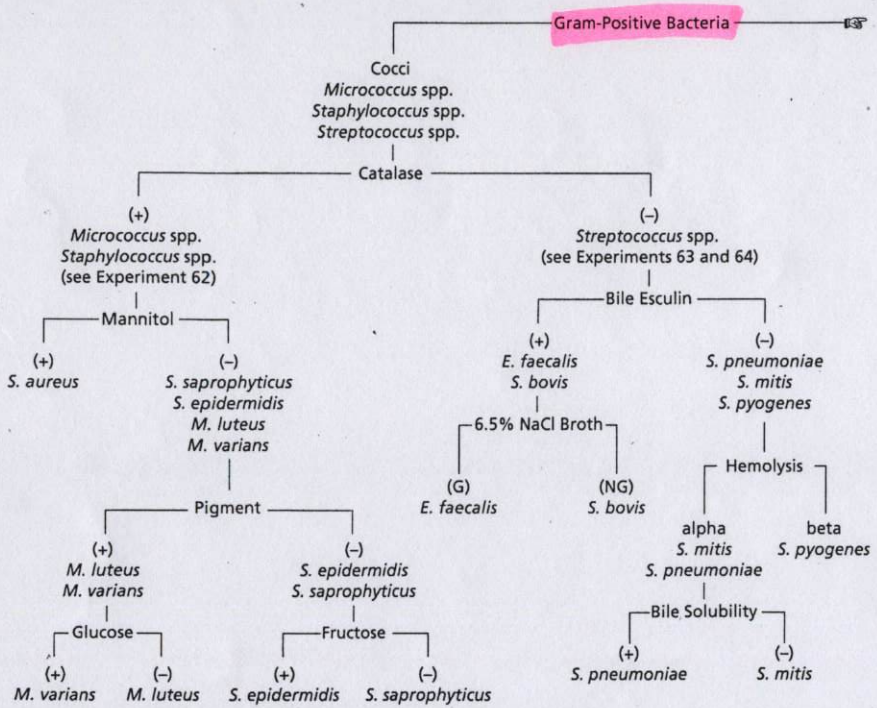

Figure 69.1 Schema for the identification of gram-positive bacteria (continued on facing page)

2. Prepare a Gram-stained smear of the original unknown culture.
3. Prepare four-way streak inoculations (see Experiment 2) on the following media for the separation of the microorganisms in the mixed cultures:
   a. Trypticase soy agar for observation of colonial characteristics.
   b. Phenylethyl alcohol agar for isolation of gram-positive bacteria.
   c. MacConkey's agar for isolation of gram-negative bacteria.
4. Incubate all the plates in an inverted position and the subculture for 24 to 48 hours at 37°C.

Session 2: Preparation of Pure Cultures

1. Isolate a discrete colony on both the phenylethyl alcohol agar plate and the MacConkey's agar plate and aseptically transfer each onto a trypticase soy agar slant (see Experiment 2).
2. Incubate the trypticase soy agar slants for 24 to 48 hours at 37°C.

Session 3: Identification of Unknown Bacterial Species

1. Prepare a Gram-stained smear from each of the trypticase soy agar slant cultures to verify their purity by means of the Gram reaction and cellular morphology.

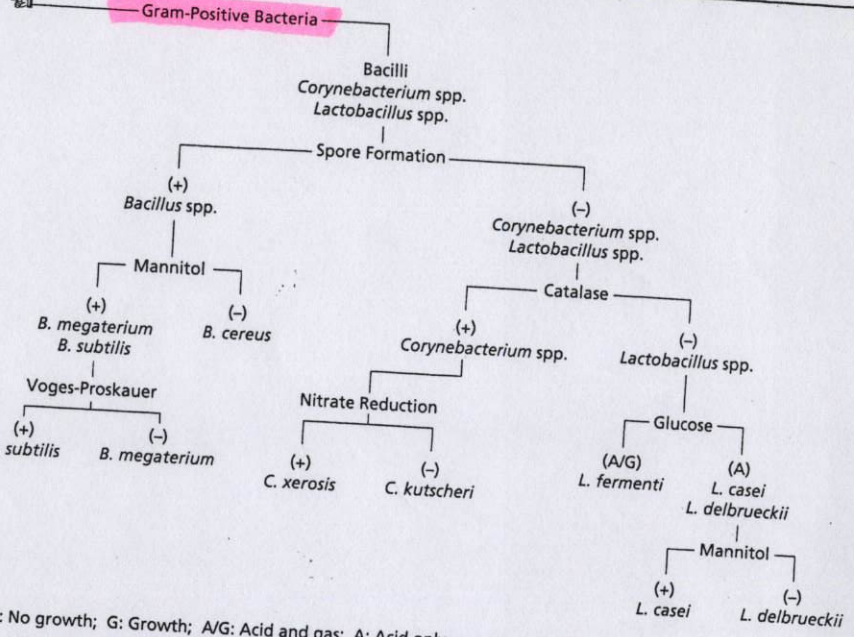

Figure 69.1 Schema for the identification of gram-positive bacteria (continued from facing page)

2. If each Gram-stained preparation is not solely gram-positive or gram-negative, repeat the steps in Sessions 1 and 2 using the refrigerated trypticase soy agar subculture as the test culture.
3. If the isolates are deemed to be pure on the basis of their cultural and cellular morphologies, continue with the identification procedure. During this period and in subsequent sessions, use the dichotomous keys in Figures 69.1 and 69.2 to select and perform the necessary biochemical tests on each of your isolates for identification of their species. Incubate all cultures for 24 to 48 hours at 37°C prior to making your observations.