addition to investigating to determine the cause of the contamination, it is also necessary to determine the acceptability of affected product(s). Further testing may be required and failure to meet any of the regulatory food safety criteria (highlighted in orange in the tables above), will mean that product cannot be sold.

Failure to meet other microbiological guideline criteria could indicate that there is a potential issue with the product, and a risk assessment to understand any potential food safety issues would be warranted. Irrespective of the outcome, such results call for corrective action to be taken.

Table 5.18 Recommended corrective actions (if failure of microbiological guideline criteria)

Immediate action
• Identify, isolate and secure affected product(s) until appropriate disposition is determined • Notify state regulator and other relevant authorities • Determine whether production needs to be stopped and/or affected process line(s) isolated in order to investigate and address the issue
Investigative action to determine root cause
• Review production and pre-operational records for affected product • Test raw materials, in-process materials, related batches (e.g. batches before and after affected batch) and finished product to assist in identifying sources of contamination • Disassemble (where practicable) and inspect affected equipment • Undertake enhanced environmental sampling (Zones A, B, C, and D) • Undertake enhanced cleaning and disinfection of implicated equipment and environment, and verify effectiveness • Identify corrective action to rectify the cause of the incident (root cause analysis). Put into effect procedures that will prevent future occurrences (preventative actions)
Follow-up action
• Determine appropriate disposition of any affected product (this may involve further testing, a recall or withdrawal) • Undertake Clearance program (see Clearance program section) • Document incident investigation, outcomes and corrective actions

Failure to meet process hygiene criteria

When process hygiene criteria are not met, this may indicate that a specific control step is not working as effectively as it should. It identifies where in the process this is occurring, thereby providing an opportunity for early intervention. Appropriate actions when process hygiene criteria are not met would include those in Table 5.19.

Table 5.19 Recommended corrective actions (if failure of process hygiene criteria)

Investigative action to determine cause
• Review all aspects of the step of the process that was being verified • Test finished product against microbiological guideline criteria to assess impact of inadequate control of that process step. Failure of microbiological guideline criteria requires action as described in a) above

5.6 Clearance program

A clearance program is undertaken when a product fails to meet microbiological guideline criteria, indicating a failure of one or more food safety controls. The expected response to a pathogen detection in product is described in the Corrective actions section 5.5. A clearance program will then allow the manufacturer to demonstrate that the actions taken were effective and sustained.

A clearance program involves extensive sampling and testing of subsequent batches of product on the implicated production line, to demonstrate that the issue has been rectified and the corrective and preventative measures put in place are sustained.

The sampling plan for a clearance program is as follows:

From the first batch after production re-start, 30 samples are taken per batch from the affected production line at the following intervals.

Day	Samples
Day 1	30 samples *
Day 3	30
Day 5	30
Day 12	30

* First batch after re-start, not first batch after contamination event

The 30 samples representing the batch will need to be of sufficient size for the laboratory to take 25g (or 25 ml) from each. Samples should be spread across the production run so they are representative of entire batch. Each sample may be tested individually or composited (for example, two lots of 15 samples).

For small manufacturers, taking 30 samples for testing may be impractical if only a small number of units are produced. In these circumstances, your regulator may consider alternative sampling protocols with a risk-based approach.

A clearance program is only considered to be complete when the results of all tests meet guideline criteria (see Microbiological criteria section). If results from any of the days tested indicate unacceptable levels of microorganisms, then the program needs to be recommenced and appropriate product control and investigation undertaken (see Corrective actions section).

It is recommended that products manufactured on day 1 are held and released when they test negative. Similarly, product made on days 2 and 3, days 4 and 5, and days 6–12 are retained until the results from days 3, 5 and 12 have tested negative, respectively. This may not be practical with short shelf-life products that cannot be held pending release to the market.

Any product from the implicated processing line that was produced prior to the original contamination (day 0) and is still available should also be tested at 30 samples per batch and withheld until cleared. This may include product within the warehouse or retained samples. Product should be tested back to the last compliant test result. Depending on the outcome of the root cause investigation, it is strongly recommended that all products from other production lines in the same processing area be tested for

the same contaminant from the day of, the day before and the day after the original contamination.

5.7 Environmental monitoring

An important means of controlling microbiological food safety hazards is preventing contamination of food from the processing environment. The effectiveness of controls (mainly cleaning and sanitation programs, plant maintenance and appropriate staff practices) are verified by environmental monitoring. Information on environmental monitoring is provided in Chapter 6.

5.8 References

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6. ENVIRONMENTAL MONITORING

6.1 Introduction

Preventing contamination of food from the processing environment is an important means of controlling microbiological food safety hazards. The effectiveness of these controls (mainly cleaning and sanitation programs, plant maintenance and appropriate staff practices) are verified by environmental monitoring. Environmental contamination often occurs sporadically and unevenly. The limitations of sampling end products (i.e. only a very small amount of product from each batch can be tested), mean that environmental monitoring is likely to be more effective and reliable than end product testing in identifying contamination or ineffective cleaning.

The aim of a routine environmental monitoring program is to identify potential and problematic contamination sources before product becomes contaminated. This proactive approach is a targeted way to verify equipment cleaning, sanitation and maintenance. It allows issues to be identified and rectified early and reduces the risk of foodborne illness incidents and product recalls. A risk-based approach that focuses on areas of highest risk of contaminating products will provide most value.

Routine microbiological testing of the processing environment can indicate how effectively it has been cleaned and sanitised. However, microbiological testing can take several days to complete. More rapid methods such as ATP testing and protein swabs can also be used, for immediate results, although these may not determine whether target organisms are present. Visual inspections (especially of dismantled equipment) are also important. While these methods indicate how clean a premises is, they do not show whether any target microorganisms are present. The combination of rapid tests and visual observations before production start-up, and confirmation by subsequent microbiological testing, is the most effective means of environmental monitoring. This section will focus on the microbiological testing component.

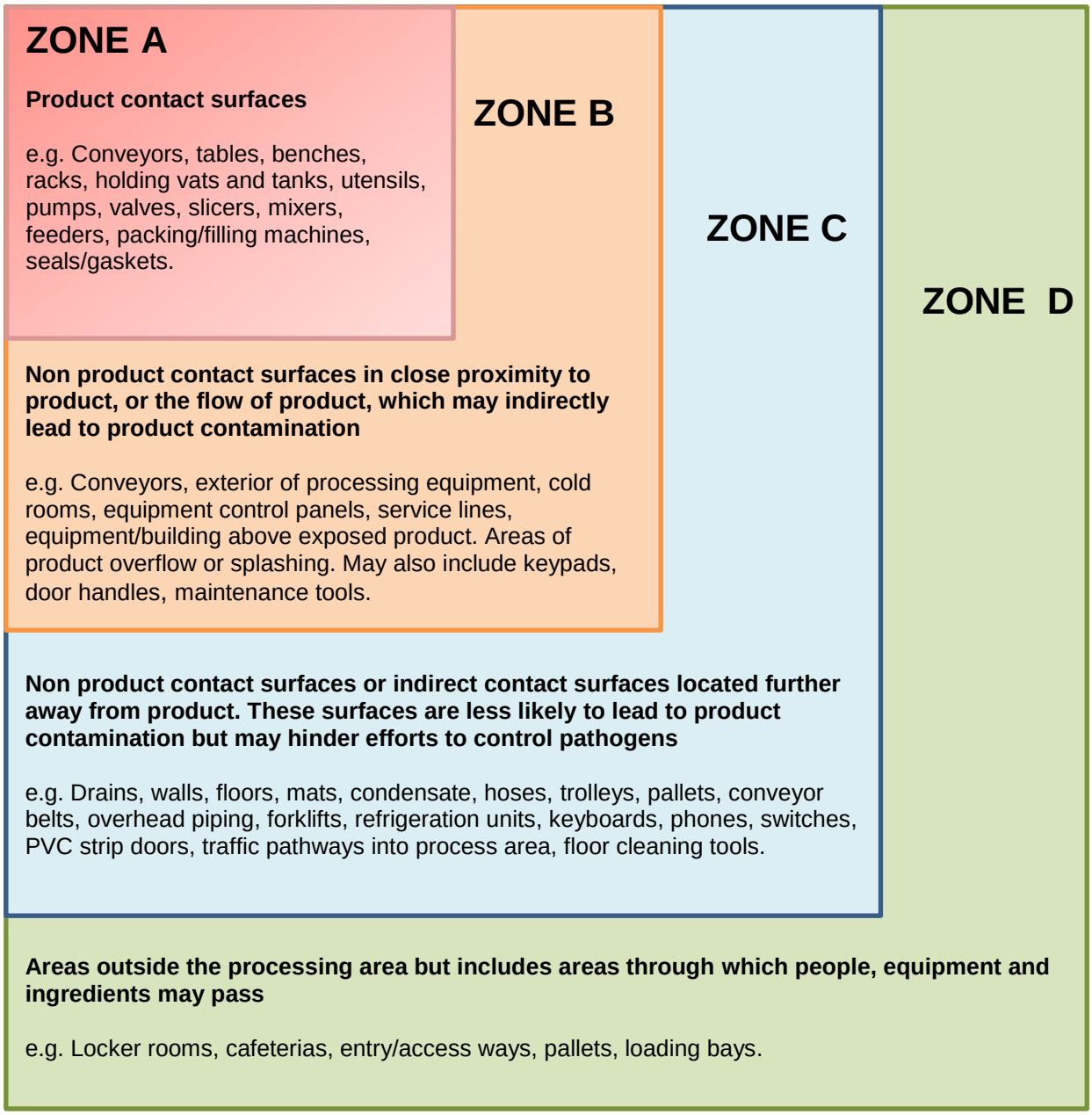
Microbiological monitoring generally involves swabbing surfaces in the environment and testing for the presence of indicator and/or specific pathogenic microorganisms. In addition to swab samples, environmental sampling may include microbiological testing of residues from products, materials or surroundings in either dry or wet form (e.g. shavings from slicing machines, condensate or liquid residues).

6.2 A typical monitoring process

The routine environmental monitoring program for every business can vary greatly due to the different equipment, processes and conditions within the premises. A typical environmental monitoring plan to verify hygiene and sanitation controls may involve the following steps:

1. Targeting zones and sampling sites – Using a detailed diagram of the manufacturing/ processing premises, identify ‘zones’ and mark the exact location of routine sampling sites.
    a. Zones are categories of area within the processing environment and are based on the potential of the area to contaminate product. At a minimum, zones should include a product contact zone and a non product contact zone. Further zones may be added according to proximity to the product and processing line. The zones are often labelled from Zone A (highest risk) onwards (to lowest risk). The figure below shows an example of four zones in a typical site. A business may choose to sample sites in any or all these zones.

Figure 6.1: Example environmental monitoring zones (e.g. dairy processing plant)
(there should be at least two zones: a product contact and a non product contact zone)



b. Identify routine sampling sites within these zones on the diagram. Selection of the most appropriate sites requires a thorough knowledge of premises, processes and equipment. Sites should be selected based on the potential of the surface to harbour microorganisms. Most benefit is obtained by selecting areas that are hard to reach and clean, and surfaces where biofilms are most likely to form. The choice of sites should be justified and documented (e.g. in a food safety program or similar document). Marking these sites on the diagram makes it quite clear where they are located and ensures the entire processing area is covered.

c. Identify which microorganisms will be tested. Most environmental monitoring programs involve testing for both pathogens and indicator organisms. Selection of appropriate organisms will also depend on the type of facility and products being manufactured. Testing for indicator organisms is cost effective and can readily

identify problematic areas. However, pathogens can have specific growth requirements and occupy unique niches, so there is value in testing for these separately. (See, for example, section 6.4 for *Listeria monocytogenes.*)

2. Sampling schedule – Develop a sampling schedule to identify when each of the routine sampling sites are to be tested. The frequency of sampling and number of samples taken in each zone will depend on the type of product, complexity of the production system and size of the facility. All sites do not need to be swabbed every time, and greater coverage of the production environment can be attained by rotating through a larger number of sampling sites. Higher risk sites, such as those in Zone A and B, are tested in higher number and frequency than sites in lower risk zones.

3. Sampling responsibilities and method – Allocate a staff member/s responsible for the swab testing and ensure they are trained in swabbing methods and aseptic techniques. Swabs should be tested as soon as possible after collection and kept cold if there is an extended period between sampling and testing. Testing costs can be minimised by compositing swabs taken from the *same* zone during routine environmental monitoring. However, a positive result will implicate multiple sites, which means further swabbing and/or investigation will be needed. Compositing samples is not appropriate during incident investigations.

4. Checking results – Review the results from environmental monitoring as soon as they are received. Plotting positive results on the diagram, as well as trending of results for particular sites or zones can identify patterns or trends that may suggest a gradual loss of control. Regular, routine monitoring over extended periods will identify 'normal' or acceptable levels for particular sites or zones and allow the business to set targets for certain areas. Acceptable results for environmental monitoring will vary for different surfaces in different areas.

5. Corrective actions – Where results of environmental monitoring exceed expected or target levels, it may indicate that cleaning and sanitation has been ineffective. This should prompt corrective action to identify and rectify the issue, including an assessment of whether any product may have been compromised. Table 6.1 provides a list of recommended actions when environmental monitoring detections exceed expected limits. Persistent detections may indicate that a particular piece of equipment may require maintenance or replacement.

6. Regular review – Review the program regularly. This may include analysing all results, trends and patterns to determine whether there are recurring issues that may require attention. It may also involve assessing whether some sites should continue to be sampled. For example, there may be little value in continually testing a site that never has a detection. Resources would be better directed towards identifying and testing other areas with greater potential to contaminate product.

The above process describes routine microbiological environmental monitoring for the purposes of verifying hygiene and sanitation controls. A similar approach can also be taken when conducting microbiological environmental monitoring as part of an investigation in response to adverse test results.

6.3 Recommended corrective actions

Recommended actions for where environmental monitoring detections exceed expected limits in different zones are listed in the table below.

Table 6.1 Recommended corrective actions

ZONE A: Product contact surfaces	**ZONE B: Non product contact surfaces in close proximity to product**
• Consider placing potentially affected product on hold • Increase sampling to pinpoint contamination sources • Review access/entry restrictions to Zone A and review staff hygiene training and knowledge • Review Zone B results and trends to identify any areas that may require control reassessment • Review cleaning and sanitising program • Reassess manufacturing and product handling procedures • Review the sanitary design of equipment • Investigate for possible equipment/maintenance failure • Clean and sanitise this zone and any suspect areas • Resample all sites to verify effectiveness of cleaning and sanitising (e.g. until three consecutive negative results for the contaminated area are obtained) • Sample and test product associated with the area manufactured on the day of, day before, and day after the positive environmental result. Corrective action is needed if any batches are positive. If no product is available, the next available batch of product manufactured after the date of the environmental positive should be tested	• Increase sampling to pinpoint contamination sources • Reassess access/entry restrictions to Zone B and review staff hygiene training and knowledge • Review Zone C results and trends to identify any areas that may require control reassessment • Reassess cleaning and sanitising program • Reassess manufacturing and product handling procedures • Review sanitary design of equipment • Investigate for possible equipment/maintenance failure • Check receipt of packaging material • Clean and sanitise this zone and any suspected areas • Resample all sites to verify cleaning and disinfection efficacy (e.g. until three consecutive negative results for the contaminated area are obtained)
ZONE C: Non product contact surfaces located further away from product	**ZONE D: Surfaces outside of the processing area**
• Increase sampling to pinpoint contamination sources • Reassess access/entry restrictions to Zone C and review staff hygiene training and knowledge • Review Zone D results and trends to identify any areas that may require control reassessment • Check pallets, trolleys and forklifts and any other items repeatedly entering the area • Reassess cleaning and disinfection program • Reassess manufacturing and product handling procedures • Clean and disinfect this zone and any suspect areas • Resample all sites to verify cleaning and disinfection efficacy	• While there is limited value in swabbing these areas, a review of sites in this zone may be warranted, giving consideration to cleanliness and the potential for contamination to be carried into the processing area

6.4 Monitoring for *Listeria monocytogenes*

L. monocytogenes in food can cause listeriosis, a potentially fatal illness. This pathogen is widespread in the environment and can persist in food processing plants and foods (see Appendix 1 for further details). This section outlines environmental monitoring for the control of *L. monocytogenes* in food processing environments.

Microbiological criteria

Schedule 27 of the Code specifies two microbiological criteria for *L. monocytogenes* in ready-to-eat (RTE) foods. Application of these criteria depends on whether a RTE food supports the growth of *L. monocytogenes* or not. Standard 1.6.1 defines RTE foods for the purpose of applying these limits and sets out the criteria against which RTE foods are considered not to support growth. If an RTE food can support the growth of *L. monocytogenes,* this increases the risk that the food could cause listeriosis – such RTE foods are considered higher risk products.

Monitoring for effective control

When *L. monocytogenes* is detected in processed RTE foods, it is often due to recontamination after processing.

The aim of a routine environmental monitoring program is to find where *L. monocytogenes* could potentially grow and/or survive, and to provide information about hygiene practices. Corrective actions can then be taken before *L. monocytogenes* can contaminate product-contact surfaces or product.

The monitoring program design will be specific to each individual business. However, the same principles as outlined in sections 6.1–6.3 should apply. The program should be risk-based, taking into account the complexity of the processing environment, process flow and the nature and intended use of the product. It should clearly identify the sampling zones, the exact site of sampling (including size and location), frequency of testing, and details of any corrective actions to be taken if a positive sample is detected. Some further factors to consider are listed below.

Target organisms – Using a broad indicator group increases the chances of identifying a specific pathogen's harbourage sites. Detection of a group such as *Listeria* spp*.* in food processing environments can signal that conditions are favourable for *L. monocytogenes*.

Sample sites – At a minimum, sampling sites can be classified into two zones: (A) those that come into contact with RTE food (food contact surfaces) and (B) those that do not (non-food-contact surfaces). Non-food-contact surfaces can be further zoned based on proximity to the product. Refer to Figure 6.1 for examples. Ideally, the presence of *Listeria* spp. in harbourage sites in Zone B (or further zones) or transfer points should be detected early and eliminated to prevent contamination of Zone A.

Sampling frequency – Sampling frequency should be increased for higher-risk products (e.g. RTE foods that support the growth of *L. monocytogenes*). Sampling days and times should be rotated to represent shifts across the entire production schedule.

Recommendations for sample numbers and frequency may be provided in industry (or other) guidance, or specified for certain sectors as part of their approved programs.

Recommended corrective actions

When *Listeria* spp. are detected, the potential cause should be investigated and corrective actions taken. The aim is to eliminate *Listeria* spp. from the environment where practical and possible, and identify any risks posed to the safety of the RTE food products. Corrective actions may depend on several factors including what zone/location the positive detection occurred in, the history of the facility and whether *L. monocytogenes* is confirmed.

Although elimination is the goal, *Listeria* can colonise areas that are inaccessible to cleaning (often through poor premise or equipment design or maintenance), making removal extremely difficult. Examples include structures such as wall cavities, electrical/ services control centres, or inefficient/ damaged drainage systems. It is important to identify and monitor such sites (through an environmental monitoring program), and develop corrective actions to monitor and contain any further spread of the organism. However, such a strategy is not applicable when detections fall within Zone A or B areas (Figure 6.1 above).

When a positive sample for *Listeria* spp. is detected, corrective actions such as those described in Table 6.1 above should be taken.

Corrective actions should escalate if *Listeria* spp. detection is a persistent or ongoing problem and if *L. monocytogenes* is confirmed.

When samples in Zone A test positive for *Listeria* spp., typing for *L. monocytogenes* should be done and potentially contaminated product held pending the result. If *L. monocytogenes* is confirmed in Zone A or B, additional action should follow to ensure potentially unsafe product is not released, the problem is fixed and the situation is monitored to ensure corrective actions have been effective.

6.5 References

Australian Meat Regulators Group (2019). Standard 4.2.3 - Guidelines for the Management of *Listeria*. https://www.safefood.qld.gov.au/wp-content/uploads/2019/02/Standard-4.2.3-Guidelines-for-the-Management-of-Listeria.pdf

Codex (2007) Guidelines on the Application of General Principles of Food Hygiene to the Control of *Listeria monocytogenes* in Foods CAC/GL 61-2007. http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

Dairy Food Safety Victoria (2016) Dairy pathogen manual. http://www.dairysafe.vic.gov.au/publications-media/regulations-and-resources/guidelines/417-pathogen-manual/file

NSW Department of Primary Industries - Food Authority (2016) Controlling *Listeria monocytogenes* in the food processing environment; Guide for the development of an environmental monitoring program. https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/controlling_listeria_monocytogenes_food_processing.pdf

APPENDIX 1 Pathogens

A number of pathogenic microorganisms can cause foodborne illness including bacteria, viruses and parasites. The significance of these agents to specific food products varies, depending on their ecology, survival and growth characteristics. Knowledge of these characteristics and typical food pathogen associations is useful to inform the choice of test(s) for microbiological examination of foods. A snapshot of relevant information on pathogenic microorganisms commonly associated with foodborne illness is provided below. This material is not intended to be comprehensive – more detailed information is available in the other resources listed.

Bacillus cereus & other *Bacillus* spp.

Description
This is a diverse group of spore-forming bacteria commonly found in the environment (e.g. soil and vegetation). The spores are able to survive harsh environments including normal cooking temperatures.

Two types of foodborne illness are associated with *B. cereus* – emetic (vomiting) and diarrhoeal. The emetic syndrome is caused by ingesting heat-stable pre-formed emetic toxin produced in the food during active growth of the bacteria. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine following ingestion of large numbers of the bacteria.

Foodborne illness is generally associated with high bacteria levels (greater than 10^5 cfu/g) in implicated foods. Onset of illness is fairly rapid (1- 5 hours for emetic syndrome, 8-16 hours for diarrhoeal) and symptoms generally mild and short-lived.

Other species of *Bacillus* that are associated with foodborne illness are from the *Bacillus subtilis* group (including *B. subtilis*, *B. licheniformis* and *B. pumilis*). Symptoms of illness and causative factors are similar to *B. cereus*.

Not all strains are associated with illness.

Associated foods
Given its distribution in the environment, low level contamination of many food commodities with *B. cereus* spores should be expected. When these foods are cooked, vegetative cells are destroyed; however spores can survive and be activated. *B. cereus* is then able to multiply if the characteristics of the food (pH, water activity etc.) allow growth and the food is not kept under temperature control.

Foods associated with the emetic syndrome are predominantly rice dishes, although other starchy foods (potato and pasta dishes) may be involved.

There are a wide range of foods associated with diarrhoeal-type food poisoning including meat, vegetable and fish dishes, particularly those incorporating spices (spices may carry a high load of *Bacillus* spp. spores).

Slow cooling and storage of large amounts of cooked foods at temperatures between 10 - 50°C favour *B. cereus* growth.

Control measures
B. cereus associated with emetic toxin production are mesophilic (optimal growth temperature is 30–40°C). To control growth and toxin production, cooked foods should be either:

- cooled rapidly and stored at 5°C or below
- held warm at 60°C or above
- displayed and handled according to the '2-hour/ 4-hour rule' (see Appendix 2 of Safe Food Australia).

Other growth characteristics

- pH

Acid tolerance varies between strains of *B. cereus*. In general, enterotoxin is produced in the range pH 5.0–9.0 and the growth range is pH 4.5–9.0.
Growth of *B. cereus* is controlled when foods are acidified to pH <4.6.

- Water activity

The minimum water activity for growth is 0.93 (where temperature and pH are optimum for growth).

The maximum salt concentration for *B. cereus* for growth is in the range 7–7.5%.

Further information on preservatives and other factors that control growth and toxin production can be found in ICMSF (1996).

Resources

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 93–96. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2013) Agents of Foodborne Illness, FSANZ. https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

FSANZ (2016) Safe Food Australia. 3rd ed, Food Standards Australia New Zealand Canberra. https://www.foodstandards.gov.au/publications/pages/safefoodaustralia3rd16.aspx

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

Jenson I, Moir CJ (2003) *Bacillus cereus* and other *Bacillus* species. Ch 14 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 445-478

New Zealand Ministry of Primary Industries (2015) *Bacillus cereus* Microbial Pathogen Data Sheet. https://www.mpi.govt.nz/dmsdocument/21545-Bacillus-cereus-spore-forming-

Campylobacter spp.

Description

Campylobacter spp. are Gram-negative, non-spore forming bacteria, generally motile with an S-shape morphology.

C. jejuni and *C. coli* are the species primarily associated with campylobacteriosis, the gastrointestinal disease caused by *Campylobacter*. *C. jejuni* accounts for most cases of human illness. Many domestic and wild animals such as cattle, sheep, poultry, dogs, wild birds and rodents carry *C. jejuni* as part of their normal intestinal flora and shed the organism in their faeces. *Campylobacter* spp. are transmitted to humans predominantly through the consumption of contaminated food or water or through direct contact with infected animals. Most cases are sporadic.

Infection by *Campylobacter* spp. has been associated with ingestion of as few as 100 cells. The incubation period before onset of disease is usually 2–5 days, with illness generally lasting for 2–10 days. The major symptoms include fever, diarrhoea (sometimes bloody), abdominal cramps, headache, nausea and vomiting.

A distinctive feature of *Campylobacter* infection is the severity of abdominal pain which may become sufficiently intense to mimic acute appendicitis. As a result of infection, a small percentage of people develop secondary conditions such as reactive arthritis or Guillain-Barré syndrome.

Campylobacter spp. are microaerophilic (growing best at 5-6% O_2) and require special incubation conditions for cell isolation and growth.

Associated foods

The major food sources linked to campylobacteriosis are:
- inadequately handled or undercooked poultry and poultry products such as livers
- raw milk
- contaminated water.

Control measures

Control measures include:
- avoiding cross contamination of raw poultry and meats to ready-to-eat (RTE) foods and food contact surfaces
- thorough cooking of poultry and poultry products
- pasteurisation of milk
- only consuming/using water that has been treated (potable).

Given poultry meat is a primary source of *Campylobacter* spp., contamination levels should be minimised through appropriate controls during primary production and processing.

Growth and survival characteristics

- Temperature

C. jejuni and *C. coli* are thermophiles, growing optimally at 42°C with a growth range of 30–45°C. *Campylobacter* spp. are unable to grow in foods (due to the levels of oxygen) but can survive refrigerated conditions. They are easily inactivated by heating such as applied during cooking or pasteurisation.

- Water activity

Campylobacter spp. are sensitive to drying and do not survive long in dry conditions (minimum water activity for growth is 0.987).

Resources

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p.14-17. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2013) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra. https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

New Zealand Ministry of Primary Industries (2018) *Campylobacter* spp. Microbial Pathogen Data Sheet https://www.mpi.govt.nz/dmsdocument/21548/direct
Wallace, B (2003) *Campylobacter*. Ch 10 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p359–380.

Clostridium botulinum

Description
Clostridium botulinum (C. botulinum) is a spore-forming anaerobic bacterium that produces a potent neurotoxin. *C. botulinum* is naturally found in soil, water and the intestines of marine animals, humans and other mammals. The spores are resistant to heat (withstanding boiling temperatures) and to freezing.

Based on serological properties of the toxins they produce*, C. botulinum* are divided into seven types A–G. Toxins A, B, E and F cause foodborne illness in humans. These strains form a diverse group with varying genetic and phenotypic characteristics. Overall, the strains causing human botulinum can be divided into two groups – Group I: proteolytic (i.e. those that break down proteins) and Group II: non-proteolytic (i.e. those that do not break down proteins). Group I includes *C. botulinum* with toxin type A, B and F. Group II includes toxin types B, E and F.

C. botulinum can cause foodborne botulism, a serious or fatal illness caused by ingesting the neurotoxin. Symptoms usually start 18–36 hours after consumption of contaminated food but can occur as early as 2 hours or as late as 8 days. Common symptoms are vertigo, nausea, dry mouth, vomiting, double vision, trouble speaking and swallowing, muscle weakness and constipation. If left untreated symptoms may progress to paralysis of the muscles, including the respiratory muscles, and can be fatal.

C. botulinum can also cause infant botulism (generally in infants under 12 months), caused by ingesting bacterial spores that colonise and produce toxin in the infant's intestine. Common symptoms of infant botulism are constipation, loss of appetite, weak sucking and crying, and muscle weakness including poor head control and breathing problems.

Associated foods

Foods associated with botulism are generally foods processed or packaged in a low-oxygen environment and include:
- vacuum-packed foods, including sous vide foods
- home-canned and -bottled foods
- fermented, salted and smoked meat and seafood
- honey (infant botulism).

Control measures
Foodborne botulism outbreaks have been associated with food processing failures and/ or temperature abuse allowing spore activation and toxin production. Given the potency of the toxin, multiple control measures are recommended. Control steps include:

- maintaining strict hygiene throughout food processing and packaging
- maintaining strict temperature protocols through all stages of food handling and storage
- controlling the level of acidity (pH) in the food product
- discarding preserved or vacuum-packed food that is out of date or appears badly damaged, bulging, or spoiled

- avoiding feeding honey to infants under 12 months old.

Growth and survival characteristics

- Oxygen level

Anaerobic conditions support growth of *C. botulinum* and can develop in food processes such as canning, vacuum packing and smoking. Growth is inhibited in the presence of oxygen.

- pH

A neutral pH (around 7.0) favours growth of *C. botulinum,* whereas growth is inhibited in acidic conditions (Group I: pH ≤4.6, Group II: pH ≤5.0). The pH of food also influences the amount of heat needed to kill *C. botulinum* spores: the higher the pH, the greater the temperature required. Note that a low pH will not degrade any pre-formed toxin.

- Temperature

Group I can grow at 13–50°C with most rapid growth at 35–40°C. Group II can grow between 3.3 and 45°C with an optimum for growth and toxin production at 28–30°C. For Group II strains, refrigeration above 3.3°C may not be a complete safeguard against botulism. Note that *C. botulinum* spores are heat-resistant, with temperatures well above 100°C needed to destroy the spores.

- Water activity (a_w)

Growth slows with reducing a_w and ceases at levels used in food preservation, usually in combination with other factors such as pH, salt and temperature. The minimum a_w for growth of Group I strains is 0.94 and for Group II is 0.97.

Resources

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p.108-12. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2016) Safe Food Australia. 3rd, Food Standards Australia New Zealand Canberra. https://www.foodstandards.gov.au/publications/Pages/safefoodaustralia3rd16.aspx

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Jeffery LA & Karim S. *Botulism*. (2020). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459273/

World Health Organization (WHO). 2000. "*Clostridium botulinum*: International Programme on Chemical Safety Poisons Information Monograph 858 Bacteria." http://www.who.int/csr/delibepidemics/clostridiumbotulism.pdf

Clostridium perfringens

Description

Clostridium perfringens is an anaerobic (or aerotolerant) spore-forming bacilli that is widely distributed in the environment, with spores persisting in soils. *C. perfringens* is a part of the normal intestinal flora of humans and other animals.

Spores are able to survive normal cooking temperatures and can germinate and multiply if warm conditions prevail. Depending on the temperature and food matrix, *C. perfringens* can have a doubling time of <10 minutes.

Illness is caused by ingestion of a large number (>10^6) of vegetative cells that multiply and sporulate in the lower small intestine, producing an enterotoxin which causes profuse diarrhoea and abdominal cramps about 16 hours after consumption. Gastrointestinal illness is generally mild and self-limiting.

There are many strains of *C. perfringens*, not all of them producing enterotoxin. *C. perfringens* enterotoxin (CPE) is most commonly produced by type A strains.

Associated foods
Because of its widespread distribution, spores of *C. perfringens* may be present in various animal or plant food products (such as spices).

Foodborne illness associated with *C. perfringens* is almost always associated with temperature abuse of cooked foods such as meats (mainly beef and poultry) and meat-containing products (e.g. gravies, stews and curries), although vegetable dishes have also been implicated in outbreaks.

C. perfringens food poisoning primarily occurs when large volumes of food are prepared and are cooled too slowly or kept at ambient temperature, so that the food is kept warm for extended periods of time. The centre of a mass of cooked food provides an anaerobic environment that allows for the growth of *C. perfringens* at these warm temperatures.

Control measures
The primary control for *C. perfringens* in ready-to-eat foods is maintaining temperatures that prevent multiplication of vegetative cells in cooked foods.

The optimum growth temperature for *C. perfringens* is generally 43°C to 47°C. Because of its fast doubling time, cooked foods prepared in advance need to be cooled rapidly to limit the time at these temperatures. Clause 7(3) of Standard 3.2.2 of the Code (Australia only**) specifies cooling requirements for cooked potentially hazardous foods which require cooling from 60°C to 21°C to be achieved within 2 hours*. Cooling from 21°C to 5°C should occur within a further 4 hours. Once cooled, cooked foods should be stored at 5°C or below.

Reheating previously cooked foods also needs to be rapid to minimise the time the food is kept at optimal growth temperatures. Reheating to above 70°C will kill vegetative cells of *C. perfringens* present.

pH and water activity
The growth of *C. perfringens* is inhibited below pH 5.5 and the minimum water activity for growth is 0.97.

* Standard 3.2.2 provides for an alternative cooling process to be used where it can be demonstrated that the microbiological safety of the food will not be adversely affected.

**Similar time–temperature requirements for the cooling of foods are also included in New Zealand legislation.

Resources
Bates, J & Bodnaruk, P (2003) *Clostridium perfringens*. Ch 15 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p479-542

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p.14-17.

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https://www.foodstandards.gov.au/publications/pages/safefoodaustralia3rd16.aspxICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.
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https://www.mpi.govt.nz/dmsdocument/11021-Clostridium-perfringens-Microbial-pathogen-data-sheet

Listeria monocytogenes

Description

Listeria monocytogenes is a Gram-positive, non-spore forming bacterium that is widespread in the natural environment and carried by many domestic and wild animals. It has been isolated from soils and vegetation, animal feeds such as silage, as well as surface and marine waters as a result of contamination from sewerage or run off.

L. monocytogenes grows at low oxygen conditions and refrigeration temperatures (<5 °C). It can survive for long periods in the environment, on foods, and in food processing plants where it has been isolated from floors, drains, wet processing areas and equipment. Post-processing contamination at food contact surfaces is a main factor for the presence of *L. monocytogenes* in RTE foods.

L. monocytogenes causes listeriosis, which may be non-invasive (a mild form of disease) or invasive. Invasive listeriosis is a relatively rare but often severe disease with fatality rates around 20-30%. Populations at risk include those with chronic disease (e.g. cancer, diabetes, malnutrition, AIDS), pregnant women (foetuses or neonates infected *in utero*), the elderly, and individuals being treated with immunosuppressive drugs (e.g. transplant patients).

Individuals infected with *L. monocytogenes* may exhibit mild flu-like symptoms such as fever and muscle aches, and sometimes gastrointestinal symptoms such as vomiting and diarrhoea. In at-risk population groups manifestations of the more severe, invasive form of the disease include bacteraemia, septicaemia, meningitis, encephalitis, miscarriage, neonatal disease, premature birth, and stillbirth.

Illness is generally associated with ingesting high numbers of *L. monocytogenes*. For invasive listeriosis, the level will vary depending on the virulence of the serotype and the general health and immune status of the host.

Associated foods

Outbreaks of foodborne listeriosis have included those associated with soft-style cheeses, delicatessen meats, cooked chicken, pre-prepared salads, pâté, smoked seafood and rockmelon.

Risk factors typically associated with foods linked to outbreaks include:

- it is ready to eat
- it has an extended shelf life at refrigeration temperatures
- it is susceptible to post-process contamination or has received no listericidal processing
- product characteristics support the growth of *L. monocytogenes* to levels that can present a risk to consumers.

Control measures

Control of *L. monocytogenes* in RTE foods includes:

- minimising contamination of raw materials during primary production
- using listericidal processes
- minimising contamination following processing
- restricting growth through limiting shelf life, maintaining the cold chain or product formulation.

For RTE foods that have received a listericidal process (such as cooking or pasteurisation), control measures should minimise post-process contamination before final packaging or during subsequent handling. These should include the design and maintenance of premises and equipment, process flow, and cleaning and sanitation programs. The Codex *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods* outlines key control measures to minimise or prevent contamination and growth of *L. monocytogenes* in RTE foods.

Foods with the following criteria are regarded as not being able to support the growth of *L. monocytogenes*:

- pH < 4.4
- aw < 0.92
- a combination of pH < 5.0 and water activity < 0.94.

Resources

Codex (2007) *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods* (CAC/GL 61 – 2007) Codex Alimentarius Commission, Geneva, Switzerland.
http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 83-86
http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2013) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.
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Sutherland P, Miles D and Laboyrie (2003). *Listeria monocytogenes*. Ch 13 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney.

Salmonella spp. (non-typhoidal)

Description

Salmonella spp. are members of the family *Enterobacteriaceae.* They are Gram-negative non-spore forming rod-shaped bacteria, generally motile.

Salmonella spp. are named and typed according to antigenic typing and subtyped further through phage typing or molecular typing, such as pulse-field gel electrophoresis (PFGE). More recently, whole genome sequencing (WGS) is being used as a sub-typing tool. Over 2500 serotypes of salmonellae have been described.

Salmonella Typhi, the agent causing typhoid fever, is the only *Salmonella* serovar for which humans are the only animal carrier. *Salmonella* spp. associated with gastrointestinal foodborne illness are termed non-typhoidal *Salmonella* (and are members of the species *Salmonella enterica*) In Australia, *S*. Typhimurium is the most commonly reported serovar of all notified *Salmonella* infections.

A primary reservoir for *Salmonella* is the intestinal tract of vertebrates, including poultry, livestock, wildlife, domestic pets and humans. Faecal shedding by animals colonised with *Salmonella* spp. leads to contamination of the surrounding environment including soil, crops and water ways. *Salmonella* can survive for long periods of time in foods and other substrates.

Salmonella spp. are transmitted via consumption of contaminated food or water, as well as person-to-person contact or from direct contact with infected animals. Gastrointestinal illness results when *Salmonella* are able to invade the intestinal epithelial cells and infect the host, producing a heat-labile enterotoxin. Low numbers of *Salmonella* may cause illness.

Symptoms of salmonellosis usually start 12 to 36 hours after infection and include nausea, vomiting, diarrhoea, cramps and fever. The duration of these symptoms is several days (4 to 7 days but sometimes longer).

Associated foods
A wide range of foods have been implicated in foodborne salmonellosis, particularly those of animal origin and foods that have been subject to faecal contamination from the environment. Examples of foods that have been attributed to outbreaks include:

- animal products such as eggs (particularly raw egg dishes), poultry, raw meat, milk and dairy products
- fresh produce (such as leafy greens, seed sprouts, melons, paw paw)
- low moisture foods such as spices, peanut butter, chocolate.

Factors contributing to salmonellosis include:

- cross contamination during food handling (from the environment or raw products)
- inadequate temperature control
- inadequate processing
- consumption of contaminated raw products.

Control measures
Control of *Salmonella* includes:

- prevention of contamination (particularly of RTE foods)
- including a processing step that will kill any *Salmonella* that may be present
- maintaining temperature control to prevent growth.

Cross contamination occurs when *Salmonella* is spread to foods via contaminated food (such as raw meat, poultry or eggs), water, animals or an infected food handler. Contamination can spread further to food contact surfaces, equipment and utensils if there is inadequate cleaning and sanitising or inadequate hygiene practices such as hand washing.

<u>Growth and survival characteristics</u>

- Temperature

The optimal growth temperature for *Salmonella* spp. is 35 to 43°C. Most serotypes do not grow at temperatures below 7°C. Growth of *Salmonella* spp. does not occur at 50°C. Foods that are high in fat and low in moisture, such as chocolate and peanut butter, may have a protective effect against heat.

- pH

Salmonella spp. will grow over a broad pH range; however, the optimum pH for growth is 7–7.5. The minimum pH at which *Salmonella* spp. can grow is dependent on temperature, presence of salt and nitrite and the type of acid present and has been reported as pH 3.8.

- Water activity

The optimum water activity for growth of *Salmonella* spp. is 0.99. The minimum water activity for growth is 0.93. *Salmonella* spp. can survive for extended periods in foods with a low water activity, such as black pepper, chocolate, peanut butter and gelatine.

Resources

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 83-86. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2013) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra. https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

Jay L,S, Davos D, Dundas M, Frankish E, Lightfoot, D (2003). *Salmonella*. Ch 8 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p207–266.

New Zealand Ministry of Primary Industries (2018) Non Typhoidal Salmonellae Microbial Pathogen Data Sheet. https://www.mpi.govt.nz/dmsdocument/1214-Non-Typhoid-Salmonellae

Shiga toxin-producing *Escherichia coli* (STEC)

Description

Escherichia coli are rod-shaped, Gram-negative bacteria that occur naturally in the gut of humans and warm-blooded animals. Some *E. coli* strains are pathogenic because they have acquired virulence factors and are grouped based on how they cause disease and the symptoms that occur. These pathogenic strains are further serotyped based on three antigens: O (somatic), H (flagella) and K (capsule) antigens. Usually the O and H antigens are enough to classify the strain.

Shiga toxin-producing *E. coli* (STEC), also known as verocytoxin-producing *E. coli* (VTEC), are strains of *E. coli* that produce Shiga toxins (Stx). These pathogenic *E. coli* are able to cause serious disease in humans including haemorrhagic colitis (HC). The highest amount of STEC infections globally are caused by STEC O157 strains (in particular O157:H7). Other strains associated with illness in Australia include O111, O26, O113, O55 and O86. The term EHEC is commonly used to refer to the subgroup of STEC that cause HC and includes the serotypes 0157:H7, 026:H11, 0111:H-, 0157:H-.

Symptoms of STEC infection include abdominal cramps, (bloody) diarrhoea, vomiting and fever. The illness develops over 3–8 days, with many patients improving in 10 days. However more serious illness may result, including haemolytic uraemic syndrome (HUS) and its associated complications. In some individuals this can lead to kidney failure and death. Children under five years of age and the elderly are more susceptible to infection and the development of serious illness.

The dose required for STEC to cause illness will depend on the serotype and virulence factors. For *E. coli* O157:H7 the infective dose is estimated to be low (10–100 cells).

The major animal reservoir of STEC is ruminants, in particular cattle and sheep. Infected animals shed the bacteria in their faeces, resulting in contamination of the environment. STEC can survive in soil, manure, water trough sediments and can also survive for extended time in water.

Direct transmission of STEC to humans is possible through contact with infected animals as well as person to person. The major transmission route is foodborne.

Associated foods

Foods that have been associated with STEC outbreaks include those of animal origin and fresh produce that has been subject to faecal contamination from the environment:

- inadequately cooked ground beef (hamburger patties)
- poorly processed uncooked fermented comminuted meat (e.g. salami)
- raw or inadequately pasteurised dairy products
- fresh produce such as leafy greens and sprouted seeds.

Control measures

The main source for STEC and entry point into the food chain is animal faeces. Primary produce can be either contaminated directly by faecal material or indirectly via contaminated water or soil. Control measures for STEC are through chain and include:

- preventing/minimising contamination of raw products at primary production by implementing good hygienic practices
- ensuring processing controls are adequate (e.g. cooking, pasteurisation, fermentation [including control of pH, water activity etc.]).
- preventing cross contamination of RTE foods from raw foods and the processing environment.

Growth and survival characteristics

- Temperature

E. coli does not grow at temperatures below 7°C but can survive in chilled and frozen food. Optimum temperatures for growth are 35–40 °C. Its sensitivity to heat depends on the composition, pH and water activity of the food; for example the heat resistance increases as the water activity decreases. It is generally recommended that foods (such as hamburger patties) are thoroughly cooked to a core temperature of 75°C.

- pH and water activity

The optimum pH range for *E. coli* growth is pH 6–7 with the minimum pH for growth being 4.4. The effect of pH on survival however depends on the acid present, for example STEC are more acid resistant when hydrochloric acid is the acidulant than when lactic acid is used. The minimum water activity permitting growth of *E. coli* is 0.95 (about 8% salt). This value increases as pH and temperature conditions become sub-optimal.

Resources

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 75–79. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2013) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.

https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspxICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

Shigella spp.

Description

Shigella spp. are Gram-negative, non-motile, non-spore forming, rod-shaped bacteria. Humans are the only natural hosts and the bacteria are often found in water polluted with human faeces. Some strains, e.g. *S. dysenteriae* serotype 1, produce enterotoxin and Shiga toxin similar to that produced by *E. coli* O157:H7 (see STEC in this chapter). *Shigella* spp. are sensitive to external environments and do not survive pasteurisation and cooking temperatures.

Shigella spp. cause the gastrointestinal illness shigellosis. It is highly infectious: as few as 10–200 bacteria may cause illness. Symptoms of shigellosis can include watery diarrhoea, abdominal cramps, nausea, vomiting and fever 8 to 50 hours after consumption of contaminated food or water. The illness is usually self-limiting and lasts from several days to weeks, with an average of 4-7 days. In more severe cases, as is the case for *S. dysenteriae* serotype 1 infection, patients can develop dysentery (characterised by frequent, painful stools containing blood and mucus), abdominal cramps, nausea and vomiting. Also, some infections can be asymptomatic and persist for months. Shigellosis can be more severe in vulnerable people such as the young, elderly and immunocompromised. It can result in reactive arthritis (following *S. flexneri* infection) and haemolytic uremic syndrome (following *S. dysenteriae* serotype 1 infection).

Associated foods

Shigella spp. do not naturally occur in a particular type of food. Food and water become contaminated either directly or indirectly from an infected person. Water contaminated with human waste and unhygienic handling by food handlers are the most common contamination causes.

Common foods associated with *Shigella* spp. contamination include foods handled extensively during preparation and consumed raw (e.g. salads and sandwiches).

Control measures

Shigellosis is largely transmitted through poor hygienic practices, so stringent personal hygiene is an essential control measure. People who are ill should not handle any food. Other measures include:

- proper cooking and pasteurisation
- avoiding cross-contamination, separating raw foods from ready-to-eat foods
- proper cleaning and sanitising of equipment and surfaces, particularly in higher risk environments like childcare centers and aged care facilities
- use of properly treated, potable water for all food handling and food contact equipment.

<u>Growth and survival characteristics</u>

- Temperature

Shigella spp. are heat sensitive and do not survive pasteurisation and cooking temperatures. The temperature range for bacterial growth is 6–47^{o}C. *Shigella* can survive extended periods in refrigeration and frozen conditions.

- pH

Shigella spp. are tolerant to low pH (optimum growth at pH range of 6-8). They can survive and in some cases grow in foods with low pH, such as some fruits and vegetables.

- Modified atmosphere

Shigella spp. can survive on produce packaged under vacuum or modified atmosphere and can also survive in water.

- Salt and preservatives

Shigella spp. in general are tolerant to salt (4-5%), but sensitive to organic acids typically used to preserve food.

Resources

Centers for Disease Control and Prevention (CDC) Shigella – Shigellosis.
https://www.cdc.gov/shigella/index.html
European Centre for Disease Prevention and Control (ECDC) – Shigellosis.
https://www.ecdc.europa.eu/en/shigellosis
FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p.22-25.
http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm
FSANZ (2013) Agents of Foodborne Illness. 2nd edition, FSANZ.
https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx
FSANZ (2016) Safe Food Australia. 3rd edition, FSANZ.
https://www.foodstandards.gov.au/publications/Pages/safefoodaustralia3rd16.aspx

Staphylococcus aureus and other coagulase-positive staphylococci

Description

Staphylococcus aureus is a Gram-positive, non-spore forming cocci bacteria that belongs to the *Staphylococcus* genus. Several staphylococcal species (coagulase-negative and coagulase-positive strains) have the ability to produce heat-stable enterotoxins that cause gastroenteritis in humans. Staphylococcal food poisoning is predominantly caused by *S. aureus.*

Staphylococci are widespread in the environment and commonly occur on the skin and mucous membranes of warm-blooded animals. Humans are a main source of enterotoxin-producing strains, with many healthy people (50% or more) carrying *S. aureus* as part of the normal microflora of the nose, throat or skin. *S. aureus* can survive for extended periods in a dry state.

Staphylococcal enterotoxins (SEs) are produced in food during the exponential phase of *S. aureus* growth. Doses of SE that cause illness are reached when *S. aureus* grows to levels of 10^5 – 10^8 cfu/g. SEs are very resistant to freezing and heating and will survive thermal processes used for low-acid canned foods.

Staphylococcal food poisoning occurs following ingestion of food containing SEs. There is generally a rapid onset of symptoms, appearing around 3 hours after ingestion (range 1–7 hours) which include nausea, vomiting, abdominal cramps and diarrhoea. While illness is acute, it is generally self-limiting and recovery is rapid (within 2 days).

Associated foods

All foods that are handled directly by humans and/or those of animal origin may be contaminated with staphylococci. Foods associated with staphylococcal food poisoning are

those that often require considerable handling during preparation and are prone to be out of refrigeration for extended periods. Such foods may include bakery products such as cream- or custard-filled pies and éclairs; sandwich fillings; meat, poultry and egg products; salads such as potato, tuna, chicken and pasta. Foods high in starch and protein are thought to favour SE production.

Control measures

Temperature

S. aureus grows in the temperature range 7–48°C, with optimal growth between 35–40°C. The production of enterotoxin is optimal between 40–45°C and does not occur at temperatures <10°C. As temperature decreases, the level of SE production also decreases. *S. aureus* is easily killed at pasteurisation or cooking temperatures.

The time food products prone to contamination by *S. aureus* are held at temperatures between 5°C and 60°C should be minimised in order to prevent the opportunity for *S. aureus* growth and toxin production. The '2-hour/4-hour rule' (Appendix2 of Safe Food Australia) provides time limits that can be applied for when RTE food is outside of temperature control.

Heat processes such as cooking and pasteurisation will destroy viable cells of *S. aureus* but will not destroy preformed staphylococcal enterotoxins.

Hygiene of food handlers

Food handlers are regarded as the main source of food contamination with *S. aureus*. Food handling controls to minimise contamination during food preparation include:

- preventing unnecessary contact with RTE food
- using gloves, tongs or other implements to handle food
- handwashing whenever direct contact with food is likely to occur
- avoiding sneezing, coughing or blowing over food or food contact surfaces.

Other growth characteristics

S. aureus is tolerant of high salt and sugar content and can grow in conditions of low water activity (a_w). Most *S. aureus* strains can grow over an a_w range of 0.83 (when other conditions are optimal) to >0.99. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of pH 6–7.

Resources

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 75–79. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

FSANZ (2016) Safe Food Australia. 3rd ed, Food Standards Australia New Zealand Canberra. https://www.foodstandards.gov.au/publications/pages/safefoodaustralia3rd16.aspx

FSANZ (2013) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra. https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

New Zealand Ministry of Primary Industries (2001) *Staphylococcus aureus* Microbial Pathogen Data Sheet. https://www.mpi.govt.nz/dmsdocument/11051-Staphylococcus-aureus-Microbial-pathogen-data-sheet

Stewart, C (2003) *Staphylococcus aureus and Staphylococcal Enterotoxins*. Ch 12 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW),Sydney, p359– 380.

Vibrio parahaemolyticus

Description

Vibrio parahaemolyticus is a Gram-negative, curve-shaped rod naturally present in coastal and estuarine waters. It is salt-tolerant and lyses almost immediately in freshwater. It is a natural contaminant of seafood (fish, shellfish and crustaceans).

Most *V. parahaemolyticus* isolates from the environment are non-pathogenic. Pathogenic strains (indicated by the Kanagawa reaction or presence of genetic markers) cause gastroenteric infections, with symptoms including diarrhoea (which can be bloody), abdominal pain, nausea and vomiting. The infectious dose is greater than 10^5 cells with onset of symptoms ranging from 4 hours to a few days.

Initial levels of *V. parahaemolyticus* in seafood depend on environmental factors at harvest, such as water temperature and salinity, which vary seasonally. Numbers naturally present in seafood are generally low (<100 cfu/g).

V. parahaemolyticus grows at 5–43°C, with optimal growth at 37°C. Generation time is 9–10 minutes at optimal temperatures, which means infective levels can be quickly reached (2–3 hours) in warm conditions. *V. parahaemolyticus* is slowly inactivated at temperatures below 7°C.

Associated foods

Foods associated with foodborne illness caused by *V. parahaemolyticus* are predominantly fish, shellfish and crustaceans (particularly raw molluscs and cooked crustacea).

Control measures

One of the main control measures for *V. parahaemolyticus* is to chill seafood quickly to <5°C after harvest and maintain them under refrigeration to prevent growth. Shellfish harvesting practices may also be implemented to ensure that shellfish is not harvested where water temperatures are in a particular range or following a rainfall event in estuarine areas.

Cooking to an internal temperature of 65°C will destroy any *V. parahaemolyticus* present. Cross contamination should be prevented by keeping raw and cooked foods separate and preventing transfer from food contact surfaces.

Resources

Centers for Disease Control and Prevention (2019) *Vibrio* Species Causing Vibriosis – Vibrio & Food. https://www.cdc.gov/vibrio/food.html and https://www.cdc.gov/vibrio/prevention.html

Desmarchelier P (2003) *Pathogenic vibrios*. Ch 11 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p333 - 358

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 75–79. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

New Zealand Ministry of Primary Industries (2001) *Vibrio parahaemolyticus* Microbial Pathogen Data Sheet https://www.mpi.govt.nz/dmsdocument/11033-Vibrio-parahaemolyticus-Microbial-pathogen-data-sheet

APPENDIX 2 Indicator microorganisms

Direct testing of pathogens is not always possible or practical. The use of indicator and index tests can be a useful and cost-effective means of assessing the microbiological status of food. These tests can be used to:

- indicate the effectiveness or otherwise of process hygiene and process controls (indicator microorganisms)
- indicate the presence of pathogenic microorganisms when direct and reliable analytical methods are not available (index microorganisms).

Information on indicator microorganisms commonly tested in foods is provided below.

Coliforms

Description

Coliforms are a group of Enterobacteriaceae (see below) that are able to ferment lactose rapidly (within 24-48 hours) producing acid and gas. They are not a well-defined taxonomic group and are often defined by the method used (e.g. ability to ferment lactose rapidly). Bacteria outside the Enterobacteriaceae group can also ferment lactose and can be falsely detected as coliforms if no other confirmatory tests are performed.

Organisms that ferment lactose (presumptive coliforms) may be inoculated into selective media at temperatures between 44-45.4 °C. If lactose fermentation occurs at these elevated temperatures, the organisms are termed faecal or thermophilic coliforms. Faecal coliforms may be tested further to determine whether they are *E. coli*.

Purpose of test

Historically, coliforms were the most common indicator group tested for by the food industry, particularly by the dairy sector for monitoring the effectiveness of hygiene measures post pasteurisation.

A high coliform count in heat-processed foods generally indicates under-processing or unsatisfactory post-process contamination.

Interpretation of results

The presence of coliforms in many foods may be expected and does not necessarily indicate unsatisfactory hygiene measures. For example coliforms are part of the normal flora of many raw foods including cereal crops and vegetables and are generally present on raw meats as well as some fermented foods.

Coliforms are able to survive and grow in food processing environments where other pathogenic Enterobacteriaceae may not. As such, their presence in food does not necessarily indicate faecal contamination. Their presence at high levels provides a warning that unhygienic food handling may have occurred or processing was not effective.

Resources

Baylis C, Uyttendaele M, Joosten H, & Davies A (2011) *The Enterobacteriaceae and their significance to the food industry*. International Life Sciences Institute (ILSI), Europe. http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/

Craven H, Eyles M, & Davey J (2003) *Enteric Indicator Organisms in Foods*. Ch 6 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 163-194.

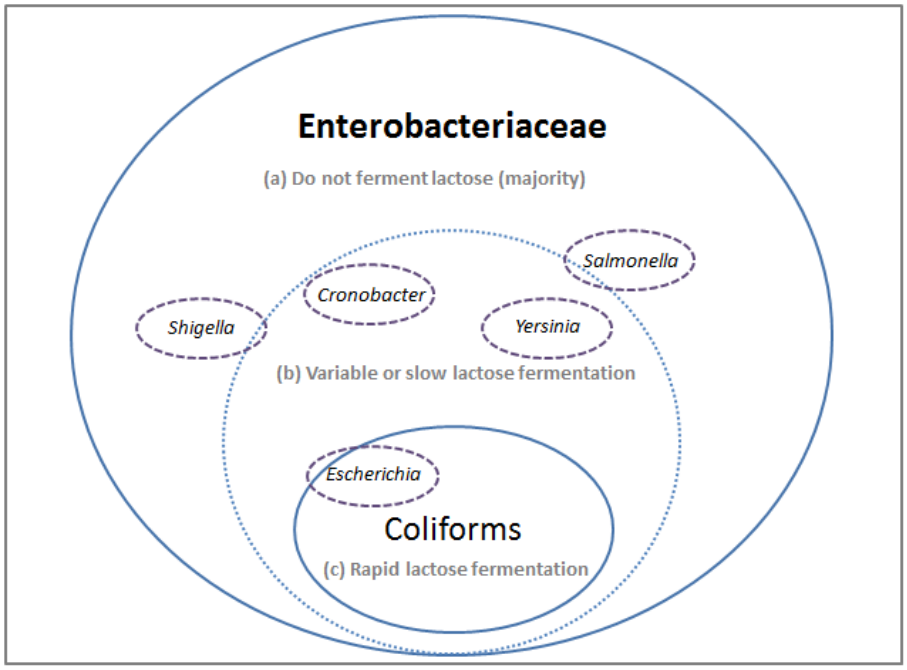
Enterobacteriaceae

Description

Enterobacteriaceae is a family of Gram-negative, non-spore forming bacteria that includes many bacteria that are found in human or animal intestinal tracts, as well as plants and the environment. The family includes a number of foodborne pathogens such as *Salmonella*, pathogenic *E. coli*, *Shigella* and *Cronobacter*, as well as non-pathogenic bacteria.

The ability of Enterobacteriaceae to produce acid and gas from the fermentation of D-glucose is a characteristic commonly used as a basis for their detection and enumeration. Enterobacteriaceae also lack cytochrome C oxidase (have a negative reaction to the oxidase test) which enables them to be differentiated from other closely related bacteria.

While most Enterobacteriaceae do not ferment lactose, some members (collectively termed coliforms) are able to ferment lactose rapidly (within 24–48 hours) producing acid and gas. Members of the Enterobacteriaceae that do not ferment lactose, or ferment it slowly include pathogens (e.g. *Salmonella*, *Shigella*, and some pathogenic *E. coli*) which aren't detected by coliform tests. The relationship between members within the Enterobacteriaceae and those in the coliform group is depicted in the diagram below.



Source: *Adapted from Baylis* et al *(2011)*

Purpose of test

Enterobacteriaceae counts are useful to assess the adequacy of processing and hygiene practices, particularly for heat-treated foods. As all Enterobacteriaceae are killed by thermal processes used in food production, their presence in pasteurised or cooked foods can indicate inadequate processing or post-process contamination.

Interpretation of results

The significance of testing results for Enterobacteriaceae will depend on the type of food being analysed. For example high levels of these bacteria are expected in some food commodities such as salad vegetables and other foods of plant origin.

There are also psychrotrophic Enterobacteriaceae that are able to multiply in chilled foods. These are widely distributed and found in a variety of foods including milk, meat and poultry.

This makes it difficult to interpret levels found throughout the shelf life of a chilled food as they do not necessarily reflect initial contamination levels or whether temperature control has been adequate. Enterobacteriaceae do provide an indication of processing and good hygiene on the day of production.

Resources

Baylis C, Uyttendaele M, Joosten H, & Davies A (2011) *The Enterobacteriaceae and their significance to the food industry*. International Life Sciences Institute (ILSI), Europe. http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/

Craven H, Eyles M, & Davey J (2003) *Enteric Indicator Organisms in Foods*. Ch 6 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 163-194.

Escherichia coli

Description

E. coli are gram-negative, facultative anaerobic rod-shaped bacteria that are a common part of the normal intestinal flora of humans and other warm-blooded animals. As such, *E. coli* is a more specific indicator of faecal contamination than Enterobacteriaceae or coliforms. Its presence in foods indicates recent contamination, either directly or indirectly by faeces or contaminated material.

E. coli can become established in processing environments and can grow on inadequately cleaned surfaces and in food. It is killed by thermal processes used in food production and can be readily removed from food processing equipment and surfaces by appropriate cleaning procedures.

Purpose of test

E. coli testing is used predominantly as an indicator of faecal contamination and measure of the effectiveness of hygiene measures. This can be useful for raw commodities as well as heat processed foods to indicate:

- good manufacturing practices (GMP)/ good hygienic practices (GHP) of meat slaughter
- potential faecal contamination of raw fruit and vegetables during growth and harvest (good agricultural practices, GAP)
- potential faecal contamination of bivalve mollusc harvest waters
- post-process contamination or inadequate processing of heat-treated foods.

E. coli has also been used as an index organism for enteric pathogens such as *Salmonella*.

Interpretation of results

E. coli is the best indicator of recent faecal contamination. It can, however, become established in factory environments such that its presence does not necessarily signify faecal contamination or the risk of enteric pathogens being present. Equally, the absence of *E. coli* does not ensure that enteric pathogens are not present as the survival and growth characteristics of different strains of *E. coli* and enteric pathogens can vary.

Interpretation of *E.coli* results should relate to the purpose of the test and the risk implied by the presence or level detected. For some foods or processes (e.g. production of uncooked comminuted fermented meat) the detection or level detected may require further testing of specific pathogens such as STEC or *Salmonella*.

Resources

Baylis C, Uyttendaele M, Joosten H, & Davies A (2011) *The Enterobacteriaceae and their significance to the food industry*. International Life Sciences Institute (ILSI), Europe. http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/

Craven H, Eyles M, & Davey J (2003) *Enteric Indicator Organisms in Foods*. Ch 6 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 163-194.

Listeria spp.

Description

The *Listeria* genus includes *L. ivanovii, L. innocua, L. welshimeri, L. selligeri* and *L. grayi* and *L. monocytogenes*. The term *Listeria* spp. is fully inclusive of all these species. *L. monocytogenes* is the only human pathogen.

Listeria spp. are Gram-positive, non-spore forming bacteria that are able to grow at refrigeration temperatures. They are widespread in the environment and carried by many domestic and wild animals. *Listeria* spp. survive for long periods in environmental niches from where they can enter the food chain (e.g. via contamination of raw commodities) and food processing facilities. Within processing environments, areas that can harbour these bacteria include drains, floors, conveyors, chilled storage areas, and in cracks and crevices of equipment.

Purpose of test

Testing for *Listeria* spp. is useful to indicate whether conditions exist which can favour *L. monocytogenes* growth or survival. Testing for the broad indicator group *Listeria* spp. increases the chances of finding these conditions and allows for early investigation and corrective action when they are detected.

Interpretation of results

Food testing

The presence of *Listeria* spp. in processed RTE foods indicates inadequate processing or cross contamination from the environment. Higher levels (>100 cfu/g) may also suggest poor temperature control or overextension of shelf life. When *Listeria* spp. are detected, specific testing for *L. monocytogenes* should be done to assess product safety and an investigation carried out to determine the route cause (e.g. whether production, processing and hygiene controls are being implemented effectively).

Environmental testing

Corrective and preventative actions should be taken any time *Listeria* spp. are identified in the processing facility (e.g. cleaning and sanitising of all suspect areas, cleaning and sanitising equipment, increased environmental testing to verify control is re-established). Corrective actions will depend on the zone or location of the detection, whether it is a persistent problem and whether *L. monocytogenes* is confirmed.

The purpose of investigation is to try to identify the root cause and eliminate the condition that may have resulted in the presence of *Listeria* spp. Where product contact surfaces test positive for *Listeria* spp. confirmation testing for *L. monocytogenes* should also be done to assess whether any associated product could be contaminated.

Environmental monitoring for *Listeria* spp. is also covered in Chapter 6.

Resources

Codex (2007) *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods* CAC/GL 61-2007. http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

Orsi, R. H., & Wiedmann, M. (2016). Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Applied Microbiology and Biotechnology*, *100*, 5273–5287. http://doi.org/10.1007/s00253-016-7552-2

Sutherland P, Miles D and Laboyrie (2003). *Listeria monocytogenes*. Ch 13 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney.

Standard plate count (SPC)

Description

The SPC, also termed aerobic plate count, total viable count or aerobic mesophilic count, provides the total number of microorganisms in a food that grow in the presence of oxygen (aerobic) and at moderate temperatures (mesophilic).

The SPC test is based on cells present forming visible colonies when mixed with agar containing appropriate nutrients, generally after incubation at 30°C. Different types of bacteria are not differentiated.

Purpose of test

A test for SPC indicates the microbiological quality of food. It does not determine the presence of pathogenic microorganisms and should not be used as a direct assessment of safety.

The significance of SPC counts varies markedly according to the type of food product and the processing it has received. If it is applied on a regular basis SPC tests can be a useful means of observing trends by comparing results over time.

Interpretation of results

Interpretation of SPC counts should take into consideration knowledge of the product and whether a high count is expected. The stage of shelf life should also be considered as the SPC will increase over the life of a food product (unless processing factors prevent growth e.g. dried food products).

For raw commodities, such as fruits, vegetables, raw meat and fish, total counts are likely to be quite high due to the bacterial flora normally present (10^6–10^7 cfu/g). Fermented foods will also have high colony counts, mainly comprising the starter culture used.

Foods that have receive heat treatments such as pasteurisation or cooking should have low SPC counts following processing ($<10^3$–10^4 cfu/g).

SPC counts in foods that undergo considerable handling such as slicing, portioning, packaging etc. will be influenced by the hygiene measures in place. Table 3 *Interpretation of results for standard plate counts (SPC) in RTE foods* in Section 3 provides further information.

Resources

Health Protection Agency (2009) *Guidelines for Assessing the Microbiological Safety of Ready-to-eat Foods Placed on the Market*. Health Protection Agency, London. https://www.gov.uk/government/publications/ready-to-eat-foods-microbiological-safety-assessment-guidelines