LESSON ASSIGNMENT

LESSON 1

Introduction.

TEXT ASSIGNMENT

Paragraphs 1-1 through to 1-22.

LESSON OBJECTIVES

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

1-1. Match the name of a symbiotic association mutualism, commensalism, or parasitism with its description.

1-2. Associate basic terms of parasitology with their definitions.

1-3. Identify the means by which bacteria reproduce.

1-4. Identify the functions of the bacterial capsule, flagellum, cell wall, and spore.

1-5. Given the description of a toxin; identify its category.

1-6. Classify bacteria according to their source of nutrients.

1-7. Given a common microscopic morphological description; identify the corresponding morphological term.

1-8. Identify the proper methods for collecting, processing, and shipping bacteriological specimens.

SUGGESTION

After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.
LESSON 1

INTRODUCTION

Section I. HOST-PARASITE RELATIONSHIPS

1-1. CAUSATION OF MICROBIAL DISEASE

Microbial disease must be understood in terms of the interrelationship among parasites, the host, and the environment. For example, the general health of the host may determine whether a disease occurs and how severe it may be. Even though a microorganism may be part of the ordinary flora, it can cause disease if the host's natural defenses are not fully effective. Some common symbiotic relationships between two organisms, such as man and a microorganism, are given below.

a. **Mutualism.** In this case, both organisms benefit.

b. **Commensalism.** Commensalism is a relationship where one organism benefits without producing harmful effects for the other.

c. **Parasitism.** In parasitism, one organism benefits at the expense of the other. The parasite may be facultative or obligatory. If the parasite is able to live independently of the host, it is considered a facultative parasite. When the parasite has an absolute requirement for a host and cannot live outside the host, it is an obligatory parasite. In the microbial association of parasitism, disease results if the host is injured by the parasite. In this regard, the parasite has certain characteristics favoring its establishment in the host. On the other hand, the host has certain characteristics that oppose the establishment of the parasite. The outcome of the host-parasite relationship depends on the active interplay between factors.

1-2. TERMINOLOGY

a. Parasites that usually cause disease are called pathogens, and pathogenicity is the term used to denote disease-producing ability. **Virulence** denotes the degree of pathogenicity; it is used to describe a particular strain or variety of a species. Pathogenicity or virulence may be due to the bacteria's invasiveness or toxigenicity. **Invasiveness** means the ability of the bacteria to enter, spread, and multiply in host tissue. **Toxigenicity** refers to the ability of the bacteria to produce toxic substances.

b. Virulence is usually expressed by the LD$_{50}$ that is, the concentration of microorganisms representing a lethal dose for 50 percent of the experimental animals under standardized conditions. The LD$_{50}$ is specific to the laboratory animal employed.

c. The process whereby a pathogenic microbe enters into a relationship with the host is known as infection. The infection may or may not result in overt-disease. In this regard, the host may overcome the infection, the host may develop latent, that is, unapparent infection, or the host may become a healthy carrier of the pathogen.
d. Bacteria reproduce asexually by splitting at right angles to their long axis—a process called binary fission.

1-3. THE INFECTIOUS PROCESS

Various steps are required for infectious process. First, an appropriate portal of entry is required. The respiratory tract, the gastrointestinal tract, direct contact, and insect bites may each represent the portal of entry. The portal of entry is usually specific for any particular pathogen. Second, the pathogen must establish itself in and reproduce within the host. This involves its spread through the host's tissues via the lymphatic and the blood stream. Third, to complete the transmission of the pathogen to a new host, a portal of exit is required. The portal of exit may be the respiratory tract, the gastrointestinal tract, direct contact, or an insect bite. Finally, to ensure perpetuation of the parasite, the pathogen must be introduced to a new host. This may occur by the ingestion of contaminated food or drink, by direct contact with infected persons, by contact with contaminated objects (fomites), or by-insect vectors.

Section II. ASPECTS OF PATHOGENICITY

1-4. CAPSULATION

Of primary consideration is the presence of a capsule on most pathogenic bacteria. Encapsulated bacteria are able to resist phagocytosis by leukocytes better than nonencapsulated bacteria. The loss of the capsule in certain bacteria (pneumococci, Friedlander's bacillus) results in the loss of virulence. The capsule is generally regarded as a factor of invasiveness.

1-5. BACTERIAL TOXINS

Bacterial toxins are known to play a role in the ability of the bacteria to cause disease. Toxins are divided into endotoxins and exotoxins.

a. Exotoxins. Exotoxins are secreted by bacteria into their environment and are potent and specific in their action upon host tissue. The ability to form exotoxins is usually attributed to certain gram-positive rods such as the clostridia and the corynebacteria. The exotoxin has been found to be a highly antigenic, heat labile, proteinaceous substance that is usually destroyed by proteolytic enzymes. However, the very potent and toxic exotoxin of Clostridium botulinum is the exception; is not destroyed by proteolytic enzymes. The toxic properties, but not the antigenic properties, of exotoxins are destroyed by formalin, heat, or prolonged storage. Such treated toxins are called toxoids and find great practical use in immunization programs.
b. **Endotoxins.** Endotoxins are found intracellularly, and are considered to be part of the bacterial cell wall. They are released upon destruction of the bacterial cell. Endotoxins are usually associated with gram-negative bacteria, and considered to be weakly antigenic. Among their other properties, endotoxins are heat stable polysaccharides that are not digested by proteolytic enzymes. Endotoxins are weakly toxic and induce a generalized reaction in the host that manifests itself in the form of a febrile response. Endotoxins may also cause shock.

1-6. **EXTRACELLULAR ENZYMES**

Certain microorganisms produce extracellular enzymes that contribute to their pathogenicity.

a. Production of the enzyme **coagulase** has been correlated to the pathogenicity of the staphylococci. Coagulase acts upon plasma causing it to coagulate. This activity results in the walling off of the site of infection and causes a layer of fibrin to form on the cell wall of the bacteria, which enables the staphylococci to resist phagocytosis.

b. Collagenase is an enzyme that is produced by some of the clostridia. It acts upon collagen, a constituent of connective tissue. The breakdown of collagen promotes the spread of bacteria in tissue.

c. Hyaluronidase is an enzyme that is known as the spreading factor. It acts upon hyaluronic acid, a constituent of connective tissue. The enzyme is produced by staphylococci, clostridia, streptococci, and pneumococci. The activity of hyaluronidase contributes to the spread of the pathogens through tissue.

d. Streptokinase, also known as fibrinolysin, is an enzyme produced by streptococci, staphylococci, and Clostridium perfringens. Streptokinase activates a proteolytic enzyme of plasma known as plasmin. Plasmin is able to dissolve coagulated plasma, and this activity may aid in the spread of the bacteria.

e. Hemolysins are a group of soluble substances produced by staphylococci, pneumococci, some clostridia, and groups A and C of the blood cells and probably streptococci. These hemolysins destroy red tissue cells.

f. Leukocidins are substances that destroy leukocytes; they are produced by streptococci and staphylococci.
Section III. HOST RESISTANCE

1-7. NONSPECIFIC FACTORS

a. Skin and mucous membrane barriers.

   (1) Physical barriers.

      (a) Intact skin.
      (b) Mucous-sticky lining.
      (c) Nasal hair.
      (d) Cilia.
      (e) Peristaltic action.
      (f) Normal flora-occupying attachment sites.

   (2) Chemical barriers.

      (a) Acid pH (stomach skin).
      (b) Bile salts (intestine).
      (c) Lysozyme (eyes).

   (3) Microbial antagonism.

      (a) Bacteriocins from normal flora.
      (b) Antimicrobial factors from serum.
      (c) Competition for nutrients.

b. Phagocytosis.

   (1) PMN, monocytes, and macrophages ingest foreign particles in the host.
   (2) Numerous enzymes act to degrade ingested particles.
   (3) Local tissue damage-inflammation may occur.

c. Emotional and nutritional states (hormones, vitamins, etc.) play an undefined role in resistance.
1-8. SPECIFIC FACTORS-IMMUNOLOGICAL RESPONSE

a. Antibodies.
   (1) Neutralize the antigen.
   (2) Opsonize the antigen.

b. Cell mediated immunity.
   (1) Antigen stimulates the release of biologically active substances called lymphokines.
   (2) Lymphokines enhance phagocytosis and killing.

c. Complement system.
   (1) Complex system of serum proteins.
   (2) Assist antibody in neutralizing the antigen.
   (3) Assist antibody in lysing bacterium.

d. Interferon.
   (1) Cell protein-local production.
   (2) Production stimulated by viruses and other microbes.
   (3) Local defense against antigens.

Section IV. THE BACTERIAL CELL

1-9. AUTOTROPHIC BACTERIA

Autotrophic bacteria obtain energy and grow on inorganic media, employing carbon dioxide (CO₂) as their sole source of carbon. Autotrophs begin with CO₂ and ammonia (NH₃) and from these build an entire protoplasmic structure of protein, fat and carbohydrates, using the oxidation of ammonia to obtain energy for their other processes.
1-10. HETEROTROPHIC BACTERIA

Heterotrophic bacteria obtain energy from organic carbon sources. Heterotrophs require the addition of sugars, amino acids, purines, pyrimidines, and vitamins to their culture media. The fermentation of sugar is their primary source of energy. Parasitic bacteria are heterotrophs. They have become adapted to an environment in which many kinds of organic materials are normally available. In many cases such organisms have lost their ability to synthesize certain complex organic substances needed for their growth. Bacterial parasites require nutrients from living organisms and may cause harm to the host. Saprophytic bacteria are heterotrophs that utilize decaying organic matter for nutrients and usually do not harm the host.

1-11. MORPHOLOGY

The size of bacteria (figure 1-1) is measured in thousandths of a millimeter. Generally, the following dimensions apply. The coccus has a diameter of approximately 1 micron (µ); the bacillus appears as a rod with a width of 0.5 µ and a length of 2 µ; and the spirochete appears as a corkscrew with a width of 0.2 µ; and a length of 10 µ. Bacteria can occur in a number of arrangements and a predominant arrangement is usually specific for a bacterium. In this regard, prefixes are added to the word indicating the shape of the bacterium.
NOTE: 1 mm = 1,000 μ; 1 μ = 1,000 nm; 1 nm = 10 Å (Å = Angstrom unit)

(Reproduced for instructional purposes from Textbook of Virology, dated 1968, 5th ed., by A. J. Rhodes and CUE. Van Rooyen, Figure 1/2/1, adapted from Endeavor, volume 15, page 153. Written consent of the copyright owner has been obtained.)

Figure 1-1. Size range of objects with different types of microscopes.
a. **Coccus (Plural, Cocci).** These are spherical bacteria occurring in any of the following arrangements:

1. Singly (coccus) (figure 1-2A).
2. In pairs (diplococcus) (figure 1-2B).
3. In chains (streptococcus) (figure 1-2C).
4. In clusters (staphylococcus) (figure 1-2D).
5. In clusters of 4 (tetrad) or 8 (cube) (figure 1-2E).

b. **Bacillus (Plural, Bacilli).** These are rod-shaped bacteria occurring in any of the following arrangements.

1. Singly (bacillus) (figure 1-2F).
2. In pairs (diplobacillus) (figure 1-2G).
3. In chains (streptobacillus) (figure 1-2H).
4. In palisades (palisade) (figure 1-2I).

c. **Spirillum (Plural, Spirilla).** These are spiral, corkscrew-shaped organisms whose long axes remain rigid while in motion. A **spirochete** is a spiral microorganism whose long axis flexes when it is in motion (figure 1-2J).

d. **Coccobacillus.** This is a short, plump bacillus with rounded ends, resembling a coccus in shape and arrangement.

e. **Vibrio.** This is a comma-shaped bacillus resembling the spirillum because of its motility.
Figure 1-2. Shapes and arrangements of bacteria.
1-12. STRUCTURE OF BACTERIA

The typical structure of a bacterial cell is shown in figure 1-3. It consists of the following general structures, found in all bacterial cells; and special structures, found in specific types of bacterial cells:

<table>
<thead>
<tr>
<th>GENERAL STRUCTURES</th>
<th>SPECIAL STRUCTURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall</td>
<td>Vacuole</td>
</tr>
<tr>
<td>Diffuse Nucleus</td>
<td>Capsule</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Flagellum</td>
</tr>
<tr>
<td>Cytoplasmic membrane</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1-3. Bacterial cell structure.

a. General Structures.

(1) Cell wall. A thin, rigid cellulose covering that encloses the protoplasm of the cell and gives rigidity to the bacterial shape.

(2) Cytoplasmic membrane. A semi-permeable membrane that is located directly beneath the cell wall and which governs osmotic activity.

(3) Cytoplasm. The protoplasmic or vital colloidal material of a cell exclusive of the nucleus.

(4) Nucleus. Diffused chromatin material responsible for replication of the cell. The bacterial cell does not have a nuclear membrane or a well-defined nucleus.
b. **Special Structures.**

(1) **Capsule.** An accumulation of high molecular weight, excretory substances (slime layer) around a bacterium or bacteria. A capsule serves as a defense mechanism against phagocytosis by white blood cells and penetration by viruses.

(2) **Flagellum.** A protoplasmic strand of elastic protein originating in the cytoplasm and extending from the body of the cell. A flagellum serves as an organ of locomotion. The arrangement of flagella (plural) is peculiar to the species.

(3) **Spore.** Metabolically resistant body formed by a vegetative bacterium to withstand an unfavorable environment. **Only bacilli form spores.** The position and size of a spore within a bacillus is peculiar to the species.

(4) **Inclusion bodies.** Vacuoles of reserve or waste materials contained within the cytoplasm.

1-13. **IDENTIFICATION OF BACTERIA**

Since there are several thousand species of bacteria, it would be impossible to identify them on the basis of appearance alone. Therefore, the bacteriologist employs a wide variety of techniques, based upon known characteristics of specific bacteria, to arrive at the identity of a given specimen. The following characteristics, which are used frequently as terms of reference, assist the microbiologist in the positive identification of bacteria as well as in eliminating them from consideration.

a. **Food Requirements.**

(1) **Natural media.**

(a) Saprophytes--grow on dead organic matter.

(b) Parasites--grow on living tissue.

(2) **Artificial media.**

(a) Grow on any culture medium.

(b) Grow only on special culture media.

(c) Will not grow on any artificial culture medium.
b. **Oxygen Requirements.**

1. Aerobes--grow in the presence of free oxygen.
2. Anaerobes--grow without free oxygen
3. Obligate-aerobes--must have free oxygen for growth.
4. Obligate aerobes--must not have free oxygen.
5. Facultative aerobes--able to adjust to an aerobic environment.
6. Facultative anaerobes--able to adjust to an anaerobic environment.
7. Microaerophiles--require small amounts of free oxygen for growth.

c. **Colony Morphology.**

d. **Microscopic Examination.**

1. Size.
2. Shape.
3. Spore formation--sporeformers or nonsporeformers.
4. Capsule formation--encapsulated or nonencapsulated.
5. Motility--motile or nonmotile.
6. Staining characteristics. Specimens are normally stained prior to microscopic examination. Various species react differently to the stains.
   (a) Gram-positive--bacteria which, when stained by the gram stain method, retain the crystal violet stain (purple or blue).
   (b) Gram-negative--bacteria which, when stained by the gram stain method, do not retain the crystal violet stain, but retain the color of the counterstain (red).
   (c) Acid-fast--bacteria which, when stained with certain dyes and then treated with an acid, followed by a counterstain, retain the color of the dye.
   (d) Nonacid-fast--bacteria which, when treated as in (c), above, retain the counterstain rather than the dye.
e. Pathogenicity.

(1) Hemolytic or nonhemolytic.

(a) Beta hemolytic--can cause complete hemolysis (dis- (solution) of red blood cells.

(b) Alpha hemolytic--cause partial hemolysis of red blood cells.

(c) Gamma forms--do not cause hemolysis.

(2) Production of toxins.

(a) Exotoxins--extremely potent poisons which are produced in bacterial cells and which diffuse freely into the cells of host tissues, causing severe systemic poisoning.

(b) Endotoxins--toxins, less potent than exotoxins, which are produced in bacterial cells and which diffuse into the host cells only after the bacterial cell disintegrates.

1-14. PATHOGENIC BACTERIA

Table 1-1 presents a list of the principal pathogenic bacteria of public health importance, organized in such a way as to illustrate the aids in identification discussed in the above paragraph.
<table>
<thead>
<tr>
<th>IDENTIFICATION GROUP</th>
<th>SPECIES</th>
<th>CAUSATIVE AGENT OF</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GRAM-POSITIVE COCCI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha hemolytic</td>
<td>Diplococcus pneumoniae</td>
<td>Lobar pneumonia</td>
<td>Produces exotoxin causing skin rash.</td>
</tr>
<tr>
<td>Beta hemolytic</td>
<td>Streptococcus pyogenes</td>
<td>Impetigo, septic sore throat, scarlet fever</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Upper respiratory infections, boils, surgical infections, food poisoning</td>
<td>Produces exotoxin causing food poisoning.</td>
</tr>
<tr>
<td><strong>GRAM-NEGATIVE COCCI</strong></td>
<td>Neisseria gonorrhoeae</td>
<td>Gonorheal conjunctivitis</td>
<td>Kidney-shaped diplococci.</td>
</tr>
<tr>
<td></td>
<td>Neiseseria meningitides</td>
<td>Epidemic cerebrospinal meningitis</td>
<td>Kidney-shaped diplococci.</td>
</tr>
</tbody>
</table>

Table 1-1. Pathogenic bacterial of public health importance (continued).
<table>
<thead>
<tr>
<th>IDENTIFICATION GROUP</th>
<th>SPECIES</th>
<th>CAUSATIVE AGENT OF</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GRAM-POSITIVE BACILLI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>Corynebacterium diphtheriae</td>
<td>Diptheria</td>
<td>Produces powerful exotoxin causing inflammation of mucosa and impairment of vital organs.</td>
</tr>
<tr>
<td></td>
<td>Bacillus anthracis</td>
<td>Anthrax (Chiefly in herbivorous animals, but also in man)</td>
<td>Forms capsule.</td>
</tr>
<tr>
<td>Aerobic, spore-forming</td>
<td>Mycobacterium tuberculosis</td>
<td>Tuberculosis (man)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. bovis</td>
<td>Tuberculosis (cattle and man)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. leprae</td>
<td>Leprosy</td>
<td></td>
</tr>
<tr>
<td>Aerobic, acid-fast</td>
<td>Clostridium botulinum</td>
<td>Food poisoning</td>
<td>Produces powerful, lethal exotoxin.</td>
</tr>
<tr>
<td></td>
<td>C. tetani</td>
<td>Tetanus (lockjaw)</td>
<td></td>
</tr>
<tr>
<td>Aerobic, spore-forming</td>
<td>Clostridium perfringens</td>
<td>Gas gangrene food poisoning</td>
<td>Produces powerful, lethal exotoxin.</td>
</tr>
</tbody>
</table>

Table 1-1. Pathogenic bacterial of public health importance (continued).
<table>
<thead>
<tr>
<th>IDENTIFICATION GROUP</th>
<th>SPECIES</th>
<th>CAUSATIVE AGENT OF</th>
<th>REMARKS</th>
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<tbody>
<tr>
<td><strong>GRAM-NEGATIVE BACILLI</strong></td>
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<tr>
<td>Saprophytic enterics</td>
<td>Escherichia coli</td>
<td>Part of the normal flora of the adult</td>
<td>All nonspore-forming.</td>
</tr>
<tr>
<td></td>
<td>Enterobacter aerogenes</td>
<td>intestinal tract, but pathogenic to</td>
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<td></td>
<td>Proteus vulgaris</td>
<td>infants or when introduced into other</td>
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<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>parts of the body.</td>
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<tr>
<td></td>
<td>Alkaligenes faecalis</td>
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<tr>
<td></td>
<td>Klebsiella pneumoniae</td>
<td></td>
<td></td>
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<tr>
<td>Pathogenic enterics</td>
<td>Salmonella typhi</td>
<td>Typhoid fever</td>
<td></td>
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<tr>
<td></td>
<td>S. paratyphi</td>
<td>Partyphoid fever</td>
<td></td>
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<tr>
<td></td>
<td>S. typhimurium</td>
<td>Acute gastroenteritis (Salmonellosis-</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>&quot;food poisoning&quot;</td>
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<tr>
<td></td>
<td>Shigella dysenteriae¹</td>
<td>Bacillary dysentery</td>
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<tr>
<td></td>
<td>S. flexnerei</td>
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<tr>
<td></td>
<td>S. sonnei²</td>
<td>²Infant diarrhea</td>
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</tr>
<tr>
<td></td>
<td>Vibrio cholerae</td>
<td>Cholera</td>
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</tr>
</tbody>
</table>

Table 1-1. Pathogenic bacterial of public health importance (continued).
<table>
<thead>
<tr>
<th>IDENTIFICATION GROUP</th>
<th>SPECIES</th>
<th>CAUSATIVE AGENT OF</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMALL GRAM-NEGATIVE BACILLI</td>
<td>Brucella abortus(^1)</td>
<td>Contagious abortion in animals</td>
<td>(^1)Occurs in cattle.</td>
</tr>
<tr>
<td></td>
<td>B. suis(^2)</td>
<td></td>
<td>(^2)Occurs in swine.</td>
</tr>
<tr>
<td></td>
<td>B. melitensis(^3)</td>
<td></td>
<td>(^3)Occurs in sheep</td>
</tr>
<tr>
<td></td>
<td>Hemophilus influenzae</td>
<td>brucellosis (undulant fever) in man</td>
<td>Encapsulated.</td>
</tr>
<tr>
<td></td>
<td>H. pertussis (Bordetella)</td>
<td>Pharyngitis, otitis, sinusitis, pneumonitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H. ducreyi</td>
<td>Whooping cough</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasteurella (yersinia)</td>
<td>Chancroid</td>
<td></td>
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<tr>
<td></td>
<td>pestis</td>
<td>Plague</td>
<td></td>
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<tr>
<td></td>
<td>F. tularensis</td>
<td>Tularemia</td>
<td></td>
</tr>
<tr>
<td>SPIROCHETES</td>
<td>Treponema pallidum</td>
<td>Syphilis</td>
<td>Does not stain with ordinary stains nor grow on artificial media.</td>
</tr>
<tr>
<td></td>
<td>Borrelia recurrentis</td>
<td>Relapsing fever</td>
<td>May be stained and cultured (chick embryo).</td>
</tr>
<tr>
<td></td>
<td>Leptospirosera ichterohemorrhagiae (also L. canicola, L. autumnalis, and L. pomona)</td>
<td>Leptospirosis (Weil's disease, infectious jaundice)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1-1. Pathogenic bacterial of public health importance (concluded).
Section V. COLLECTING AND PROCESSING OF BACTERIOLOGICAL SPECIMENS

1-15. BASIC PRINCIPLES

The improper collecting and processing of bacteriological specimens is often responsible for the failure to isolate and identify the bacterial agent of disease. Certain basic principles, therefore, should be followed at all times.

a. First, instruments, containers, and other equipment in direct contact with specimens must be sterile. The cartons for the collection of stool specimens are an exception to this rule.

b. Second, material for culture must not come in contact with chemicals, disinfectants, or germicidal agents.

c. Third, material for culture should be obtained before the patient receives antibiotic therapy. If this is not possible, the type of therapy should be indicated on the bacteriology request form. Such substances as penicillinase should then be added to the media if penicillin is indicated; para-aminobenzoic acid should be added if sulfonamides are indicated.

d. Fourth, specimens should be properly labeled and dated, and should be delivered to the bacteriological section of the clinical laboratory immediately after collection. Specimens should be inoculated to media immediately after delivery to the bacteriology section.

e. Fifth, if anaerobic cultures are requested, the specimen should be inoculated to fluid thioglycollate medium at the time the specimen is collected, or as soon thereafter as possible. Exposure to atmospheric oxygen should be avoided completely if possible. (See discussion of anaerobic methods in Lesson 2, Section IV).

f. Sixth, to avoid contamination, always culture the specimen before making smears or performing special tests.

g. Staining and testing procedures mentioned in the following pages on processing will be explained more completely in the following chapters.

1-16. COLLECTING AND PROCESSING OF BLOOD CULTURES.

NOTE: Refer to figure 1-4.

a. Circulatory Infections. Isolation of microorganisms from blood is of value to the medical officer in the diagnosis and treatment of various infections. Blood in man is normally sterile, and the presence of bacteria in the bloodstream is a pathogenic condition. This condition is called septicemia or bacteremia.
Figure 1-4. Technique for processing blood specimens.
b. **Timing of Collections.** Many blood cultures yield negative results because samples are not collected during the proper stage of the disease concerned. Specimens of blood should be drawn when symptoms are indicative of circulatory involvement, (chills, fever, convulsions, and so forth). Negative results from a single blood culture never rule out septicemia.

c. **Preparation of Venipuncture Site.** Preparation of the site for venipuncture is crucial. Swab the site with cotton-tipped applicator sticks saturated with iodine tincture, USP. Allow to dry. Remove with sponges saturated with 70 percent isopropyl alcohol. Then repeat the entire process.

d. **Processing of Specimen.** There are many methods for the culturing of blood specimens. Regardless of the methods of choice, the blood should be aspirated by venipuncture using the strictest aseptic technique. Blood may be immediately inoculated into broth at the bedside of the patient, or the blood may be drawn and transported to the laboratory in sterile, capped test tubes containing anticoagulant.

e. **Prepared Culture Bottles.** Prepared culture bottles are available commercially, which make it possible to withdraw blood and introduce it into the culture medium within a sterile, closed system. One needle is inserted into the vein. The blood passes through a vinyl tube to a needle at the other end of the tube. This other needle has been used to puncture the rubber cap of a vacuum bottle containing the medium. When sufficient blood has passed into the bottle (about 1 ml of blood for each 10 ml of medium), the vinyl tube is pinched to stop the blood flow, the tourniquet is removed, and the needle is removed from the vein. This system can be used both aerobically and anaerobically. For aerobic culture, filtered air is allowed to enter the bottle after blood collection. Frequently included in the medium is the desirable anticoagulant called sodium polyanethol sulfonate (Liquoid).

f. **Culture of Specimen-Infusion Broth Technique.**

(1) Inoculate a volume of approximately 5 ml of blood to each of two bottles or flasks containing the infusion broth. Each bottle or flask should contain 50 ml of trypticase soy broth or other suitable infusion broth.

(2) Incubate one broth aerobically, under CO₂ tension at 37°C.

(3) Incubate the second broth anaerobically at 37°C.

**NOTE:** Trypticase soy broth in vacuum bottle with CO₂ incorporated is available commercially. If inoculated and incubated according to the manufacturer's instructions, this medium will yield excellent results.
g. **Culture of Specimen-Castaneda Bottle Technique.**

(1) Inoculate a volume of approximately 5 ml of blood to each of two Castaneda bottles (these are bottles containing both a broth and an agar surface).

(2) Incubate one bottle aerobically, under CO₂ tension at 37°C.

(3) Incubate the second bottle anaerobically at 37°C.

**NOTE:** This method is desirable when colonial characteristics are to be studied. Bottles prepared with thioglycollate broth will support growth of anaerobes.

h. **Culture of Specimen--Pour Plate Technique.**

(1) Prepare trypticase soy agar pour plates using the method in Lesson 2, Section II.

(2) Inoculate the desired volume of the patient’s blood to the liquid state in the pour plate agar.

(3) Incubate the plate under CO₂ at 37°C. Aerobic and anaerobic conditions will be provided in the pour plate.

**NOTE:** This method is desirable when hemolysis is to be studied.

i. **Care of Sample.** The blood culture media should be placed in the incubator as soon as possible. Both CO₂ tension and anaerobic conditions (see chapter 4) should be incorporated into the incubation to promote growth and reproduction of the organisms most frequently encountered in septicemia. Blood culture specimens should be incubated at 37°C, and should remain in the incubator and be observed for at least 21 days before being reported as negative and discarded. Blood cultures should be observed daily for signs of growth. At the first sign of turbidity, gas formation, pigmentation of media, "cotton-ball" formation, coagulum formation, or growth on the agar slant, the culture should be checked for the presence of bacteria.

j. **Checking the Culture.**

(1) To check a blood culture for the presence of bacteria, first mix the culture well by gentle swirling. Using a sterile syringe and needle and aseptic technique, remove an aliquot of 1 to 2 ml from the blood culture. Use a portion of the sample to inoculate the appropriate media for subculture, usually blood agar, chocolate agar, and thioglycollate broth. Place another portion of the sample on a clean glass slide and allow to air dry. Perform a gram stain on this slide. (Gram stain procedures are explained in Lesson 3.) If there is any visible evidence of growth in the blood culture, and if organisms are demonstrated on the gram stain cell, call the requesting physician immediately.
(2) If visible signs of growth do not occur in a blood culture, the specimen should still be checked for growth at least twice a week during the three-week incubation period. The procedure to check these cultures is the same as the method used to check cultures with visible signs of growth.

(3) A negative blood culture usually remains quite clear; however, it may develop cloudiness after prolonged incubation. This is usually due to shaking the bottle during each observation with the development of turbidity from fibrin or agar particles. Many times this type of turbidity occurs immediately above the red cell layer and it may suggest bacterial growth. In any case, turbidity alone is not a reliable guide for cellular growth, and a gram stain should always be made to determine the presence of microorganisms.

(4) Failure to examine blood cultures often enough or to hold the cultures long enough may result in delay or failure in recognizing the presence of microorganisms. On the other hand, excessively frequent opening of the culture bottles may result in contamination of the culture. It is necessary that a strictly aseptic technique always be followed in the handling and processing of any culture, particularly a blood culture.

1-17. COLLECTING AND PROCESSING OF SPECIMENS FROM THE RESPIRATORY TRACT

a. Importance of Throat and Nasopharyngeal Specimens. Throat and nasopharyngeal cultures are important in the diagnosis of such infections as streptococcal sore throat, scarlet fever, diphtheria, and whooping cough. They are also useful in determining the focal point of infection in such diseases as rheumatic fever and acute glomerulonephritis. In epidemiological studies, these cultures have been essential for the detection of carriers of beta hemolytic streptococcus, staphylococcal infections, Corynebacterium diphtheriae, and other potential pathogens.

b. Collecting and Primary Care.

(1) Collect specimens under good lighting using a sterile cotton-tipped applicator stick (see figure 1-5). Depress the tongue with a tongue blade and pass the swab gently over the crypts and tonsils. Also move the swab across the back surface of the pharynx depending on which areas appear to be red and swollen or suggestive of infection by displaying white patchy areas or lesions. Be careful to avoid touching the swab to the tongue, cheek, or teeth as this will result in a culture highly contaminated with a large amount of normal throat flora, and thereby reduce the chances of recovering a true pathogen if one is present.

(2) Place the swab in a sterile test tube and immediately transport the specimen to the clinical laboratory. The sterile tube may contain sterile culture broth to prevent drying of the specimen. Thioglycollate, Trypticase soy, or Todd-Hewitt broth is satisfactory for this purpose.
c. **Processing.** See figure 1-6 for the basic technique for processing throat and nasopharyngeal specimens.

1. Group A beta hemolytic streptococci are better obtained from throat swabs than from any other infected area. For this reason the blood agar streak plate should be carefully examined after 18-24 hours of incubation at 37°C for the presence of colonies of beta hemolytic streptococci. Any colony that appears to exhibit beta hemolysis should be subcultured by stabbing with an inoculating needle and streaked on a sheep blood agar plate. If a pure culture is not obtained, the process can be repeated. Further identification of these colonies and/or sensitivity studies may then be carried out as indicated.

2. Normal throat cultures show a predominance of alpha hemolytic streptococci and neisseria. If a culture shows a predominance of organisms that normally occur only in small numbers, this could be of significance and should be reported.
(3) Pneumococci do occur occasionally in the throats of normal individuals; however, if a large number of the organisms are present, especially in specimens taken from the pediatric age group, it may be of etiological significance and should be reported. A diagnosis of pneumococcal pneumonia may be dependent upon the isolation of pneumococci from a throat or nasopharyngeal culture.

(4) Bordetella pertussis (whooping cough) may also be isolated from the respiratory tract. Cough plates containing a special medium are often utilized; however, nasopharyngeal cultures usually give a higher proportion of positive cultures.
(5) A diagnosis of diphtheria may also be made from throat cultures: however, physicians often expect a definitive diagnosis within 10 minutes based on a direct smear of the throat lesion. Nonpathogenic diphtheria-like (diphtheroid) bacilli may be indistinguishable from the very pathogenic C. diphtheriae. One cannot distinguish these species from a direct smear. The immediate diagnosis of diphtheria is a clinical problem and should rest with the attending physician, with a positive or negative confirmation coming from the bacteriology section after the culture has been processed. Inoculate the specimen on blood agar medium for the growth of aerobes. Inoculate the specimen into thioglycollate medium for the growth of anaerobes and aerobes. Inoculate the specimen on potassium tellurite agar and/or Loeffler's serum slant if Corynebacterium diphtheriae is suspected. Direct smears may be of value if Vincent's angina is suspected. Incubate the blood agar medium under O₂ tension for 18-24 hours at 37°C. Incubate the other media under normal incubation atmosphere for 18-24 hours at 37°C. On potassium tellurite agar, Corynebacterium diphtheriae usually requires 48 hours of incubation at 37°C. If growth is observed in thioglycollate liquid medium, subculture this growth under anaerobic and aerobic conditions.

d. **Sputum Specimens.** Sputum for bacteriological examination (figure 1-7) should be collected in sterile, wide-mouth, screw-capped jars or with the Falcon sputum collection kit. In the case of pneumonia, one sputum specimen is usually sufficient for the examination. This specimen should be collected as soon as the patient awakes in the morning. In the case of pulmonary tuberculosis, a 24-hour collection of sputum or a 3-day collection should be submitted for the examination. Sputum should be processed as soon as possible after it is collected.

(1) **Technique for routine organisms and pneumococci.**

(a) Inoculate the specimen on blood agar medium for the growth of aerobes. If pneumococci are suspected, place an Optochin disc (ethylhydrocupreine hydrochloride) on the initial streak.

(b) Inoculate the specimen into thioglycollate liquid medium for the growth of aerobes and anaerobes.

(c) Direct smears should be gram-stained and examined for the presence of *Staphylococcus*, *Pneumococci*, yeast cells, etc.

(d) Incubate the media for 18-24 hours at 37°C. CO₂ tension may be incorporated to facilitate aerobic growth on the blood agar medium. If pneumococci are present, a zone of inhibited growth will be seen around the Optochin disc after incubation.

(e) If liquid anaerobic growth is observed in the thioglycollate liquid medium, subculture this growth under anaerobic conditions.
(2) Technique for acid-fast bacilli.

(a) Prepare smears from grayish or yellowish cheesy masses or purulent blood-tinged portions of the specimen.

(b) Acid-fast stain smears should be prepared and examined. (These are explained in para 2-2)

(c) If the specimen is to be cultured, inoculate a portion of the specimen or Lowenstein-Jensen, Petragnani’s, or other suitable media for the cultivation of acid-fast bacilli. A concentrated sediment is generally more reliable than using direct smears. If there is only a small amount, the entire specimen could be used for inoculating the medium. Also, animal virulence tests may be required.

Figure 1-7. Technique for processing sputum specimens.
1-18. COLLECTING AND PROCESSING URINE SPECIMENS

a. Importance. Urine cultures (figure 1-8) are of value in diagnosing primary infections of the anterior urinary tract (urethritis), bladder (cystitis), and kidneys (nephritis). Urine cultures are also important in diagnosing certain systemic infections, for the etiological agents are often excreted via the urinary tract. Staphylococcus species, a Streptococcus species, and Neisseria gonorrhoea are among the primary etiological agents of urethritis while Escherichia, Proteus, and occasionally Pseudomonas species are among the chief causative agents of cystitis. Any one or more of these organisms may be the cause of a bacterial nephritis. Yet, many of the above mentioned organisms may be a common urine contaminant. A medical officer usually does not make a diagnosis on the basis of one urine culture. Rather, repeated isolation of large numbers of a particular organism from a series of urine specimens is evidence for pathogenicity of the organism concerned.

Figure 1-8. Technique for processing urine.
b. Collection. "The clean-catch" method of collecting urine is generally preferable. Since the periurethral area (tip of the penis, vulva, and labial folds) is generally contaminated, it must be carefully cleaned prior to collection. Wash carefully with plain soap and water, and rinse. Repent this process, and then rinse well with warm sterile water. The first portion of urine is voided and discarded; this flushes the urethra. A subsequent portion is collected aseptically for culture. All urine specimens for culture should be collected in sterile urine bottles or sterile wide-mouth, screw-capped jars. Delay between the collecting and culturing of urine specimens may contribute to a change in pH and other characteristics of the specimen, resulting in a high death rate of the organisms in question. Urine must be processed within an hour of collection or stored in a refrigerator at 4ºC until it can be cultured.

c. Culture for Routine Organisms.

(1) Centrifuge the urine specimen and inoculate the concentrated sediment on blood agar, eosin-methylene blue (EMB) or MacConkey's agar, and thioglycollate liquid medium.

(2) If Neisseria is suspected, inoculate a portion of the sediment to supplemented chocolate agar.

(3) Gram-stained smears of the sediment may be helpful.

(4) Incubate the media for 18-24 hours at 37ºC. Increased CO₂ tension should be incorporated to facilitate growth on the blood and chocolate agar media.

(5) If growth is observed in the thioglycollate liquid medium subculture this growth under anaerobic and aerobic conditions.

d. Culture for Acid-Fast Bacilli.

(1) A 24-hour pooled specimen maybe necessary to demonstrate the presence of acid-fast bacilli in urine. To process this 24-hour collection, add 2-3 grams of tannic acid to the total 24-hour pooled volume and shake well. Place the collection in the refrigerator overnight or for an equivalent period of time. A brown precipitate will form. Decant the supernatant and concentrate the sediment.

(2) The concentrated sediment may be inoculated to suitable media, injected into test animals, and/or spread on clean slides and dried for acid-fast staining. Lowenstein-Jensen or Petragnani's medium is satisfactory for culturing acid-fast organisms.
1-19. COLLECTING AND PROCESSING OF FECAL SPECIMENS

a. Importance. The bacteriological examination of fecal specimens (figure 1-9) aids in the diagnosis of gastrointestinal infections manifested by diarrhea and/or dysentery. Stool cultures, along with blood and urine cultures, are important aids for diagnosing typhoid and paratyphoid fevers. Since many diseases are spread by human carriers through food and drink, properly performed stool cultures on all food-handling personnel strongly supplement public health control measures.

![Diagram of fecal specimen processing]

Figure 1-9. Technique for processing fecal (stool) specimens.

(1) The organisms most frequently involved in enteric infections are the Salmonella and Shigella species of the Enterobacteriaceae family. The normal intestinal flora of the adult is composed primarily of anaerobic, gram-negative rods. The biochemical activities of this normal flora can harm species of the family Enterobacteriaceae, which are the focus of clinical testing. Pseudomonas aeruginosa, Alcaligenes faecalis, and Proteus species are sporadically present in the intestine and may be called transient saprophytes. Saprophytic organism (including E. coli, Morganella morganii, and Providencia) have been implicated as etiological agents of infant diarrhea.
(2) In culturing specimens of intestinal origin, the basic problem is the isolation of pathogenic agents from specimens heavily contaminated with saprophytic organisms. To accomplish this, special differential, selective, and inhibitory media are available.

b. Collection. Fecal specimens should be collected in clean, wide-mouth containers with tight fitting lids: screw-capped jars are satisfactory. These containers do not have to be sterile but should always be very clean. If rectal swabs are obtained, these should be transported to the clinical laboratory in clean, cotton-plugged test tubes. Rectal swabs may be useful when dealing with infants or large numbers of patients. Fecal material should be cultured as soon as possible after, collection; if culturing is delayed, the isolation of causative agents, particularly Shigella species will be jeopardized.

c. Culture for Routine Organisms.

(1) Inoculate sufficient fecal material into selenite F broth (or tetrathionate broth) to produce a heavy suspension. Incubate the broth for 8-12 hours at 37°C. This procedure will boost the growth and reproduction of enteric pathogens while inhibiting the growth and reproduction of enteric saprophytes for approximately 8-12 hours.

(2) After 8-12 hours of incubation, subculture from the selenite F into the following media:

(a) Differential medium, that is, EMB, MacConkey's, or deoxycholate media.

(b) Selective medium, that is, Salmonella-Shigella (SS) or deoxycholate-citrate media.

(c) Inhibitory brilliant green medium, that is, bismuth sulfite or brilliant green media.

(3) Incubate the differential, selective, and inhibitory media aerobically at 37°C.

(4) After 12-16 hours of incubation, examine the differential, selective and brilliant green media for colonies of lactose-nonfermenting organisms (encompassing possible pathogens). These media should be incubated for 24 hours before being discarded as negative.

(5) After 12-24 hours of incubation, examine the bismuth sulfite medium for typical colonial growth of pathogens, particularly Salmonella typhi. The bismuth sulfite medium should be incubated for 48 hours before being discarded as negative.
(6) Isolation of lactose-fermenting organisms from fecal material is usually considered insignificant, with the exception of isolates from infant diarrhea.

d. Culture for *Streptococcus faecalis*.

(1) *Streptococcus faecalis*. (SF) medium should be immediately inoculated if the enterococcus *Streptococcus faecalis* is suspected.

(2) Incubate SF medium for 18-24 hours at 37°C. This medium is specific for the growth and reproduction of *Streptococcus faecalis*.

1-20. COLLECTING AND PROCESSING BODY FLUIDS

NOTE: See figure 1-10.

![Figure 1-10. Technique for processing body fluids.](image)

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MD0856 1-32
a. **Importance.** Body fluids include spinal, synovial, pleural, pericardial, and peritoneal fluid. Examination of spinal fluids is important in determining the cause of meningitis. In many cases, emergency therapeutic agents are administered on the basis of results from microscopic examination (gram-stained smear) of concentrated spinal fluid. The genera of microorganisms most frequently responsible for meningitis are *Neisseria*, *Diplococcus*, *Streptococcus*, *Staphylococcus*, *Haemophilus*, and occasionally *Mycobacterium*. One of the fungi chiefly responsible is *Cryptococcus neoformans*. *Neisseria* may be encountered in specimens of synovial fluid from arthritic patients.

b. **Collection.** When a medical officer submits a body fluid for culture, the source and the provisional diagnosis should be entered on the request form. This will aid in the selection of the proper media for inoculation. Generally, all body fluids are aspirated by a medical officer and transported to the laboratory in sterile, screw-capped test tubes. Culture body fluids as soon as possible after collection since fibrinogen coagulates the fluids, making them difficult to transfer. Use extreme care when handling body fluids because these fluids often contain highly infectious organisms.

c. **Culture for Routine Organisms.**

   (1) Mix the specimen well and centrifuge at 2,500 rpm for approximately 15 minutes.

   (2) Decant the supernatant into a jar of phenol. Inoculate a portion of the concentrated sediment to two blood agar plates, one supplemented chocolate agar plate, and one tube of thioglycollate liquid medium.

   (3) Prepare a direct smear from the sediment: gram stain and observe for predominating organisms.

   (4) Incubate one blood agar plate and the chocolate agar plate under CO₂ tension for 18-24 hours at 37°C.

   (5) Incubate the second blood agar plate anaerobically for 18-24 hours at 37°C.

   (6) Incubate the thioglycollate liquid medium under normal incubation atmosphere for 18-24 hours at 37°C.

   (7) If growth is observed in the thioglycollate liquid medium, subculture this growth under anaerobic conditions and aerobic conditions.
d. Culture for Acid-Fast Bacilli.

(1) The sediment from the centrifuged specimen need not be further concentrated, providing the specimen is free from mucus, debris, and contaminating growth.

(2) The processed sediment may be inoculated to suitable media, injected into test animals, and/or spread on clean slides and dried for acid-fast staining. Lowenstein-Jensen or Petragnani's medium is satisfactory for culturing acid-fast organisms.

1-21. COLLECTING AND PROCESSING EXUDATE SPECIMENS

NOTE: See figure 1-11.

![Diagram of exudate processing]

Figure 1-11. Technique for processing exudates.
a. **Importance.** An exudate is material that has passed through the walls of vessels into adjacent tissues or areas of inflammation. Exudates may be obtained from boils, wounds, ear or mastoid infections, eye infections, and skin lesions. A great variety of microorganisms may be isolated from such areas. Boils may yield *Staphylococcus* species, *Streptococcus* species, and occasionally gram-negative rods (*Escherichia*, *Pseudomonas*, or *Proteus*). Deep wounds, especially puncture wounds, and those with severe tissue damage provide ideal living conditions for anaerobes, especially *Clostridium* species. Infected wounds that exhibit a greenish or bluish purulent discharge may reveal *Pseudomonas* infection. Exudates from surgical or postoperative infections may reveal anaerobic *Streptococcus* species. Ear and mastoid infections are often caused by *Pseudomonas*, *Staphylococcus*, or *Streptococcus* species. Eye infections may yield *Haemophilus* or possibly *Neisseria* species. Purulent discharges from the urethra frequently reveal *Neisseria gonorrhoeae*. A gram-stained smear of exudate from chancre or soft chancre usually reveals small gram-negative rods (*Haemophilus ducreyi*). A dark field examination of exudate from the true or hard change usually reveals the spirochete *Treponema pallidum*.

b. **Collection.** Exudate specimens are usually collected on sterile cotton-tipped swabs by a medical officer and sent to the laboratory in sterile cotton-plugged test tubes. Exudate material in the form of purulent drippings from cases of urethritis may be collected on sterile cotton-tipped swabs or on sterile wire loops and inoculated directly on or into culture media.

c. **Culture.**

1. Inoculate the specimen on blood agar, EMS or MacConkey's and thioglycollate liquid medium.

2. If *Neisseria* is suspected, inoculate a supplemented chocolate agar plate.

3. Prepare direct smears, gram-stain, and examine microscopically for predominating organisms.

4. Incubate the media for 18-24 hours at 37°C. Place the blood and chocolate agar plates under CO₂ tension for the period of incubation.

5. If growth is observed in the thioglycollate liquid medium, this growth under anaerobic conditions and aerobic conditions.

6. Prepare gram-stained smears from any growth in the thioglycollate medium.
1-22. PROCESSING OF SPECIMENS FOR SHIPMENT

NOTE: See table 1-2.

It is frequently necessary to ship specimens to reference laboratories for analysis or confirmation. Acceptable shipping containers and procedures for packing specimens are described below:

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>PROCESSING FOR SHIPMENT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascitic fluid for bacterial and fungal culture.</td>
<td>Place aseptically in sterile containers without preservative.</td>
<td>Where tuberculosis is suspected, see directions for processing specimens from suspected tuberculosis patients.</td>
</tr>
<tr>
<td>Pure cultures of bacteria for identification, confirmation, and sensitivity tests.</td>
<td>Grow in pure culture on beef infusion agar in screw-cap tubes. Prior to shipment, tighten screw-caps and secure with adhesive tape or by dipping in melted paraffin.</td>
<td>Plant subcultures on agar slants and incubate 12-18 hours. A duplicate of each culture should be retained in the laboratory of origin until a final report is rendered.</td>
</tr>
<tr>
<td>Blood or urine for culture.</td>
<td>Collect 3 ml. of blood aseptically before serum, chemical or antibiotic therapy and transfer to rubber-stoppered bottle containing hemoglobin-tryposephosphate broth.</td>
<td>Prior to shipment, incubate specimens for 12-24 hours.</td>
</tr>
<tr>
<td>Serum for bacterial and cold agglutinins, antibiotic levels, and complement-fixation tests.</td>
<td>Collect blood aseptically in sterile evacuated tubes, filling to capacity.</td>
<td>Allow firm clot to form before packing for shipment. Blood for cold agglutination tests must not be refrigerated until cells are separated from serum.</td>
</tr>
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</table>

Table 1-2. Collection and processing of bacteriological specimens for shipment (continued)
<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>PROCESSING FOR SHIPMENT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal fluid for bacterial culture, and antibiotic and sulfonamide level.</td>
<td>A minimum of 3 ml. of fluid is collected aseptically and inoculated into hemoglobin-tryptosephosphate broth.</td>
<td>Where tuberculosis is suspected, see directions for processing such specimens. Retain broth culture under incubation 12-24 hours prior to shipment.</td>
</tr>
<tr>
<td>Throat cultures for <em>C. diphtheriae</em>.</td>
<td>Innoculate Loeffler's serum slant with a fresh throat swab.</td>
<td>Mail specimen immediately.</td>
</tr>
<tr>
<td>Joint or other body fluids or bacterial and fungal cultures.</td>
<td>Collect joint and other body fluids and transfer aseptically to suitable sized sterile container without preservative. Plant fluids for mycological culture on Sabouraud's media, or place in sterile screw-capped containers without preservative.</td>
<td></td>
</tr>
<tr>
<td>Sputum for pneumococcus culture or typing.</td>
<td>Collect aseptically before serum, chemical, or antibiotic therapy is started and place in sterile container without preservative.</td>
<td></td>
</tr>
<tr>
<td>Swabs for culture from open lesions.</td>
<td>Swab lesion with broth-soaked swab and place aseptically in sterile screw-capped test tubes.</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis, specimens for smear, culture, and animal inoculation (other than sputum and gastric contents).</td>
<td>Collect fluids, tissue, or feces in suitable sized sterile containers without preservative.</td>
<td>Specimens include ascitic fluid, cerebrospinal fluid, joint fluid, pleural fluid, pus, urine, lymph node, and other tissue, and feces.</td>
</tr>
</tbody>
</table>

Table 1-2. Collection and processing of bacteriological specimens for shipment (continued)
<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>PROCESSING FOR SHIPMENT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis, sputum specimens.</td>
<td>Collect all sputum coughed up on three consecutive mornings after first having patient wash teeth and rinse mouth thoroughly with boiled water. Place entire 3-day specimen in a sterile wide-mouthed screw-capped bottle; add an equal volume of 20% trisodium phosphate.</td>
<td>During collection, keep jars out of direct sunlight or the tubercle bacillus will be rendered nonviable and, therefore, unsuitable for culture or animal inoculation. Do not use cardboard sputum cups for collection.</td>
</tr>
<tr>
<td>Tuberculosis, gastric contents.</td>
<td>Through sterile stomach lavage tube introduce 200-300 ml. of sterile saline solution into fasting stomach. Evacuate stomach and place entire specimen in suitable sterile container.</td>
<td>Prior to placing specimen in shipping container, neutralize it to litmus with 20 percent sodium bicarbonate.</td>
</tr>
<tr>
<td>Exudates for bacterial and fungal cultures.</td>
<td>Place swabs aseptically in sterile screw-capped containers without preservative.</td>
<td></td>
</tr>
<tr>
<td>Fecal specimens for bacterial culture (except tuberculosis).</td>
<td>Emulsify a 5 g. sample, aseptically in a sterile screw-capped vessel containing one part C.P. glycerin and three parts 0.6 percent saline solution.</td>
<td>In food poisoning outbreaks, feces should be collected from patients with diarrhea and all food handlers associated with the outbreak.</td>
</tr>
</tbody>
</table>

Table 1-2. Collection and processing of bacteriological specimens for shipment (continued)
<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>PROCESSING FOR SHIPMENT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimens for bacteriological examination following food poisoning outbreaks.</td>
<td>(1) Any suspected canned foods should be shipped unopened in original container.</td>
<td>Complete epidemiological history should accompany any specimens taken from food poisoning outbreaks.</td>
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<tr>
<td></td>
<td>(2) Well mixed representative samples of suspected prepared food should be placed aseptically in suitable containers without preservative.</td>
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<tr>
<td></td>
<td>(3) Vomitus from all cases in which vomiting is present should be placed aseptically in suitable sized sterile containers without preservative.</td>
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</tr>
<tr>
<td></td>
<td>(4) Swabs from lesion taken from food handlers should be placed in nutrient broth using aseptic technique and transferred to sterile screw-capped test tubes.</td>
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</table>

Table 1-2. Collection and processing of bacteriological specimens for shipment (concluded).

a. **Noninfectious Specimen.**

(1) Enclose the specimen in a sterile glass container and cap. A screw-shaped test tube or jar is satisfactory.

(2) Place the glass container in standard double shipping containers.

(3) Pad the spaces between the containers to guard against breakage.

(4) The properly packed container of noninfectious material will have the following layers, from specimen outward: specimen, glass, padding, metal, padding, and heavy cardboard (figure 1-12A).
b. **Infectious Specimen.**

1. Enclose the specimen in a sterile, stout glass tube and seal the ends of the tube by fusion of the glass.

2. Place the glass tube containing the specimen in a stout glass container that can be sealed by an insulated screw cap, rubber stopper, or by fusion of the glass.

3. Add formalin to the outer container so that the inner glass container is completely surrounded by formalin; then seal the glass container with wax. This procedure provides for disinfection if the inner container should break.

4. Pack this double glass container in standard double shipping containers.

5. Pad the spaces between the containers to guard against breakage.

6. The properly packed container of infectious material will have the following layers, from specimen outward: specimen, glass, formalin, glass, padding, metal, padding, and heavy cardboard (figure 1-12B).

![Figure 1-12. Microbiological specimens packed for shipment.](image-url)
c. **Labeling.**

(1) The appropriate request form, properly completed, must accompany the specimen.

(2) The request slips should be placed between the two outer shipping containers to prevent the slips from being damaged or contaminated if breakage occurs.

(3) The following notation must be made on the outside label of any mailing container of bacteriological specimens: Specimen for bacteriological examination; this package shall be packaged with letter mail.

*Continue with Exercises*

*Return to Table of Contents*
EXERCISES, LESSON 1

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. Mutualism is a microbial association in which:
   a. The host and microorganism both benefit.
   b. The microorganism derives significant benefit at the expense of the host.
   c. Neither the microorganism nor the host derives any benefit, or harms the other.
   d. The host derives significant benefit at the expense of the microorganism.

2. Commensalism is a microbial association in which:
   a. The host and microorganism both benefit.
   b. The microorganism derives significant benefit at the expense of the host.
   c. The microorganism derives significant benefit without harming or greatly benefiting the host.
   d. The host derives significant benefit at the expense of the microorganism.

3. The LD$_{50}$ is a standardized means of expressing:
   a. Invasiveness.
   b. Virulence.
   c. Pathogenesis.
   d. Infectiousness.
4. Bacteria commonly reproduce by:
   a. Spore germination.
   b. Conjugation.
   c. Budding.
   d. Binary fission.

5. A toxin that is secreted by bacteria into their environment is known as:
   a. An exotoxin.
   b. An endotoxin.
   c. A fomite.
   d. A proteolytic enzyme.

6. A toxin that is found in the cell wall of a living organism is:
   a. An endotoxin.
   b. An enterotoxin.
   c. An exotoxin.
   d. A pseudotoxin

7. Bacteria that is able to obtain energy and grow on inorganic media and utilize carbon dioxide as their sole source of carbon are known as:
   a. Heterotrophic bacteria.
   b. Autotrophic bacteria.
   c. Parasitic bacteria.
   d. Saprophytic bacteria.
8. Heterotrophic bacteria require __________ media.
   a. Organic.
   b. Inorganic.

9. Bacteria are measured in microns, which are:
   a. 1/10 of a millimeter.
   b. 1/100 of a millimeter.
   c. 1/1,000 of a millimeter.
   d. 3.1416 parts of an inch.

10. Spherical bacteria arranged in chains are called:
    a. Spirilla.
    b. Diplococci.
    c. Streptococci.
    d. Staphylococci.

11. The outer layer that gives rigidity to a bacterial cell is known as the:
    a. Cell wall.
    b. Cytoplasmic membrane.
    c. Nuclear membrane.
    d. Cytoplasm.
12. Flagella are:
   a. Gummy envelopes surrounding organisms.
   b. Internal structures formed for protection.
   c. Thread-like appendages for locomotion.

13. One would expect to find spores in:
   a. ALL OF THE BELOW.
   b. Cocci.
   c. Bacilli.
   d. Spirilla.

14. The formation of spores by bacteria is generally considered as a means of:
   a. Reproduction.
   b. Toxin formation.
   c. Survival.
   d. Resistance to phagocytosis.

15. Two media suitable for initial culture of blood specimens are:
   a. EMB agar and MacConkey's agar.
   b. Thioglycollate broth and Trypticase soy broth.
   c. Brilliant green agar and bismuth sulfite agar.
   d. Deoxycholate agar and Salmonella-Shigella agar
16. Blood cultures should be observed daily. They should be checked for the presence of bacterial growth at the first sign of:

a. ANY OF THE BELOW.
b. Turbidity.
c. Gas formation.
d. Coagulum formation.

17. Blood cultures should be observed for at least __________ before being reported as negative and discarded.

a. 1 week.
b. 2 weeks.
c. 3 weeks.
d. 4 weeks.

18. Normal throat cultures usually show a predominance of

a. Alpha hemolytic streptococci and neisseriae.
b. Corynebacteria.
c. Bordetella.
d. Beta hemolytic streptococci.

19. If diphtheria is suspected, a throat culture should be inoculated on which of the following special media in addition to those used routinely?

a. Chocolate agar.
b. Potassium tellurite agar.
c. Petragnani’s agar.
d. Lowenstein-Jensen agar.
20. The disk is used as a screening test for:
   a. Pneumococci.
   b. Streptococci.
   c. Staphylococci.
   d. Corynebacteria.

21. The generally preferred method of urine collection is:
   a. Catheterization.
   b. 24-hour collection.
   c. Two-container collection.
   d. The clean-catch method.

22. When urine specimens cannot be processed within 1 hour of collection, they must be stored at what temperature?
   a. Below 0°C.
   b. 4°C.
   c. 25°C.
   d. 37°C.

23. For the initial suppression of most enteric saprophytes in stool specimens being cultured for enteric pathogens, which of the following should be used?
   a. Blood agar.
   b. Nutrient broth.
   c. Trypticase soy agar.
   d. Selenite F broth.
24. A subculture on bismuth sulfite agar of suspected *Salmonella typhi* organisms should be incubated at 37°C for how long before being discarded as negative:
   a. 12 hours.
   b. 24 hours.
   c. 48 hours.
   d. 72 hours.

25. SF medium is specific for the growth and reproduction of:
   a. *Sarcophaga fuscicauda*.
   b. *Shigella flexneri*.
   c. *Siphunculina funicola*.
   d. *Streptococcus faecalis*.

26. Examination of spinal fluids is important in determining the cause of:
   a. Meningitis.
   b. Pleurisy.
   c. Pneumonia.
   d. Urethritis.

27. Which of the following would be considered an exudate rather than a body fluid?
   a. Synovial fluid.
   b. Pericardial fluid.
   c. Peritoneal fluid.
   d. Purulent discharge from a wound.
28. Which of the following would be considered an exudate?
   a. Fluid from the peritoneum.
   b. Fluid from the pericardium.
   c. Fluid from the joints.
   d. Fluid from a boil.

29. If Neisseria is suspected in exudate material, the specimen should be plated on blood agar, EMB, thioglycollates and:
   a. Brilliant green agar.
   b. Chocolate agar.
   c. Selenite F broth.
   d. Tetrathionate broth.

Check Your Answers on Next Page
SOLUTIONS TO EXERCISES, LESSON 1

1. a (para 1-1a)
2. c (para 1-1b)
3. b (para 1-2b)
4. d (para 1-2d)
5. a (para 1-5a)
6. a (para 1-5b)
7. b (para 1-9)
8. a (para 1-10)
9. c (para 1-11; figure1-1)
10. c (para 1-11a(3))
11. a (para 1-12a(1))
12. c (para 1-12b(2))
13. c (para 1-12b(3))
14. c (para 1-12b(3))
15. b (para 1-16f, g)
16. a (para 1-16i)
17. c (para 1-16i)
18. a (para 1-17c(2))
19. b (para 1-17c(5))
20. a (para 1-17d(1))
21. d (para 1-18b)
22. b (para 1-18b)
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