17 The Genus *Streptococcus*

The genus ***Streptococcus*** includes a broad group of gram-positive, facultatively anaerobic cocci that form chains of varying length. Species of streptococci may be located on the human skin as well as in the mouth, upper respiratory tract, and intestine. Cultivation in a rich medium such as blood agar is often necessary because the organisms lack certain enzyme systems for nutrition. This exercise is concerned with the isolation of streptococci from the upper respiratory tract and oral cavity, and with the study of some of their properties. Special precautions should be taken because the bacteria isolated may be pathogenic.

A. Streptococci from the Upper Respiratory Tract

PURPOSE: **to distinguish hemolytic streptococcus isolated from the throat.**

Streptococci are an important component of the microbiota (normal flora) of the upper respiratory tract. The organisms may be cultivated on an enriched medium that contains blood and is incubated in an environment rich in carbon dioxide and low in oxygen. Certain streptococci will destroy the red blood cells in the medium completely and form a clear zone around the colonies. These are **beta-hemolytic streptococci,** as typified by pathogenic *Streptococcus pyogenes.* Other streptococci cause incomplete destruction of the blood cells, and their colonies are surrounded by an olive green or brown discoloration of the medium. These are **alpha-hemolytic streptococci.** They include *S. mitis* and *S. pneumoniae*, the agent of bacterial (lobar) pneumonia. A final group, the **nonhemolytic streptococci,** cause no hemolysis of red blood cells. *S. lactis*, the organism in yogurt, is a member of the group.

In this section, streptococci will be isolated from the upper respiratory tract, and the various types of hemolysis will be examined.

Special Materials

- Blood agar plates
- Sterile swabs and tongue depressors
- Candle jar (optional)
- Bacitracin disks (0.04 units per disk)
- Gram stain reagents

Procedure

1. If blood agar has not been prepared before the laboratory session, the instructor will demonstrate its preparation. A blood agar base such as trypticase soy agar and defibrinated whole sheep blood will be used. A **5% blood agar** medium will be prepared.
2. Select or prepare a plate of blood agar and, using a felt marker or wax marking pencil, label the bottom side with your name, the date, the name of the medium, and the designation "throat swab." Obtain a sterile swab and sterile tongue depressor.
3. Have a fellow student swab your pharynx (throat) using the sterile swab and tongue depressor. The light should permit good visibility of the pharynx, and the swab should be rolled across the posterior membranes in the region of the tonsils. Care should be taken to avoid touching the tongue or other tissues. Refer to **Figure 17.1** for the proper technique.

When taking a throat culture, be certain the swab and tongue depressor are sterile. They should come from an unopened package or from a container designated "sterile."

4. Apply the bacteria from the swab to one area of the blood agar plate by rubbing the swab on the agar gently as you turn it. Discard the swab in the disinfectant. Then perform the **streak plate technique** as follows: Take a sterile loop and pass it a couple of times across the swabbed area, then continue streaking back and forth into a second area of the plate, as described in Exercise 2A and explained by the instructor. Sterilize the loop and continue the streaking in order to obtain isolated colonies, as explained in Exercise 2A. As a final step, the loop may be stabbed into the agar in a place of high bacterial concentration. This will force bacteria into the medium, and if hemolysis occurs during incubation, it will be particularly visible in this area.
5. Invert the plate and place it in the incubator at 37° C for 24 to 48 hours. Alternately, to obtain an environment rich in CO_2, a wide-mouthed **candle jar** may be used. Place the plate in the jar and stand a candle on the plate.

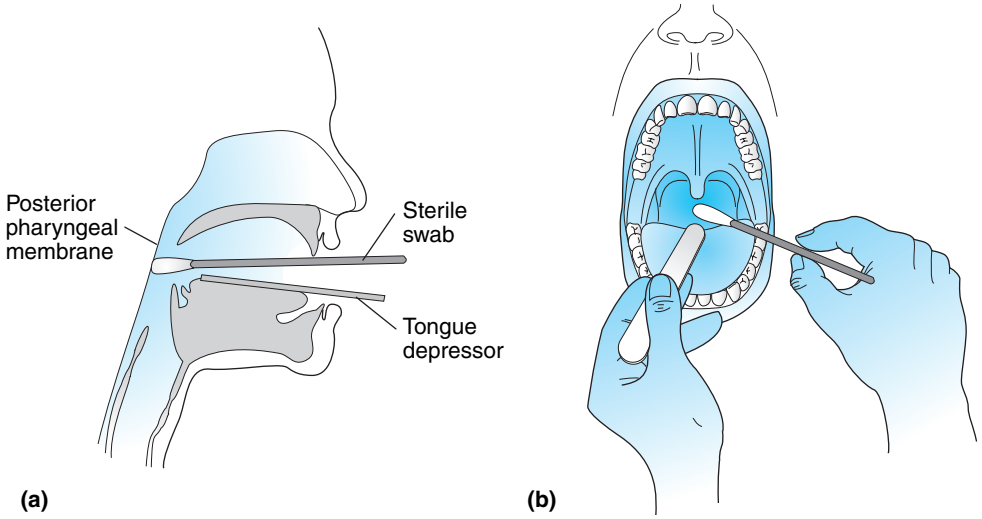


FIGURE 17.1
Two views of the procedure for obtaining a throat swab.

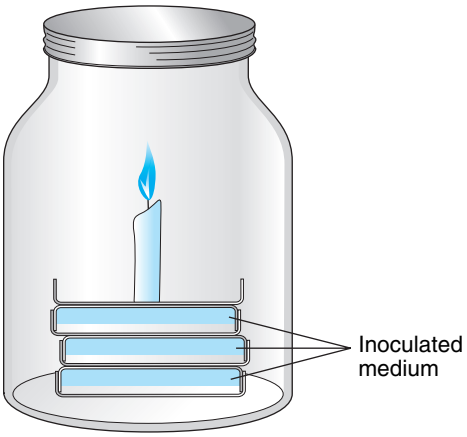


FIGURE 17.2
A candle-jar apparatus made from a wide-mouthed jar.

Then light the candle and replace the lid tightly (**Figure 17.2**). When the candle burns out, the oxygen level will have been reduced, and the carbon dioxide level will be increased. The jar may then be placed in the incubator at 37° C.

6. Following the incubation, examine the plate for evidence of alpha-hemolytic and beta-hemolytic colonies as described in the introductory paragraph to this exercise. To verify the presence and purity of streptococci, prepare air-dried, heat-fixed smears of prospective streptococci and stain them by the **Gram stain technique** (Exercise 6). Gram-positive chains of cocci should be evident. Bacterial capsules may be detected by performing the capsule stain technique described in Exercise 7B. Enter your representations of the plate and smears in the appropriate spaces of the Results section.

7. If a beta-hemolytic species of *Streptococcus* has been isolated, evidence for its grouping may be obtained by performing a **bacitracin disk sensitivity test** as follows: Obtain a sample of bacteria, and streak it on the surface of a blood agar plate using the lawn technique described in Exercise 8.2. Aseptically apply a disk containing 0.04 units of bacitracin (Taxo A disk) to a heavily streaked area, and incubate the plate as above. The presence of a clear ring around the disk indicates that the streptococci have been killed by the antibiotic and that they are probably Group A beta-hemolytic streptococci known as *Streptococcus pyogenes*. The absence of a ring provides evidence that they belong to another group. Enter your results in the appropriate space in the Results section.

B. Streptococci from the Oral Cavity

PURPOSE: **to identify streptococci using selective and enrichment media.**

Many species of streptococci, especially *S. mitis* (alpha-hemolytic) and *S. salivarius* (nonhemolytic), are dominant species in the microbiota of the oral cavity where they are found in the spaces between the teeth and under

the gums (the gingival crevices). Another nonhemolytic species, *S. mutans*, ferments sucrose to acid and is an important cause of tooth decay. Streptococci such as these will be isolated using mitis salivarius agar. This is a selective medium because it contains crystal violet, potassium tellurite, and trypan blue, which inhibit most gram-negative bacilli and gram-positive bacteria other than the streptococci. Todd-Hewitt broth is an enrichment medium containing glucose buffers, sodium carbonate, and disodium phosphate, which favor the growth of hemolytic streptococci.

Special Materials

- Plates of mitis salivarius agar
- Tubes of Todd-Hewitt broth
- Sterile swabs
- Gram stain reagents

Procedure

1. Select one plate of **mitis salivarius agar** (or pour one plate) and one tube of **Todd-Hewitt broth**, and label them as described in Part A.
2. Use a sterile swab to rub vigorously in the gingival crevice and space between the teeth. Then apply the swab to one small area of the mitis salivarius agar plate. Use a sterile loop to streak for isolated colonies, as outlined in Exercise 2A. Incubate the plate at 37° C for 24 to 48 hours. A candle jar may be used to decrease the oxygen concentration (as in Part A).
3. Take a fresh sterile swab and rub an area of the gingival crevice and space between the teeth. Place the swab in the tube of Todd-Hewitt broth and break off the end, thereby allowing the swab to fall into the medium. The instructor may demonstrate. Incubate the tube at 37° C for 24 to 48 hours.
4. Observe the mitis salivarius plate for evidence of streptococcal colonies. *S. mitis* will appear as flat, blue colonies with dark centers. *S. salivarius* produces large, blue domed-shaped colonies with a gum-drop appearance. *S. mutans* can be recognized by blue colonies having a domed brown center. Gram-negative and non-streptococcal gram-positive bacterial growth is inhibited in this agar medium. Gram stains should be prepared from the colonies to verify the presence and purity of streptococci. Representations may be placed in the appropriate space together with your detailed observations.
5. Observe the Todd-Hewitt broth for evidence of growth as a sediment at the bottom of the tube. The broth is used primarily to enrich the growth of pathogenic (beta-hemolytic) streptococci. Since streptococci form long chains in broth, Gram stained smears should reveal characteristic streptococcal formations. Be careful to avoid cotton fibers from the swab when taking samples. Drawings should be entered in the Results section.

Questions

1. What positive role may be played by the nonpathogenic streptococci normally found between the teeth and in the gingival crevice?
2. List the precautions that should be taken when obtaining a pharyngeal (throat) swab.
3. When strep throat is suspected, the physician often will recommend that a pharyngeal (throat) swab be taken and cultivated on blood agar. Why?
4. Is the mitis salivarius agar a selective or differential medium for streptococci? Explain.
5. Explain how beta-hemolysis may be distinguished from alpha-hemolysis.

Name _____

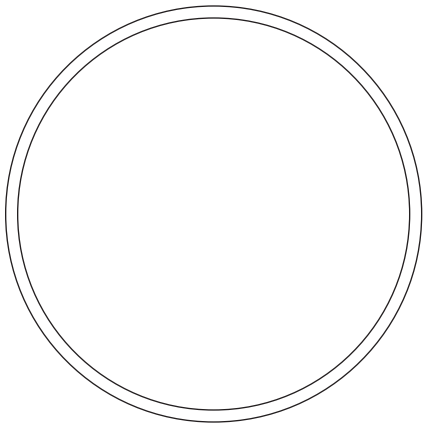
Date _____ Section _____

Exercise 17 Results

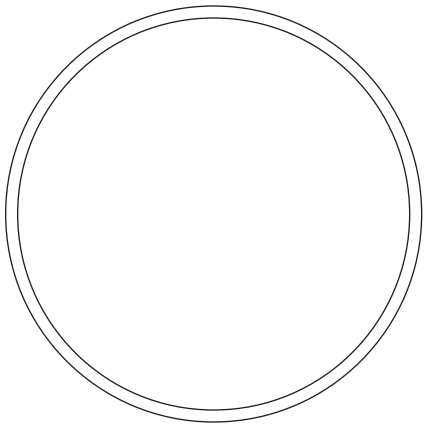
The Genus *Streptococcus*

A. Streptococci from the Upper Respiratory Tract

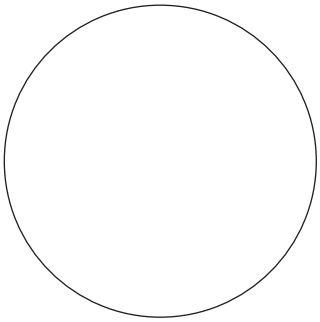
Blood Agar Plate

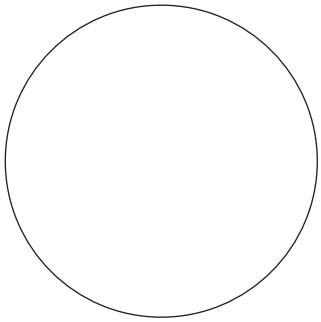


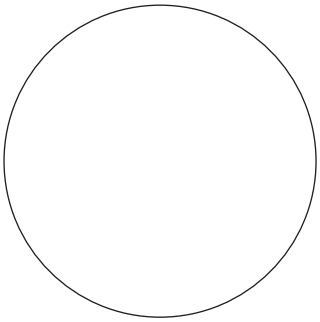
Bacitracin Disk Plate



Stained Smears of Streptococci







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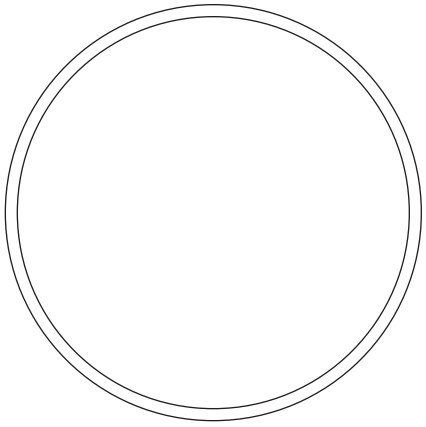
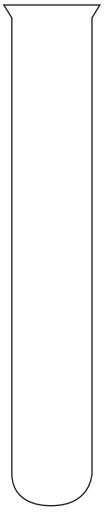
B. Streptococci from the Oral Cavity

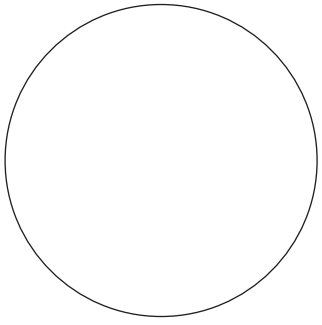
Mitis Salivarius Agar Plate

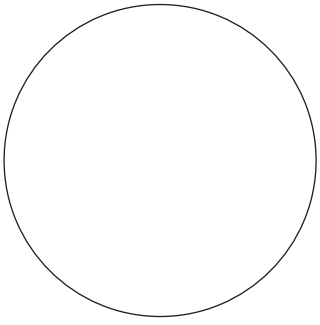


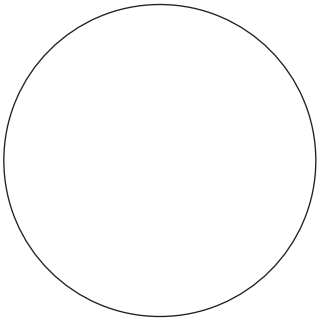
Todd-Hewitt Broth



Stained Smears of Streptococci







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Observations and Conclusions: _____

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