LESSON ASSIGNMENT

LESSON 3
Common Tests and Gram-Positive Cocci.

TEXT ASSIGNMENT
Paragraphs 3-1 through 3-32.

LESSON OBJECTIVES
Upon completion of this lesson, you should be able to:

3-1. Make correct paired associations among the name of the test, its principle, its diagnostic significance, possible results, and interpretation of results for each of the following:

a. Bile solubility test.
b. Carbohydrate fermentation test.
c. Catalase test.
d. Citrate test.
e. Coagulase test.
f. Ferric chloride test.
g. Gelatin liquefaction test.
h. Hydrogen sulfide test (lead acetate paper test).
i. Indole test.
j. Methyl red test.
k. Nitrate reduction test.
l. Oxidase test.
m. Urease test.
n. Voges Proskauer test.

3-2. Make correct paired associations among names of organisms and discriminating characteristics of colony morphology, gram morphology, biochemical reactions, biochemical reactions, pathogenicity, and interpretation of results for:

a. Staphylococcus aureus and staphylococcus epidermidis.
b. Hemolytic groups and Lancefield groups of streptococci.
c. Streptococcus (Pneumococcus) pneumoniae.

SUGGESTION
After studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.
LES S ON 3

COMMON TESTS AND GRAM-POSITIVE COCCI

Section I. COMMON TESTS

3-1. BILE SOLUBILITY TEST

a. Principle. Pneumococci (Streptococcus pneumoniae) are lysed in the presence of bile salts; other streptococci are not lysed. This test is used to distinguish between pneumococci and other alpha hemolytic streptococci.

b. Reagents and Media.

   (1) Two percent sodium deoxycholate (bile salt). Dissolve 2 g of sodium deoxycholate in a small amount of distilled water. Add sufficient distilled water to make a total volume of 100 ml.

   (2) Physiological saline. Dissolve 8.7 g of sodium chloride (NaCl) in approximately 100 ml of distilled water. Add enough distilled water to make a total volume of 1,000 ml. As required, pour into flasks, plug, and sterilize in an autoclave.

   (3) Phenol red indicator.

   (4) 0.1N sodium hydroxide. Using a pipet, transfer 1 ml of 10N or 40 percent sodium hydroxide to a 100-ml volumetric flask and add enough distilled water to make 100 ml.

   (5) Thioglycollate broth or Todd-Hewitt broth.

c. Technique.

   (1) Inoculate a tube of thioglycollate broth or a tube of Todd-Hewitt broth with the bacteria in question.

   (2) Incubate the broth culture for 18-24 hours at 37° C.

NOTE: An alternative procedure is to prepare a heavy suspension of the bacteria to be tested in 1.0 ml of physiological saline and proceed to step 3.

   (3) Because of the fermentation of glucose and the resulting lactic acid formation in streptococcal or pneumococcal cultures, adjustment of pH to neutral is necessary before proceeding with the test. Adjust the pH of the broth culture (or saline suspension) by adding one drop of phenol red indicator (or other suitable indicator) to the latter and then titrating with a few drops of 0.1N sodium hydroxide until a neutral pH is obtained. If phenol red is used, the neutral pH is manifested by a pink color.
(4) Place 0.5 ml of the neutralized suspension into each of two clean Kahn tubes.

(5) Label one tube test and add to this tube 0.5 ml of 2 percent of sodium deoxycholate.

(6) Label the second tube control and add to this tube 0.5 ml of physiological saline.

(7) Place both tubes in the incubator at 37º C and observe at 1, 2, and 3 hours for clearing in the tube labeled test (the tube containing the sodium deoxycholate).

d. Interpretation.

(1) If the organism is Streptococcus pneumoniae, clearing should occur in the tube containing the sodium deoxycholate within the 3-hour incubation period. Other alpha streptococci cultures remain turbid like the control.

(2) Regard as negative all tubes that do not clear within the 3 hours of incubation.

(3) The cultures must be neutralized (step (3)) because sodium deoxycholate in an acid solution may form a precipitate or gel that interferes with the reading of the test.

3-2. CARBOHYDRATE FERMENTATION TEST

a. Principle. In media containing carbohydrates, certain bacteria form acids. Some of these bacteria will produce gases, in addition to acids, by furthering the fermentation process.

b. Reagent and Medium. Use purple broth base containing 1 percent concentration of the desired carbohydrate. (This medium contains bromcresol purple as an indicator.)

c. Technique.

(1) Inoculate the organism to a small tube containing an inverted Durham tube in approximately 5 ml of the 1 percent carbohydrate base.

(2) Incubate the tube for 18 to 48 hours at 37º C.

(3) Observe for evidence of acid and gas production.
d. **Interpretation.** Fermentation to acid is evidenced by the medium's being converted from purple to distinct yellow. Yellow broths should be examined carefully for the possible presence of gas, which is observed as a bubble within the inverted Durham tube.

### 3-3. CATALASE TEST

a. **Principle.** Staphylococci produce the enzyme catalase which, when mixed with hydrogen peroxide, will liberate oxygen from the hydrogen peroxide with the occurrence of vigorous bubbling.

b. **Reagent.** Use 30 percent hydrogen peroxide (superoxol).

c. **Interpretation.**

   (1) Immediate bubbling in the drop of hydrogen peroxide signifies a positive test.

   (2) Staphylococci are catalase positive. Absence of immediate bubbling signifies a negative test. Streptococci and pneumococci are catalase negative.

   (3) If the loop digs into blood agar medium, blood cells from blood agar may produce a false-positive reaction.

   (4) Platinum wire may produce Nichrome wire can be used satisfactorily.

d. **Technique.**

   (1) Place a drop of hydrogen peroxide on a clean glass slide.

   (2) Pick the colony (colonies) in question from an agar plate by means of a wire loop.

   (3) Touch the loop containing the bacteria to the drop of hydrogen peroxide and observe for immediate bubbling.

   (4) Similarly test a known *Staphylococcus* specifies to verify hydrogen peroxide activity.
3-4. CITRATE TEST

a. **Principle.** This test is based on the ability of certain bacteria to utilize sodium citrate as the sole available source of carbon in a chemically defined medium.

   b. **Medium.** Use Simmons citrate agar.

   c. **Technique.**

      (1) Lightly inoculate, by streaking only, an agar slant of Simmons citrate agar.

      (2) A positive reaction (growth) is accompanied by an alkaline reaction resulting in a change in the green color of the medium to a deep blue color.

      (3) No change in the indicator or the absence of blue color in the green medium indicates a negative test.

      (4) A light inoculum must be used to insure that no nutrients are transferred to the chemically defined medium.

3-5. COAGULASE TEST

a. **Principle.** Coagulase activity is essentially confined to staphylococci and is related to the pathogenic species of staphylococci. Coagulase activity is demonstrated when a species of staphylococci capable of producing the enzyme coagulase is added to human or rabbit plasma and a thrombus or clot is formed. This procedure can be performed on a glass slide or in a test tube.

   b. **Reagents.**

      (1) Plasma, human or rabbit, fresh or dehydrated.

      (2) Physiological saline (para 3-1b(2)).

   c. **Slide Methods.**

      (1) Place a drop of physiological saline on a clean glass slide and prepare a rather heavy, even suspension of staphylococci in the drop of saline.

      (2) Place a loopful of fresh or recently reconstituted dehydrated plasma in the suspension of staphylococci. Mix and then withdraw the loop.

      (3) Immediately observe for the formation of a clot. This usually occurs within a few seconds with coagulase-positive species.
d. **Tube Methods.**

(1) Transfer 0.5 ml of a 24-hour broth culture of staphylococci or transfer a large loopful of growth from an agar plate of staphylococci to 0.5 ml of human or rabbit plasma in a glass tube.

(2) Incubate the tube at 37º C preferably in a water bath for 3 hours. Observe approximately every 30 minutes for clotting. (Since a few strains do not clot within this period, the reading should be checked after 24 hours' incubation.)

e. **Interpretation.**

(1) Pathogenic species of staphylococci usually give a positive reaction that is evidenced when the plasma is coagulated and produces a visible clot.

(2) In negative reactions the suspension remains homogeneous and coagulation does not take place.

(3) A positive reaction, whether it be on a glass slide or in a tube is decisive. A negative slide test must be confirmed by a tube test.

3-6. **FERRIC CHLORIDE TEST**

a. **Principle.** This test detects the hydrolysis of sodium hippurate to benzoic acid in the presence of group B beta hemolytic streptococci. The benzoic acid combines with ferric chloride to form an observable, permanent precipitate.

b. **Reagent and Medium.**

(1) 12 percent ferric chloride. Dissolve 12.9 of ferric chloride (Fe C₁₃) in less than 100 ml of 2 percent hydrochloric acid. Add enough 2 percent hydrochloric acid to make a total volume of 100 ml.

(2) 1 percent sodium hippurate broth,

c. **Technique.**

(1) Add 0.2 ml of the reagent to 0.8 ml of a 24-hour culture prepared in sodium hippurate broth.

(2) Mix immediately and observe after 5-10 minutes.

d. **Interpretation.**

(1) The formation of a permanent precipitate after approximately 10-15 minutes indicates a positive reaction.
(2) It is important to measure accurately the amounts of reagent and medium used in the test to prevent the redissolving of benzoic acid in an excess of the reagent.

(3) If evaporation of the broth medium occurs during incubation, replace the evaporated volume with distilled water to restore the original concentration of sodium hippurate.

3-7. GELATIN LIQUEFACTION TEST

a. Principle. Some bacteria possess the ability to produce proteolytic enzymes that can liquefy gelatin. Gelatin is a protein substance lacking essential amino acids and it is normally a liquid above 25º C.

b. Medium. Use ordinary extract or infusion broth containing 10 to 15 percent gelatin.

c. Technique.

(1) Inoculate a tube of solidified (refrigerated) nutrient gelatin by stab technique.

(2) Incubate the inoculated tube and a control gelatin tube at 37º C at which temperature the gelatin will liquefy.

(3) At the end of each 24-hour incubation period, place the inoculated tube and the control tube of liquefied gelatin in a refrigerator, for a sufficient length of time to determine whether digestion of gelatin has occurred. Check the tubes for liquefaction in this manner for as long as two weeks unless digestion occurs sooner.

d. Interpretation.

(1) If a portion of gelatin liquefies, this indicates a positive test.

(2) If the gelatin remains solid, this indicates a negative test.

(3) The test is frequently used to distinguish *Enterobacter cloacae*, which usually liquefies gelatin (delayed positive), from other members of the Enterobacteriaceae, primarily the genus *Klebsiella*, which is negative.

(4) Coagulase-positive staphylococci usually liquefy gelatin.
(5) An alternate procedure less frequently used is to proceed from step (1) to an incubation at 20º C at which temperature the gelatin will remain solid. In this method, the shape of the portion of gelatin being liquefied can be observed. The procedure outlined above appears to be more popular because the shape of the liquefied portion is less important than the time lost during the slower incubation at 20º C.

3-8. HYDROGEN SULFIDE TEST (LEAD ACETATE PAPER TEST)

a. **Principle.** The production of hydrogen sulfide can be detected in bacterial cultures by observing blackening of the medium that is produced when hydrogen sulfide comes into contact with certain metals, i.e., lead, iron, and bismuth, producing the sulfides of these metals. In triple sugar iron (TSI) medium, ferrous sulfate is incorporated as an indicator for hydrogen sulfide production. An alternative procedure consists of testing a growing culture for hydrogen sulfide by using lead acetate paper. The latter technique is described below.

b. **Reagent--10% Lead Acetate.** Dissolve 10 g of lead acetate Pb(C₂H₃O₂)₂•3H₂O in less than 100 ml of distilled water. Add enough distilled water to make a total volume of 100 ml.

c. **Technique.**

   (1) Cut a sheet of filter paper into strips approximately 6x65 mm in size.

   (2) Soak the strips of filter paper in 10 percent lead acetate solution and allow them to air-dry.

   (3) Autoclave the lead acetate paper at 121º C, 15 pounds pressure, for 15 minutes in Petri dishes.

   (4) Place one of the strips in the mouth of each culture tube so that a portion of the strip projects well below the cotton plug.

   (5) Incubate the tubes at 37º C and examine daily to determine if blackening of the strip has occurred.

d. **Interpretation.**

   (1) A positive test is indicated by blackening of the strip.

   (2) No evidence of blackening of the strip indicates that the test reaction is negative.
(3) The filter paper technique is especially useful for detection of Brucella species and for distinguishing among the species of Brucella. When an attempt is made to distinguish among the Brucella species, the filter paper strips must be changed daily after the first appearance of blackening.

3-9. INDOLE TEST

a. **Principle.** This test is based on the ability of certain bacteria to split the amino acid tryptophan into alanine and indole. The liberated indole combines with paradimethyaminobenzaldehyde in Kovac's reagent to give a deep red color.

b. **Reagents and Media.**

   (1) Kovac's reagent. Dissolve 5 g of paradimethyaminobenzaldehyde (chemically pure) in 75 ml of amyl or butyl alcohol. Add enough concentrated hydrochloric acid (HCl) to make a total volume of 100 ml.

   (2) Tryptophan broth OR Trypticase nitrate broth.

c. **Technique.**

   (1) Add 0.5 ml of Kovac's reagent to 3 to 5 ml of a 24 to 48 hour broth culture of the organism.

   (2) Observe for the appearance of a red color at the interphase between the reagent and the broth culture.

d. **Interpretation.**

   (1) The presence of the red color at the interphase between the reagent and the broth culture signifies that indole has been liberated.

   (2) The absence of the red color signifies a negative test.

3-10. METHYL RED TEST

a. **Principle.** When they ferment glucose, some organisms produce a small quantity of acids that are then converted to neutral end products. This test is designed to differentiate those organisms which, in contrast, produce a high acidity.

b. **Reagent and Medium.**

   (1) Methyl red indicator. Dissolve 0.02 g of methyl red in 50.0 ml of 95 percent ethyl alcohol and add 50.0 ml of distilled water.

   (2) MR-VP (methyl red-Voges-Proskauer) broth (Clark and Lubs medium).
c. **Technique.** Add about 5 drops of methyl red indicator to a 5 ml portion of a 2 to 7 day-old culture, prepared in MR-VP broth.

d. **Interpretation.**

(1) A positive reaction occurs when the culture is sufficiently acid to turn the methyl red indicator to a distinct red color.

(2) A yellow color indicates a negative test.

3-11. **NITRATE REDUCTION TEST**

a. **Principle.** Bacteria are grown in a broth culture containing potassium nitrate to determine the ability of the bacteria to reduce nitrate to nitrite.

b. **Reagents and Medium.**

(1) Solution A. Dissolve 8 g of sulfanilic acid in less than 1,000 ml of 5N acetic acid (this consists of 1 part of glacial acetic acid and 2.5 parts of distilled water). Add enough 5N acetic acid to make a total volume of 1,000 ml.

(2) Solution B. Place 6 ml of N,N-dimethyl-1-naphthy-lamine, in a 1-liter flask and fill to the 1,000-ml mark with 5N acetic acid. **CAUTION:** Avoid aerosols, mouth pipetting, and contact with skin due to possible carcinogenicity. (A similar chemical, alpha-naphthylamine, is listed as a carcinogen.)

(3) Zinc dust.

(4) Mix 5 g of tryptone, 5 g of neopeptone, and 1,000 ml of distilled water. Boil and adjust the pH to 7.3-7.4. Add 1 g of potassium nitrate (reagent grade) and 0.1 g of glucose. Dispense 5 ml per tube and sterilize at 121°C, 15 pounds pressure, for 15 minutes.

c. **Procedure.**

(1) Add 1 ml of solution A to approximately 5 ml of a 24-hour culture grown in nitrate broth.

(2) Add, dropwise, 1 ml of solution B and observe for a light pink to deep red color.

d. **Interpretation.**

(1) A light pink to deep red color is a positive result. Nitrate has been reduced to nitrite by the bacteria.
(2) The absence of color is an equivocal result. Add a small amount of zinc dust to the colorless mixture of culture and reagents.

(a) If the solution is colorless after the addition of zinc, the result is positive. Nitrates have been reduced first to nitrites and then further reduced.

(b) If the solution is red after the addition of zinc, the result is negative. Nitrates have been reduced by the zinc but not by the bacteria.

3-12. OXIDASE TEST

a. Principle. This test detects the enzyme oxidase produced by members of the genus Neisseria and the genus Pseudomonas. Oxidase reacts with the reagent to produce a colored compound.

b. Reagent. Add 0.1 g of tetramethyl-p-phenylenediamine hydrochloride to 10 ml of distilled water. Allow to stand for 15 minutes before using, but use within 2 hours or it will begin to lose its activity.

c. Technique.

(1) Place 2 to 4 drops of reagent on a strip of filter paper.

(2) Remove a portion of the colony with a sterile platinum loop. (Iron-containing wire may give a false-positive reaction.)

(3) Rub the portion of the colony on the impregnated paper.

(4) A positive reaction occurs if the moist paper near the bacteria turns dark purple within 10 seconds.

d. Alternate Method.

(1) Use a 1 percent solution (0.1 g in 10 ml) of dimethyl-p-phenylenediamine hydrochloride with the same time restrictions stated in b above. This reagent is less sensitive and more toxic than the one above.

(2) Place 2 to 3 drops of suspected colonies of the culture plate.

(3) Oxidase-positive colonies develop a pink color that gradually turns maroon, then dark red, and finally black.

e. Note that the oxidase test does not interfere with the gram reaction. A gram stain must be performed on all oxidase-positive colonies; gram-negative diplococci that are oxidase positive may be tentatively identified as Neisseria.
3-13. UREASE TEST

a. **Principle.** The splitting of urea to ammonia and carbon dioxide by the enzyme urease may be employed to help differentiate Proteus species from other members of the enteric group. Proteus species and other organisms hydrolyze urea and release ammonia, which is indicated by a reddening of the indicator phenol red, included in the medicine.

b. **Medium.** Use either urea agar (Christensen) slants or urease test broth (Rustigian and Stuart).

c. **Technique.**

   (1) Transfer a heavy inoculum to the urea medium. (The broth should be shaken to suspend the bacteria.)

   (2) Incubate at 37º C.

   (a) Agar slants should be observed at 2 hours, 4 hours, and after overnight incubation. Negative tubes should be observed daily for 4 days for delayed reactions due to non-Protéeus species.

   (b) Broth cultures should be read after 10 minutes, 60 minutes, and 2 hours. Longer incubation is required for non-Protéeus species.

d. **Interpretation.** Protéeus species give a red color, a positive test in 2 to 4 hours. Other urease-positive organisms produce a red color much more slowly. If no red color is produced, the test is negative.

3-14. VOGES-PROSKAUER (VP) TEST

a. **Principle.** This test detects the production of the neutral end product acetyl methylcarbinol during fermentation of dextrose. A positive test, a red color, is characteristic of Enterobacter cloacae, Enterobacter aerogenes, and Klebsiella species. A negative test is characteristic of E. coli, Shigella, and Salmonella.

b. **Reagents and Medium.**

   (1) Five percent alpha-naphthol. Dissolve 5g of alpha -naphthol in less 100 ml of absolute ethyl alcohol. Add enough absolute alcohol to make a total volume of 100 ml.
(2) Forty percent potassium hydroxide (KOH). Because of its hygroscopic nature, 40 g of potassium hydroxide must be rapidly weighed. Dissolve in less than 100 ml of distilled water. (The container may be placed in a sink with cold circulating water to control temperature.) Cool and add sufficient distilled water to make a total volume of 100 ml.

(3) MR-VP (methyl red-Voges-Proskauer) broth (Clark and Lubs medium).

c. Technique.

(1) Add 0.6 ml of 5 percent alpha-naphthol in absolute ethyl alcohol to 1 ml of a 24 to 48 hour broth culture prepared in MR-VP broth.

(2) To the same culture, add 0.2 ml of 40 percent potassium hydroxide.

(3) Shake the tube and allow it to stand for 5 to 10 minutes before interpreting results.

d. Interpretation.

(1) A positive test is indicated by the development of a pink or red color.

(2) A yellow color is regarded as a negative.

(3) Occasionally, some difficulty is experienced in obtaining a positive VP result with organisms that should produce acetyl methylcarbinol. This may be overcome by gently heating the culture after adding the test reagents. Strains that do not produce acetyl methylcarbinol (VP-negative) will still yield a negative result despite the application of gentle heat.

Section II. GRAM-POSITIVE COCCI: STAPHYLOCOCCI

3-15. GENERAL COMMENTS ABOUT GRAM-POSITIVE COCCI

The organism comprising the gram-positive cocci are chiefly the staphylococci, streptococci, and pneumococci. Collectively, these organisms are responsible for a variety of human infections. Such infections range from relatively simple involvements of the skin and mucous membranes to more serious diseases that may be manifested in pneumonia, septicemia, rheumatic fever, acute glomerulonephritis, or deep tissue abscesses. An identification schema for aerobic gram-positive bacteria is provided in figure 3-1.
Figure 3-1. Identification schema for aerobic gram-positive bacteria.
3-16. PATHOGENICITY OF STAPHYLOCOCCI

a. **Common Manifestations.** The staphylococci (figure 3-2) are ubiquitous in nature. They occur as normal inhabitants on the skin and in the respiratory and gastrointestinal tracts of man. The majority of such forms are the comparatively avirulent organisms. *Staphylococcus epidermidis* and the related forms of *Micrococcus* and *Sarcina* are saprophytes frequently isolated from the skin and mucous membranes. *Staphylococcus aureus* strains are usually responsible for the staphylococcal diseases of man. These forms occur especially in the upper respiratory tract of asymptomatic individuals. The asymptomatic carrier is of considerable importance in transmitting these organisms. Staphylococcal diseases are most commonly manifested in localized suppurations that may be in the form of simple pustules, hair follicle infections, boils, or extensive carbuncular conditions that may progress to form metastatic abscesses in any tissue. The latter results from the spread of the organism via the blood stream. A majority of cases of osteomyelitis, enterocolitis, otitis media, and sinusitis are of staphylococcal etiology. Pneumonia, meningitis, and endocarditis are relatively infrequent manifestations of the staphylococci.

![Figure 3-2. Gram-stained smear of *Staphylococcus* species from broth culture.](image)

b. **Enzyme Secretions.** The virulent staphylococci excrete a variety of substances that account for their ability to invade tissue and cause disease in man. Coagulase is an enzyme produced by pathogenic staphylococci. Coagulase causes a clotting of plasma which results in the formation of a layer of fibrin around a given staphylococcal lesion. Although this fibrin wall may confine the infection to a localized process, it also serves as a protective barrier for the organism against phagocytic activity and the action of antimicrobial drugs. This, then, is one of the very valuable clinical laboratory tests used to identify *Staphylococcus aureus*. 
c. **Toxic Production.** A variety of toxins may appear in cultures 1 to 3 days old when grown in a semisolid infusion agar of veal or beef. These cultures must be incubated in an atmosphere of 20 to 40 percent carbon dioxide. The exotoxins may be destroyed by heating to 55º to 60º C. Any of the following may be present in such a filtrate: lethal toxin, hemolysin, leukocidin, and dermonecrotic toxin.

d. **Hospital Staphylococci.** Staphylococci have eventually become resistant to practically every antibiotic introduced to combat its presence. Each succeeding generation seems to be more resistant to drugs than the previous parental strain. This has been primarily due to the promiscuous use of antibiotics to treat everything from a cold to a sore toe. Since the advent of penicillin, when there were almost no drug-resistant strains, certain staphylococcus species have been particularly adept at developing drug resistance. Since antibiotics were originally administered in low doses for such widely divergent ailments, those strains of organisms which were not eliminated by use of drugs have been able to develop into strains which are particularly pathogenic and resistant to almost every antibiotic that has been developed. This has led to the necessity of determining a series of tests to discover which antibiotic is effective for each patient. The antibiotic that is more effective against one strain of cocci for one person may not be best for another person with a different strain. Some larger laboratories perform phage tying of hospital staphylococci for epidemiologic purposes.

### 3-17. CULTURAL CHARACTERISTICS OF STAPHYLOCOCCI

a. Staphylococci are nonmotile, nonsporogenous, and usually do not form capsules. The exception to capsule formation is in very young broth cultures after a few hours' incubation. These bacteria always stain gram-positive. Those cocci not staining gram-positive are due to old and dying cultures, organisms phagocytized by white cells, and those in the center of clusters.

b. The staphylococci grow readily on ordinary nutrient media without the presence of special enrichments. The more commonly occurring species are facilitative organisms. A few staphylococci, which may be weakly pathogenic, are strict anaerobes. The staphylococci isolated from human disease grow well at 37º C. Abundant growth usually takes place in 18 to 24 hours of incubation.

c. The colony morphology of *Staphylococcus* species is usually characteristic. After 1.8 hours of incubation at 37º C on an agar media, staphylococci form rather large colonies ranging from 2 to 4 mm in diameter. The colonies are opaque, round, smooth, raised, glistening and with an entire (even) margin. The colonies may develop a characteristic golden, porcelain-white, or lemon-yellow pigment. The colony is soft or "butter-like" in consistency.
d. Pigmentation is more evident after plates of the cultivated organism have been exposed to room temperature overnight. Young colonies of staphylococci are not pigmented and appear colorless. As growth continues, a pigmentation results which will not diffuse into the surrounding medium. This is what gives pus and sputum a faint golden yellow color and usually indicates possible infection with staphylococci. All of the characteristics given up to this point relate to the genus *Staphylococci*.

e. Colonies of *Staphylococcus aureus* typically have a golden pigmentation on initial isolation while colonies of *Staphylococcus epidermidis* are usually white when cultivated on blood agar. When grown on blood agar, *Staphylococcus aureus* usually causes beta hemolysis (complete lysis of the RBC’s), resulting in a clear zone around the colony, while *Staphylococcus epidermidis* generally does not hemolyze the RBC’s.

### 3-18. LABORATORY IDENTIFICATION OF STAPHYLOCOCCI

a. It must be emphasized that on the basis of gram morphology *Staphylococcus aureus* and *Staphylococcus epidermidis* cannot be differentiated; however, the genus can usually be established on the basis of gram stain morphology. *Staphylococcus* species are gram-positive. Members of the genus *Staphylococcus* are spherical or oval cocci measuring approximately 1 micron in diameter. Most species are typically arranged in irregular clumps resembling grapelike clusters. In broth cultures in particular, single cocci, pairs, and occasional short chains may also be seen. Related organisms belonging to the genus *Gaffkya* and the genus *Sarcina* are morphologically similar to the *staphylococci* except that: they usually occur in uniform groups of four and eight cells respectively.

b. Inoculation and incubation of blood agar would reveal the typical colony morphology. Again, no species differentiation is possible since hemolytic and pigmentation patterns are variable.

c. A medium that is especially useful when working with clinical specimens suspected of containing *Staphylococcus* species is mannitol salt agar (MSA). This medium contains 7.5 percent sodium chloride, and this concentration of salt is inhibitory to most bacteria. *Staphylococcus* species are halophilic (salt-loving), and are therefore able to grow on MSA. The medium also contains the carbohydrate mannitol that is usually fermented by *Staphylococcus aureus*, thus yielding acid conditions. *Staphylococcus epidermidis* does not usually ferment this sugar. The indicator phenol red is also incorporated into this medium. Under acid conditions, phenol red will give a yellow color; under alkaline conditions, a red color will result. *Staphylococcus aureus* usually ferments mannitol with the development of a yellow color in the medium around the colonies. *Staphylococcus epidermidis* does not usually ferment mannitol, and the indicator imparts a redder shade to the medium.

d. The most important differentiating test for species identification is the coagulase test. This test positively differentiates the pathogenic *Staphylococcus aureus* from the nonpathogenic *Staphylococcus epidermidis*. In terms of result, *Staphylococcus aureus* is coagulase positive while *Staphylococcus epidermidis* is coagulase negative.
3-19. CONFIRMATORY PROCEDURES

To establish identity, select an isolated Staphylococcus-like colony and proceed with the following studies.

a. **Gram Stain Smear.** Prepare gram-stained smears and examine for the typical gram-positive cocci in grape-like clusters.

b. **Catalase Test.** Perform the catalase test (Lesson 5) to distinguish staphylococci from streptococci. Staphylococci produce the enzyme catalase, which when mixed with hydrogen peroxide will liberate oxygen from the hydrogen peroxide to produce vigorous bubbling. Immediate bubbling in the drop of hydrogen peroxide signifies a positive test. Staphylococci are catalase positive. Absence of immediate bubbling signifies a negative test. Streptococci and pneumococci are catalase negative. If the loop digs into blood agar medium, blood cells from blood agar may produce a false-positive reaction.

c. **Coagulase Test.** Pathogenic species of staphylococci usually give a positive reaction that is evidenced when the plasma is coagulated and produces a visible clot. In negative reactions the suspension remains homogeneous and coagulation does not take place. A positive reaction, whether it be on a glass slide or in a tube is decisive. A negative slide test must be confirmed by a tube test. See Lesson 5 for details.

d. **Mannitol Salt Agar.** Inoculate a mannitol salt agar plate and observe for growth and fermentation of the mannitol at 18 to 24 hours of incubation at 37º C. If the mannitol is fermented, the plate will turn from a reddish-pink color to yellow.

e. **Liquefaction.** Inoculate a tube of gelatin by stabbing some growth into the medium. Incubate at room temperature for 3 to 5 days and observe for liquefaction.

f. **Primary Means of Distinguishing S. aureus from S. epidermidis.** Although pigmentation, hemolysis, and gelatin liquefaction are all demonstrable features of most Staphylococcus aureus strains, the index of their pathogenicity is coagulase production and mannitol fermentation.

Section III. GRAM-POSITIVE COCCI: STREPTOCOCCI

3-20. INTRODUCTION TO THE STREPTOCOCCI

a. The streptococci (figure 3-3) are spherical to ovoid cocci ranging between 0.8 and 1.0 micron in diameter. The cocci predominantly occur in chains; however, paired or single cells may also be observed. Characteristic chains are more typical in smears from broth cultures. The streptococci are nonmotile and nonsporeforming. They are typically stained gram-positive, although gram-negative forms are occasionally observed in specimens of old cultures.
b. Certain *Streptococcus* species produce polysaccharide capsules, while others produce a capular substance composed of hyaluronic acid. The presence or absence of capsules is not a distinct feature for use in routine identification of streptococcal forms.

c. Several schemes for classifying the streptococci have been devised. The Lancefield classification has as its basis the antigenic structure of the organisms. The structure of a carbohydrate antigen ("C" substance) is different for each group in this series. The groups are designated by the letters A through O. According to this classification, group A strains are the most common human pathogens.

d. A second classification, devised by Sherman, has as its basis both the physiologic and immunologic characteristics of the streptococci. The Sherman classification is composed of the pyogenic streptococci, the viridans group, the enterococci, and the lactic streptococci. The human pathogens are in the pyogenic group.
3-21. HEMOLYTIC PATTERNS

The most useful method for preliminary differentiation of streptococci is the pattern of hemolysis on blood agar.

a. The hemolytic action of *Streptococcus* species is influenced by the type of blood used in blood agar. The blood of choice for study of hemolysis is defibrinated sheep blood in a concentration of 5 percent. Glucose should be excluded since it may obscure hemolytic reactions. If sheep blood is not available, use rabbit or horse blood.

b. The pour plate method (see Lesson 2, Section II) is by far the best method for studying hemolytic activity; it provides subsurface colonies of streptococci whose hemolytic activity is not greatly affected by oxygen. For convenience, a general practice is to study the hemolytic patterns of surface colonies. It must be remembered, however, that the hemolytic activity of such surface colonies may not be completely typical.

c. The four types of hemolytic patterns, which are best observed under low power (100X) magnification, are defined as follows:

1. **Alpha.** An indistinct zone around a colony in which the red cells are partially destroyed. There is often a greenish or brownish discoloration of the medium near the colony.

2. **Beta.** A clear, colorless zone around a colony that indicates complete lysis of the red cells. This is best seen in deep colonies in a pour plate since oxygen affects the activity of hemolysins and may make surface colonies appear to be alpha or nonhemolytic.

3. **Gamma.** No apparent hemolytic activity or discoloration around the colony.

4. **Alpha prime or wide zone alpha.** A small halo of intact or partially lysed cells immediately surrounding the colony, with a zone of complete hemolysis extending further into the medium. Without a microscope, this can be composed with beta hemolysis.

3-22. PATHOGENICITY OF THE STREPTOCOCCI

a. The majority of streptococcal infections of man are caused by beta hemolytic streptococci. A variety of diseases are manifested such as: puerperal fever, erysipelas, septic sore throat scarlet fever, impetigo and acute bacterial endocarditis. Of these infections, septic sore throat is, by far, the most common clinical entity. Approximately 2 to 3 weeks following recovery from a beta streptococcal pharyngitis, acute glomerulonephritis or rheumatic fever may develop not as a direct effect of disseminated bacteria, but due to tissue hypersensitivity.
b. The alpha hemolytic streptococci, especially those that are normal inhabitants of the upper respiratory and intestinal tracts, can cause disease if normal resistance is reduced. Alpha hemolytic members from the respiratory tract may cause subacute bacterial endocarditis, and Group D streptococci commonly cause urinary tract infections.

c. The anaerobic streptococci commonly encountered in the normal vaginal flora, in the mouth, and in the intestine are capable of giving rise to suppurative lesions. Infections of these organisms produce pus with a foul odor.

d. The nonhemolytic streptococci are practically all saprophytic forms that have been isolated from milk and various dairy products. A few strains have been implicated as causing subacute bacterial endocarditis (SBE).

3-23. CULTURAL CHARACTERISTICS OF THE STREPTOCOCCI

Culturally, streptococci grow on rich, artificial media. For primary isolation, use blood peptone agar or peptone agar with blood serum or ascitic fluid for best results. The addition of 0.5 percent glucose increases growth but interferes with hemolysis. An ordinary meat infusion medium, such as nutrient agar or broth, will generally not support the growth of *Streptococcus* species. The standard blood agars and such broth media as thioglycollate, Todd-Hewitt, and Trypticase soy are successfully used in cultivating most species. The majority of the streptococci are facultative anaerobes, although some strains, especially those forms isolated from deep tissue infections, are obligate anaerobes. Growth is best at 37º C for pathogenic, hemolytic forms. After 24 hours’ incubation on peptone blood agar, the streptococci generally appear as small (approximately 1 mm or less), slightly granular, circular, convex, translucent colonies. Some streptococci give rise to matt or mucoid colonies on primary isolation. Such colonies possess M protein antigen. After repeated transfers on laboratory media, rough or smooth colonies all eventually develop which are avirulent and void of M protein. Restoration of M protein and virulence to rough or smooth forms may be accomplished by repeated, rapid animal passage.
3-24. GENERAL IDENTIFICATION STREPTOCOCCI

Gram-stained smears of throat and nasopharyngeal materials and sputum are of limited value since saprophytic streptococci are usually present. Smears of spinal fluid are of definite value in revealing gram-positive cocci predominantly in chains. Smears of urinary sediments of exudates from localized lesions, wounds, or deep abscesses (peritonitis) may enable intelligent selection of culture procedures. The streptococci are such a heterogeneous group of organisms that neither morphological, physiological, nor immunological studies alone have been completely successful in classification of all species encountered. Hemolytic reaction is the criterion upon which streptococci are classified and reported. Clinical evidence coupled with the savings in resources supports the hemolytic criterion as being both practical and acceptable. The type of hemolysis exhibited on blood agar (alpha, beta, or gamma) affords tentative separation of streptococcal species. In all cases, suspected colony types should be verified as being streptococci by gram-staining. The catalase test is useful in differentiating culturally similar beta hemolytic streptococci and hemolytic staphylococcal forms. Streptococci are catalase negative.

3-25. BETA HEMOLYTIC STREPTOCOCCI

The beta hemolytic streptococci are identified on the basis of cultural and physiological characteristics. Table 3-1 lists some significant biochemical reactions that will usually enable identification of those groups responsible for the majority of human infections.

a. Bacitracin Susceptibility. A useful means for presumptive identification of group A, beta hemolytic streptococci uses a 0.04-unit bacitracin disk. The following precautions are advised.

(1) Be sure the bacitracin disk is manufactured for differential studies, not sensitivity studies.

(2) Use a heavy inoculum of a pure culture.

(3) Be sure the colony is beta hemolytic.

(4) Periodically check commercial disks with control strains.

(5) Any zone of inhibition is positive.

b. Sodium Hippurate Broth. Sodium hippurate broth is used to enable differentiation of group B streptococci from groups, A, C, and D. The medium is inoculated with the unknown and incubated for 48 hours. The broth is then tested for the possible presence of benzoic acid formed by the organism’s hydrolysis of sodium hippurate.
<table>
<thead>
<tr>
<th></th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C (Human)</th>
<th>GROUP D (Enterococci)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface colonies</strong></td>
<td>Greyish-white, opaque to translucent; hard, with tendency for whole colony to move on probing; hemolytic zones approximately 2 mm.</td>
<td>Grey translucent, with soft texture; slight hemolytic zone.</td>
<td>Indistinguishable from group A.</td>
<td>Grey, somewhat translucent, soft; hemolytic zones slightly wider than the colonies.</td>
</tr>
<tr>
<td>blood agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subsurface colonies</strong></td>
<td>Lens or disc shaped with 2-mm zone of hemolysis.</td>
<td>0.5-mm zones of hemolysis after 24 hours; 0.1-mm zone after 48 hours. Double ring hemolysis following refrigeration overnight.</td>
<td>Indistinguishable from group A.</td>
<td>Hemolytic zones approximately 3-mm.</td>
</tr>
<tr>
<td>blood agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SF medium</strong></td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>Growth, with acid reaction.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sodium hippurate</strong></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bile esculin medium</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bactracin susceptibility</strong></td>
<td>Susceptible</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>6.5 percent NaCl broth</strong></td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>Growth</td>
</tr>
</tbody>
</table>

Table 3-1. Identification of beta hemolytic streptococci (continued).
<table>
<thead>
<tr>
<th></th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C (Human)</th>
<th>GROUP D (Enterococci)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease</strong></td>
<td>Erysipelas, impetigo, septic sore throat, scarlet fever, rheumatic fever, acute glomerulonephritis, puerperal sp.</td>
<td>Urinary tract infections, peritonitis endometritis, wound infections, rarely septicemia.</td>
<td>Suppurative lesions of man, usually in mixed infections. Throat infections.</td>
<td>Same as group B.</td>
</tr>
</tbody>
</table>

Table 3-1. Identification of beta hemolytic streptococci (concluded).

c. **SF Broth.** When dealing with a beta *Streptococcus* species, a most important laboratory procedure is that of determining whether the organism is an enterococcus (group D). This is successfully accomplished using SF (*Streptococcus faecalis*) broth. SF broth is selective in that only *Streptococcus faecalis* and other members of group D streptococci are able to grow in it. The concentration of sodium azide in this medium will prevent the growth of other bacteria. The suspected pathogenic *Streptococcus* species should be inoculated to SF broth from a pure colony. After 24 hours' incubation at 37º C, the broth is examined for the presence or absence of growth. Visible growth is observed, coupled with an indicator change from purple to a yellowish brown.

d. **Bile Esulin Medium (BEM).** Considered to be more reliable than SF broth, the test with bile esculin medium is recommended for presumptive identification of group D streptococci. BEM contains both bile (oxgall) and esculin. It is selective for growth of group D streptococci (also Enterobacteriaceae and *Listeria monocytogenes*). When esculin is hydrolyzed, a dark brown or black color results. The medium is inoculated with a pure culture and incubated for 48 hours at 37º C. A brownish color is positive.

e. **Infusion Broth With 6.5 Percent.** This medium is selective for Group D enterococci. (Group D streptococci that are not enterococci will not grow.)
3-26. DIFFERENTIATION OF OTHER STREPTOCOCCI

Alpha and gamma streptococci, other than enterococci, are usually differentiated on the basis of colonial characteristics and reactions obtained from biochemical media. From a practical standpoint, it is only necessary to differentiate *Streptococcus faecalis* from other gamma and alpha streptococci. This is necessary because *Streptococcus faecalis* is usually resistant to penicillin, streptomycin, and sulfonamides, while the other gamma and alpha strains are sensitive to these therapeutic agents. It is also important to differentiate *Streptococcus pneumoniae* from alpha streptococci. Since pneumococcal strains form green hemolytic colonies similar to colonies of alpha streptococci on blood agar, it is necessary to employ the bile solubility test and other studies for their distinction.

Section IV. GRAM-POSITIVE COCCI: PNEUMOCOCCI

3-27. MORPHOLOGY OF PNEUMOCOCCI

The pneumococci (*Streptococcus pneumoniae*, formerly *Diplococcus pneumoniae*) are small, slightly elongated cocci arranged in pairs (diplococci). In young cultures or specimens the diplococci are frequently lancet-shaped on each end with adjoining sides flattened or slightly curved. The organisms (figure 3-4) may occur singly or in short chains. The pneumococci are typically gram-positive but rapidly become gram-negative with the tendency to lyse spontaneously (autolysis). The organisms are nonmotile and nonsporeforming. Virulent pneumococcal cells are enveloped with a well-defined polysaccharide capsule that is prominent when the organisms are observed in tissue exudates. The capsules of avirulent pneumococci are less demonstrable. When appropriately stained, the capsule normally appears as a clear halo around the cells.

3-28. GROWTH REQUIREMENTS OF PNEUMOCOCCI

a. In terms of growth requirements pneumococcal strains are fastidious. Enriched media is need for cultivation, and blood agar is recommended as the isolation medium. The optimum temperature for growth is 37° C. Although the pneumococcus is classified as a facultative anaerobe in regards to its oxygen requirements, growth is enhanced by increased CO₂ tension. This increased CO₂ tension is readily supplied by use of a candle jar or CO₂ incubator.

b. Animals may be used for isolation and determination of pathogenicity of pneumococci. White mice are injected intraperitoneally with the sample. This is the most rapid and reliable method for obtaining pure cultures of the pneumococci, and for determining strain pathogenicity. It is possible to use Avery's artificial mouse in place of live animals for easy isolation of pneumococci. The artificial mouse is a meat infusion broth that includes 1 percent glucose and 5 percent defibrinated rabbit blood with a final pH of 7.8. Hiss capsule stain of *Streptococcus pneumoniae* from initial isolation in broth culture.
3-29. COLONY CHARACTERISTICS OF PNEUMOCOCCI

a. On blood agar, after 18 to 24 hours' incubation, typical virulent strains or pneumococci form smooth, flat, transparent, slimy or mucoid colonies (0.5-1.5 mm in diameter), which may possess a central depression and ringed periphery. The colonies are alpha hemolytic. They resemble the other alpha hemolytic streptococci, but the pneumococci are usually more flattened and moist. In peptone broth there is rapid growth in 24 hours, with a faint, uniform clouding of the fluid. Growth is improved by the addition of 2 to 4 percent peptone, or adding 1 part blood serum or ascitic fluid to 3 parts broth.
b. The majority of pathogenic pneumococci are encapsulated, which accounts for the formation of smooth or "S" colony types. The nonencapsulated organisms may become the predominating cells when the organisms are cultivated on an agar medium containing their respective type-specific antipolysaccharide serum. Nonencapsulated cultures developed in this manner exhibit rough or "R" type colonies. Loss of the capsule from the pneumococcal strain is accompanied by a loss of virulence and antigenic (type) specificity since the capsular substance influences these capabilities.

3-30. PATHOGENICITY OF PNEUMOCOCCI

a. **Disease Manifestations.** Pneumococci are responsible for approximately 80 percent of the cases of lobar pneumonia and roughly 1.5 percent of bronchial pneumonia in man. These infections are characterized by sudden onset, with accompanying chills, fever, and sharp pleural pain. Sputum from affected individuals is usually bloody or rusty; possessing a thick, viscous consistency. In the early stages of pneumococcal pneumonia, bacteremia may be present. Pneumococci may infect other tissues, either as complications of pneumonia or as independent and primary infections. From the respiratory tract, the organisms most frequently spread to the sinuses and middle ear. Meningitis may result from blood stream infection following pneumonia. Localized infectious may occur in any part of the body. Virulent pneumococci are generally spread by asymptomatic carriers. The pneumococci are opportunistic pathogens in that they apparently do not invade except when an individual's general resistance is lowered. Pneumococcal pneumonia has hastened the fatal termination of such diseases as viral influenza, tuberculosis, congestive heart failure, and cancer. The virulence of these organisms is in part dependent upon the capsule, which functions as a barrier against ingestion by phagocytes in the animal host.

b. **Neufeld Quellung Reaction.** Various pneumococcus strains possess antigenically distinct polysaccharide capsules, each inducing the formation of specific antibodies in the serum of animals upon tissue contact. The antipolysaccharide antibodies are specific, in that once they are formed, they will react typically with only the particular type of capsular polysaccharide that initiated their production. Such reactions result in destruction and removal of the capsule from the cell wall, thereby rendering the pneumococcus vulnerable to phagocytosis. This characterizes the immune state possessed by individuals following recovery from pneumococcal infections. Variations in the molecular makeup of capsular polysaccharides throughout the pneumococci amount for 84 known immunologically distinct pneumococcal types. The various types have been determined by animal inoculation with purified capsular extracts of pneumococcal cells (specific soluble substance, or SSS) and subsequent demonstration of specific antibody formation in the animal's serum. Pneumococcal types are arbitrarily designated by Roman numerals or lower case letters. The serological identification of pneumococci is accomplished in vitro using slide tests that entails exposing the organisms to type-specific antisera against the strains most commonly responsible for human infections. When a particular pneumococcus type is mixed with its specific antipolysaccharide antibody on a glass slide, the capsule appears to swell and becomes markedly defined upon microscopic observation. This is known
as the quellung reaction; however, it is not used as a routine test for the identification of the pneumococci.

3-31. LABORATORY IDENTIFICATION OF PNEUMOCOCCI

a. Gram-stained smears of sputum specimens may reveal the typical gram-positive, lancet-shaped diplococci. The organisms may also be observed in spinal fluid sediments, pleural fluids, or other exudates from infected tissue. The presence of capsules may be noted in gram-stained smears as thick halos around the cells when observed under reduced light. Demonstration of the capsule is better accomplished by preparing a capsule stain or an India ink preparation as previously discussed.

b. On blood agar plates pneumococcal colonies are generally well formed after 18 to 24 hours' incubation in a candle jar at 37º C. Plates should not be discarded before 48 hours' incubation time since primary growth to certain types develops slowly. Colony growth may be obtained only on blood agar plates streaked from primary broth cultures.

c. Although typical colonies of pneumococci are rather distinctive, the growth cannot be grossly distinguished with certainty from that of alpha hemolytic streptococci. No report should be rendered as to the presence of pneumococci or alpha streptococci without performing confirmatory studies.

3-32. CONFIRMATORY STUDIES

Isolated colonies suggestive of pneumococcus (table 2-3) should be examined as follows.

a. **Gram Stain Smears.** Prepare gram-stain smears and examine for gram-positive diplococci that may be lancet-shaped.

b. **Bile Solubility Test.** Perform the bile solubility test (Section I) and observe for dissolution of growth. Pneumococci are lysed in the presence of bile salts; other streptococci are not lysed. This test is used to distinguish between the alpha hemolytic streptococci and pneumococci, which are also alpha hemolytic. If the organism is *Streptococcus pneumoniae*, clearing should occur in the test tube containing the sodium deoxycholate within the 3-hour incubation period. Other alpha hemolytic streptococci cultures remain turbid like the control.

c. **Optochin Sensitivity Test.** Inoculate a blood agar plate for determination of Optochin sensitivity. The Optochin sensitivity test may be performed in lieu of the bile solubility test. Optochin (ethylhydrocupreine hydrochloride) discs are placed on the heavily inoculated plate. After 18 to 24 hours' incubation, pneumococcal growth will exhibit a large, clear zone of inhibition. The results of the bile solubility test (or the Optochin sensitivity test) along with careful observation of colonies and microscopic studies are usually sufficient criteria for identification of pneumococci.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Hemolysis</th>
<th>Colonial Morphology</th>
<th>Capsule Reaction In Type-Specific Antiserum</th>
<th>Solubility In Sodium Deoxcholate</th>
<th>Optochin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumococci</td>
<td>Alpha</td>
<td>Small, flat shiny or mucoid colonies with concentric rings and depressed centers.</td>
<td>Present</td>
<td>Reactive</td>
<td>Soluble</td>
</tr>
<tr>
<td>Alpha Streptococci</td>
<td>Alpha</td>
<td>Small, raised, dome-shaped, smooth colonies which are translucent or opaque.</td>
<td>Absent</td>
<td>Nonreactive</td>
<td>Insoluble</td>
</tr>
</tbody>
</table>

Table 3-2. Differentiation of pneumococcal strains from alpha streptococci.

Continue with Exercises
EXERCISES, LESSON 3

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Unlike other alpha hemolytic streptococcus, pneumococci are lysed in a solution of:
   a. Sodium chloride.
   b. Sodium hydroxide.
   c. Sodium deoxycholate.
   d. Hydrogen peroxide.

2. In the bile indicated bile solubility test, the presence of pneumococci is indicated by ________ in the tube labeled test.
   a. Clearing.
   b. Turbidity.
   c. Reddening.
   d. Decreased viscosity.

3. Fermentation of a carbohydrate in purple broth is indicated by a change in color from purple to:
   a. Red.
   b. Green.
   c. Brown.
   d. Yellow.
4. Which of the following organisms is catalase positive?
   a. Alpha hemolytic streptococci (other than pneumococci).
   b. Gamma hemolytic streptococci.
   c. Pneumococci.
   d. Staphylococci.

5. A rapid test to distinguish a suspected staphlococcal colony from streptococci is:
   a. Gelatin liquefaction.
   b. The catalase test.
   c. Mannitol fermentation.
   d. Growth on 7.5 percent sodium chloride.

6. The citrate test is based on an organism's ability to utilize citrate as the sole source of:
   a. Hydrogen.
   b. Iron.
   c. Nitrogen.
   d. Carbon.

7. Coagulase is an enzyme produced by pathogenic:
   a. Streptococci (other than pneumococci).
   b. Pneumococci.
   c. Organisms of all types.
   d. Staphylococci.
8. A positive coagulase test is indicated by:
   a. ALL OF THE BELOW.
   b. Clotting of plasma.
   c. Clearing of test solution.
   d. Change to a green color.

9. A positive ferric chloride test indicates the beta presence of group __________ hemolytic streptococci.
   a. A.
   b. B.
   c. C.
   d. D.

10. A positive ferric chloride test is revealed by the formation of a:
    a. Red color.
    b. Clear solution.
    c. Colorless solution.
    d. Permanent precipitate.

11. If the lead acetate paper test for hydrogen sulfide production is positive, the strip turns:
    a. Red.
    b. Green.
    c. Black.
    d. Yellow.
12. The lead acetate paper test is especially useful for differentiation of species of:
   a. Brucella.
   b. **Streptococcus**.
   c. **Mycobacterium**.
   d. **Enterobacteriaceae**.

13. A positive indole test is indicated by which color at the interphase between reagent and broth culture?
   a. Red.
   b. Blue.
   c. Green.
   d. Yellow.

14. A positive methyl red test indicates that an organism produces a:
   a. Low acid content from glucose.
   b. Low acid content from lactose.
   c. High acid content from glucose.
   d. High acid content from lactose.

15. A positive methyl red test is indicated by which color?
   a. Red.
   b. Blue.
   c. Green.
   d. Yellow.
16. The nitrate reduction test utilizes which of the following?
   a. Ten percent ferric chloride.
   b. Sulfanilic acid and amyl alcohol.
   c. Potassium hydroxide and alpha-naphthylamine.
   d. Sulfanilic acid and N-N-dimethyl-l-naphthylamine.

17. Which of the following indicates a negative nitrate reduction test?
   a. Red color without the addition of zinc.
   b. Red color after the addition of zinc.
   c. No color after the addition of zinc.

18. In a typically positive oxidase test (alternate method), the colonies should finally:
   a. Clot.
   b. Dissolve.
   c. Turn pink.
   d. Turn black.

19. Unrease-positive organisms hydrolyze __________ to form the compound __________.
   a. Urea; ammonia.
   b. Acetamide; acetic acid.
   c. Urea; ammonium cyanate.
   d. Phenol red; carbon dioxide.
20. Which of the following is usually Voges-Proskauer-positive?
   a. E. coli.
   b. Shigella.
   c. Klebsiella.
   d. Salmonella.

21. A positive Voges-Proskauer test is indicated by what color?
   a. Blue.
   b. Green.
   c. Yellow.
   d. Pink or red.

22. On initial isolation, colonies of Staphylococcus aureus typically show a ____ pigmentation.
   a. White.
   b. Pinkish.
   c. Golden.
   d. Reddish.

23. When grown on blood agar, Staphylococcus aureus colonies usually cause:
   a. No hemolysis of the surrounding RBC's.
   b. An alpha type of hemolysis of the surrounding RBC's.
   c. A gamma type of hemolysis of the surrounding RBC's.
   d. Complete hemolysis of the surrounding RBC's.
24. **Staphylococcus epidermidis** is similar to **Staphylococcus aureus** in:
   a. Gram morphology.
   b. The ability to produce coagulase and ferment mannitol.
   c. The ability to produce toxins.
   d. The ability to hemolyze erythrocytes.

25. The typical morphological arrangement of the staphylococci is:
   a. Irregular clumps resembling grape-like clusters.
   b. Long chain formations.
   c. Pairs of slightly elongated cocci.
   d. Tetrads.

26. The primary means of distinguishing **Staphylococcus aureus** from **Staphylococcus epidermidis** is that **Staphylococcus aureus**:
   a. Ferments mannitol but does not produce coagulase.
   b. Hemolyzes RBC's producing an alpha hemolysis.
   c. Is nonhemolytic.
   d. Ferments mannitol and produces coagulase.

27. The streptococci are characterized as being:
   a. Nonmotile and nonsporeforming.
   b. Motile and sporeforming.
   c. Spore forming in broth cultures.
   d. Motile in broth cultures.
28. In the Lancefield classification of beta hemolytic streptococci, which group is most often associated with communicable diseases in humans?
   a. A.
   b. B.
   c. C.
   d. D.

29. The most useful method for preliminary differentiation of species of Streptococcus is:
   a. Gram staining reaction.
   b. Cellular morphology.
   c. Size of colonies on blood agar.
   d. Pattern of hemolysis produced on blood agar.

30. The true hemolytic reaction of a streptococcal strain may be altered by the incorporation of ________ in the agar.
   a. Blood.
   b. Glucose.
   c. Peptone.
   d. Ascitic fluid.

31. The formation of a green zone around certain strains of streptococci on blood agar usually indicates what type of hemolysis:
   a. Alpha.
   b. Beta.
   c. Gamma.
   d. Alpha prime.
32. A clear, colorless zone around a colony on blood agar usually indicates what type of hemolysis?
   a. Alpha.
   b. Beta.
   c. Gamma.
   d. Alpha prime.

33. Gamma forms of streptococci produce __________ on blood agar.
   a. A clear zone of hemolysis.
   b. A green zone of hemolysis.
   c. No hemolysis.
   d. A pink zone of hemolysis.

34. Beta hemolytic streptococci that are susceptible to bacitracin probably belong to group:
   a. A.
   b. B.
   c. C.
   d. D.

35. Beta hemolytic streptococci that are sodium-hippurate-positive probably belong to group:
   a. A.
   b. B.
   c. C.
   d. D.
36. Beta hemolytic streptococci that are bile-esculin-positive probably belong to group:
   a. A.
   b. B.
   c. C.
   d. D.

37. Beta hemolytic streptococci that are bacitracin-resistant, sodium-hippurate negative, and bile-esculin-negative probably belong to group:
   a. A.
   b. B.
   c. C.
   d. D.

38. Beta hemolytic streptococci that grow in an infusion broth with 6.5 percent NaCl probably belong to which of the following groups?
   a. A.
   b. B.
   c. C.
   d. D streptococci.
   e. D enterococci.
39. The pneumococci are slightly elongated gram-positive cocci characteristically arranged:
   a. Pairs.
   b. Clusters.
   c. Long chains.
   d. Packets of four.

40. Due to their hemolytic reaction, pneumococcal colonies growing on blood agar could very easily be mistaken for other:
   a. Beta hemolytic streptococci.
   b. Gamma hemolytic streptococci.
   c. Alpha hemolytic streptococci.
   d. Hemolytic staphylococci.

41. When people speak of "pneumonia" they are generally referring to lobar pneumonia. The most common cause of lobar pneumonia is:

   a. *Streptococcus (Diplococcus) pneumoniae*.
   b. *Neisseria meningitidis*.
   c. *Mycobacterium tuberculosis*.
   d. *Corynebacterium diphtheriae*.

*Check Your Answers on Next Page*
SOLUTIONS TO EXERCISES, LESSON 3

1.  c. (paras 3-1a, b(1))
2.  a (paras 3-1a, d(1))
3.  d (para 3-2d)
4.  d (para 3-3c(2))
5.  b (para 3-3c(2))
6.  d (para 3-4a)
7.  d (para 3-5a)
8.  b (para 3-5e(1))
9.  b (para 3-6a)
10. d (para 3-6d)(1))
11. c (para 3-8d(1)
12. a (para 3-8d(3))
13. a (para 3-9c(2))
14. c (para 3-10a,d(1))
15. a (para 3-10d(1))
16. d (paras 3-11b(1), (2))
17. b (para 3-11d(2)(b))
18. d (para 3-12d(3))
19. a (para 3-13a)
20. c (para 3-14a)
21. d (para 3-14d(1))
22. c (para 3-17e)
23. d (para 3-17e)
24. a (para 3-18a)
25. a (para 3-18a)
26. d (para 3-19f; fig. 3-1)
27. a (para 3-20a)
28. a (para 3-20c)
29. d (para 3-21)
30. b ((para 3-21a))
31. a ((para 3-21c(1))
32. b (para 3-21c(2))
33. c (para 3-21c(3))
34. a (para 3-25a)
35. b (para 3-25b; table 3-1)
36. d (para 3-25d; table 3-1)
37. c (table 3-1)
38. e (para 3-25e)
39. a (para 3-27)
40. c (para 3-31c)
41. a (para 3-27; 3-30a)

End of Lesson 3