ASSISTING IN MICROBIOLOGY AND IMMUNOLOGY

SCENARIO

Infectious diseases are a continuing threat for everyone. Anna McIntyre, CMA (AAMA), knows that some diseases have been effectively controlled with the help of modern technology, but new diseases are constantly appearing, such as the avian flu and West Nile virus infection. In addition, other familiar infectious diseases, such as malaria, tuberculosis, and bacterial pneumonias, are appearing in forms that are resistant to drug treatment. Anna knows that it is important to identify pathogens quickly so that the proper treatment can begin. The identification of pathogens, she has discovered, can involve many different tests, many of which can be performed in the physician’s office laboratory (POL) where she works.

While studying this chapter, think about the following questions:

- How can Anna protect herself and other patients in the practice from infectious microorganisms?
- How can body fluids or other samples be tested for the presence of pathogenic organisms?
- How are pathogenic organisms differentiated from normal, nonpathogenic species?
- What role do laboratory healthcare workers play in the identification and treatment of infections caused by microorganisms?

LEARNING OBJECTIVES

1. Define, spell, and pronounce the terms listed in the vocabulary.
2. Apply critical thinking skills in performing the patient assessment and patient care.
3. Cite the protocols for the collection, transport, and processing of specimens.
4. Identify the elements needed for microbial growth.
5. Compare bacteria with viruses.
6. Describe the characteristics of common viral diseases.
7. Describe the bacterial structures used in identification.
8. Compare bacteria with fungi, parasites, and protozoa.
9. Describe various bacterial morphologies.
10. Explain the characteristics of common diseases caused by bacteria.
11. Describe the unusual characteristics of Chlamydia, Rickettsia, and Mycoplasma organisms.
12. Identify the characteristics of common diseases caused by fungi, protozoa, and parasites.
13. Perform patient education on the collection of a stool specimen for ova and parasite testing.
14. Describe the equipment needed in a microbiology laboratory.
15. List the different growth media used for culturing.
17. Perform the procedure for inoculating a blood agar plate.
18. Perform a urine culture.
19. Perform a screening urine culture test.
20. Prepare a direct smear or culture smear for staining.
21. Compare and contrast the throat culture for Streptococcus pyogenes with the rapid strep test.
22. Perform a rapid strep test.
23. Describe three microbiologic tests that use a rapid identification technique.
24. Describe the method used for antimicrobial susceptibility testing.
25. Explain how pinworm testing is done and why it must be performed.
26. Perform a cellulose tape collection for pinworms.
27. Discuss the purpose of immunologic testing.
28. Describe three rapid immunologic tests that could be done in the physician’s office laboratory.
29. Perform the Mono-test for mononucleosis.
30. Discuss legal and ethical issues involved in laboratory testing.
Microorganisms get a lot of publicity. Bioterrorism became a reality in the United States in the fall of 2001, when Bacillus anthracis spores sent through the mail caused anthrax, killing three people. Products line our grocery store shelves declaring their ability to keep us germ free. The evening news reports on the latest outbreaks of “flesh-eating bacteria,” contaminated water supplies, and antibiotic-resistant microbes. No wonder most people have the impression that all microorganisms are harmful. In reality, fewer than 1% of known microorganisms are pathogens. In fact, without microorganisms, we could not survive.

Microorganisms are responsible for decomposition of waste and natural recycling. The organisms that are normally present on and in our bodies ensure that our food is digested, that our blood clots properly as a result of vitamin K production by the organisms inhabiting our intestines, and that pathogens are prohibited from invading our skin, mucous membranes, and gastrointestinal and genitourinary tracts. When the normal flora are disrupted, such as by antibiotic use or hormonal changes, certain organisms that are present normally in low numbers overgrow, causing a superinfection. For example, vulvovaginal candidiasis, a yeast infection of the vaginal tract, is common in women taking broad-spectrum antimicrobial agents.

The study of immunology, or the immune system, is closely tied to microbiology. Microorganisms induce an immune response, leading to the production of a variety of molecules that come to our defense. These molecules include antibodies and mediators of inflammation such as interleukin, prostaglandins, and interferon. Often a bacterial or viral infection must be diagnosed indirectly by testing for antibodies to the infectious agent rather than by isolating the pathogen itself.

As a medical assistant, you need to understand the role of microorganisms in both health and disease. The main objective of microbiology procedures is to identify the organisms responsible for illness so that the physician can properly treat the patient. In addition, your responsibilities will include preventing nosocomial infections and assisting with infection control in the physician’s office laboratory (POL) and in the patients the POL serves. Microbiology procedures may be performed in the POL or in the microbiology department of a medical referral laboratory.

Chapter 27 discussed the chain of infection and how it can be broken using infection control procedures such as proper hand sanitization, antiseptics and disinfectants, and sterilization methods. This chapter covers the cultivation of microorganisms; detection of infecting organisms by microscopy; detection of specific products of infecting organisms using chemical, immunologic, or molecular techniques; and detection of antibodies produced by the patient in response to an infecting organism (immunodiagnosis).

**SPECIMEN COLLECTION AND TRANSPORT**

Specimen collection and handling are among the most critical considerations in patient care, because any results the laboratory generates are directly dependent on the quality of the specimen and its condition on arrival in the laboratory. Specimens for
microbiology testing must be collected in such a way as to prevent the introduction of any contaminating microorganisms. This means not only using special sterile collection and transport devices, but also taking steps to prevent environmental and patient contamination. Such steps include using antiseptics on the skin before drawing blood, instructing a patient in the collection of a urine sample using the clean catch midstream (CCMS) technique (see Chapter 52), and avoiding the teeth and tongue when collecting a throat culture specimen on a swab.

When collecting specimens for microbiologic analysis, medical assistants should ask themselves two questions: “In what ways can I prevent contamination of this sample?” and “What can I do to protect myself from becoming infected while I collect this sample?” Answers to the first question include cleaning the area to be sampled with an antiseptic, opening sterile containers only when necessary, and never touching a sterile swab or collection device to a nonsterile surface. Answers to the second question include wearing gloves and a disposable surgical mask or face shield while collecting a throat or sputum culture and wearing gloves when receiving a urine specimen from a patient who has just voided.

Ideally, specimens should be collected during the acute phase of an illness and before antibiotics are prescribed. Many types of samples can be collected. Sterile swabs can be used to collect samples from wounds and the upper respiratory tract. Serum or whole blood can be used to test for infectious organisms. Urine and feces can be collected in containers by patients at home. If patients are expected to collect specimens, it is crucial that they receive clear instructions on how to perform the procedure without contaminating the sample. The referral laboratory is responsible for providing a manual of written instructions to the POL, and the POL is responsible for providing clear instructions (preferably written) to the patient, especially if the patient will be collecting the sample in private or at home.

The transport of specimens is also crucial. Many different types of transport devices are available, and close attention must be given to their proper use. Microorganisms are living organisms and must be provided with conditions that permit their survival but do not permit their multiplication. If microorganisms are allowed to multiply after specimen collection, the culture results will not reflect the true disease state. Specialized transport media, such as modified Stuart's medium or Amie's medium, often are used in the swabbing devices used for specimen collection (Figure 55-1). These collection devices typically are made of a plastic tube that encases a sterile Dacron swab and a sealed vial of transport medium. After the specimen is obtained on the swab, it is placed in the plastic tube and the transport medium is released, usually by crushing the internal vial. It is essential to follow the manufacturer's directions to prevent drying of the swab and specimen.

Ideally a specimen should be transported to the laboratory and cultured immediately after collection. In most situations, however, this is not possible, and the transport device must be handled by a courier en route to a referral laboratory or held in the POL until it can be cultured. For specimens that will be transported by a courier, make sure the specimens are safely packaged in leakproof containers marked with warning labels (Figure 55-2). Proper temperature and time of storage are crucial. Most pathogenic organisms prefer temperatures around 37°C (98.6°F) and will remain viable for up to 72 hours if held at room temperature or refrigerator temperature (4°C [39.2°F]). However, some organisms die if exposed to cold temperatures. Always check the referral laboratory's procedure manual for directions on duration and temperature of storage before plating. Likewise, microorganisms have oxygen requirements: some, called aerobes, require oxygen to stay alive; others, called anaerobes, die if exposed to oxygen. Devices are available for both

![Figure 55-1](https://example.com/figure551.png)

**Figure 55-1** Culturette collection and transport system. (From Bonewit-West K: Clinical procedures for medical assistants, ed 7, St Louis, 2008, Saunders.)

![Figure 55-2](https://example.com/figure552.png)

**Figure 55-2** A, Appropriate containers for transport. B, Appropriate packaging for transport.
# Table 55-1 Collection, Transport, and Processing of Specimens Commonly Submitted to the Physician’s Office Laboratory

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Container</th>
<th>Patient Preparation</th>
<th>Special Instructions</th>
<th>Storage Before Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Blood culture media set or Vacutainer brand blood culture tube with SPS</td>
<td>Disinfect venipuncture site with alcohol swab and Betadine</td>
<td>Draw blood during febrile episodes; draw two sets from right and left arms</td>
<td>Deliver to laboratory within 2 hr; incubate at 37°C (98.6°F) on receipt in the laboratory</td>
</tr>
<tr>
<td>Body Fluids (e.g., peritoneal, synovial, pleural)</td>
<td>Sterile, screw-cap container or anaerobic transporter</td>
<td>Disinfect aspiration site with alcohol swab and Betadine</td>
<td>Needle aspirations are preferable to swab collections</td>
<td>Transport immediately and plate specimen immediately on receipt in the laboratory</td>
</tr>
<tr>
<td>Eye</td>
<td>Aerobic transport swab</td>
<td></td>
<td>Moisten swab with Amie’s or Stuart’s medium before collection</td>
<td>May be stored up to 24 hr at room temperature</td>
</tr>
<tr>
<td>Stool</td>
<td>Clean, leakproof container</td>
<td></td>
<td>Transport to laboratory within 24 hr if storing at 4°C (39.2°F)</td>
<td>Plate within 72 hr if storing at 4°C (39.2°F)</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>Swab placed directly in enteric transport medium</td>
<td>Wipe away exudate before obtaining culture specimen with swab</td>
<td>Insert swab approximately 1 inch past anal sphincter</td>
<td>Store at 4°C (39.2°F), transport within 24 hr to laboratory and plate within 72 hr</td>
</tr>
<tr>
<td>Gonorrhea culture</td>
<td>Jemtec transport system or transport device with Stuart’s or Amie’s medium</td>
<td>Urogenital swabs preferred; necessary to obtain epithelial cells, not exudate</td>
<td>Do not refrigerate</td>
<td>Transport to laboratory within 2 hr</td>
</tr>
<tr>
<td>Chlamydia culture</td>
<td>Specialized Chlamydia transport medium containing antibiotics</td>
<td></td>
<td>Transport immediately on ice to laboratory</td>
<td>Store up to 24 hr at 4°C (39.2°F); inoculate cultures within 15 min of collection if swab is not on ice</td>
</tr>
<tr>
<td>Skin scraping</td>
<td>Clean, screw-top tube</td>
<td>Wipe skin with alcohol prep pad</td>
<td>Scrape skin at leading edge of lesion</td>
<td>Can be held indefinitely at room temperature but best to process within 72 hr of collection</td>
</tr>
<tr>
<td>(fungal culture)</td>
<td></td>
<td></td>
<td>Have patient collect from deep cough; do not collect saliva</td>
<td>Store at 4°C (39.2°F), and plate within 24 hr</td>
</tr>
<tr>
<td>Sputum</td>
<td>Sterile, screw-cap container</td>
<td>Patient should rinse or gargle with mouthwash before collection</td>
<td>Swab pharynx and tonsils, not mouth, tongue, or teeth</td>
<td>Transport and plate within 24 hr; room temperature storage</td>
</tr>
<tr>
<td>Throat</td>
<td>Transport swab</td>
<td></td>
<td>Swab pharynx and tonsils, not mouth, tongue, or teeth</td>
<td>Store at room temperature and deliver to laboratory within 24 hr</td>
</tr>
<tr>
<td>Ova and parasite (O&amp;P)</td>
<td>O&amp;P transport device (with formalin and PVA)</td>
<td>Three specimens collected every other day at a minimum for outpatients</td>
<td>Wait 7-10 days if patient has been taking Pepto-Bismol, Kapectate, or Milk of Magnesia</td>
<td>Hold at 4°C (39.2°F) and plate within 24 hr</td>
</tr>
<tr>
<td>Urine</td>
<td>Sterile, screw-cap container</td>
<td>Instruct patient in clean catch midstream collection</td>
<td>Hold at 4°C (39.2°F) and deliver to laboratory within 24 hr</td>
<td>Hold at 4°C (39.2°F) and plate within 24 hr</td>
</tr>
<tr>
<td>Superficial wound</td>
<td>Aerobic transport swab</td>
<td>Wipe area with sterile saline or alcohol prep pad before collection</td>
<td>Moisten swab with Amie’s or Stuart’s medium before collection</td>
<td>Transport and plate within 24 hr; room temperature storage</td>
</tr>
<tr>
<td>Deep wound or abscess</td>
<td>Anaerobic transport device</td>
<td>Wipe area with sterile saline or alcohol prep pad before collection</td>
<td>Aspirate material, excise tissue, or insert swab deep into wound</td>
<td>Transport and plate within 24 hr; room temperature storage</td>
</tr>
</tbody>
</table>


SPS, Sodium polyanethosulfonate; PVA, polyvinyl alcohol.
aerobic and anaerobic collection. Table 55-1 lists the collection, transport, and storage of specimens commonly collected or handled by medical assistants. Figure 55-3 shows some commonly used collection devices.

**CRITICAL THINKING APPLICATION 55-1**

Aaron Mitchell, age 9, was brought into the clinic this morning at 9 o'clock with scabbing sores on his upper lip. Dr. Chowdry suspects impetigo and orders a wound culture. How will Anna collect this culture? What device might she use? How should she store this specimen until the courier, who does not come until 3 pm, arrives? Anna knows that impetigo is highly contagious. How can she protect herself from becoming infected?

**CLASSIFICATION OF MICROORGANISMS**

Once the specimen reaches the microbiology laboratory, it is analyzed for the presence of infectious microorganisms or their components. Although the medical assistant is not responsible for identifying microorganisms, a working knowledge of the terminology used in the classification of microorganisms is essential.

Most cultures handled by the medical assistant have been ordered to diagnose bacterial infections. Bacteria are one type of microorganism; other microorganisms include fungi and protozoa. Parasitic worms are studied in the microbiology laboratory but are not considered microorganisms.

Many microbiologists do not consider viruses to be microorganisms simply because they are not, by definition, alive. Viruses consist of a core of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) covered by a protein shell. Alone, they neither metabolize nor reproduce; however, once inside a host cell, viruses use the host cell's organelles and macromolecules to multiply.

Because of this absolute need for a host cell for replication, viruses are called obligate intracellular parasites, and they cannot be cultured on artificial media such as those used to culture bacteria.

Viruses must be cultured in fertilized eggs or in tissue culture, which is done by referral or hospital laboratories. Often, instead of culturing a specimen for a virus, antigenic products of the virus or

**FIGURE 55-3**

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>VIRUS</th>
<th>TRANSMISSION</th>
<th>SYMPTOMS</th>
<th>TESTS</th>
<th>PREVENTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox</td>
<td>Variola major</td>
<td>Direct contact; fomites</td>
<td>Vesicles on entire body, including soles and palms</td>
<td>Eradicated (vaccine is still available)</td>
<td></td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>Epstein-Barr virus</td>
<td>Direct and airborne</td>
<td>Sore throat, fever, malaise, lymph gland involvement; hepatitis, enlarged spleen</td>
<td>Serology testing for heterophile antibodies; CBC</td>
<td></td>
</tr>
<tr>
<td>Influenza</td>
<td>Myxovirus — influenza A and B</td>
<td>Droplet and fomites</td>
<td>Fever, body aches, cough</td>
<td>Nasopharyngeal swab, nasal wash</td>
<td></td>
</tr>
<tr>
<td>Warts (verruca)</td>
<td>Human papilloma virus</td>
<td>Direct and indirect contact</td>
<td>Circumscribed outgrowths on skin; most common on hands and feet</td>
<td>Immunization for the old, young, and debilitating</td>
<td></td>
</tr>
<tr>
<td>Rabies</td>
<td>Rhabdovirus</td>
<td>Contact with saliva of infected animal (dog, cat, skunk, fox, bat are usual)</td>
<td>Fever, uncontrollable excitement, spasms of the throat, profuse salivation</td>
<td>DFA from brain or hair follicle tissue; Vaccine available; vaccinate pets</td>
<td></td>
</tr>
<tr>
<td>Mumps</td>
<td>Paramyxovirus — mumps virus</td>
<td>Direct contact</td>
<td>Pain, swelling of salivary glands; fever</td>
<td>Acute and convalescent titers; MMR vaccine</td>
<td></td>
</tr>
<tr>
<td>Measles</td>
<td>Paramyxovirus — measles virus</td>
<td>Direct contact; droplets</td>
<td>Fever, nasal discharge, red eyes; Koplik’s spots, rash</td>
<td>Serologic titer; MMR vaccine</td>
<td></td>
</tr>
<tr>
<td>Rubella (German measles)</td>
<td>Rhinovirus and many others</td>
<td>Direct contact; droplets; congenital</td>
<td>Rash, swollen lymph glands; causes severe birth defects</td>
<td>Serologic titer; MMR vaccine</td>
<td></td>
</tr>
<tr>
<td>Common cold</td>
<td>Rhinovirus and many others</td>
<td>Direct; droplets; fomites</td>
<td>Headache, fever, runny nose, congestion</td>
<td>Good hygiene (hand washing)</td>
<td></td>
</tr>
<tr>
<td>Polio</td>
<td>Poliovirus</td>
<td>Direct contact; carriers enter via mouth</td>
<td>Fever, headache, stiff neck and back, paralysis of muscles</td>
<td>IPV; SC or IM; four doses</td>
<td></td>
</tr>
<tr>
<td>Molluscum contagiosum warts</td>
<td>Molluscipox virus</td>
<td>Direct contact with infected individual</td>
<td>Small pink or white domes found in clusters</td>
<td>Microscopic evaluation; Avoid contact with infected individual; have existing warts removed</td>
<td></td>
</tr>
</tbody>
</table>

**Critical Thinking Application 55-2**

While preparing to collect the specimen from Aaron, Anna receives a telephone call from BioStatLab, the referral laboratory the clinic uses. The results from Ms. Tina Walker’s urine culture end from Mr. Robert Livore’s abscess culture are complete.

- Anna listens carefully to the technician’s report on Mr. Livore’s abscess culture, and she jots down “staphylococcus” on the reporting form. She asks the technician what species of “staph.” Why is this important?
Typical Pathogenic Bacteria

Bacteria are single-celled prokaryote organisms that reproduce by binary fission, a process that involves duplication of the chromosome and subsequent fission (splitting in half) of the cell. This process of asexual reproduction results in tremendous numbers of bacteria from a single cell and explains why bacterial infections can quickly overwhelm a person's immune system. Some bacteria reproduce in as little as 14 minutes, whereas others take days to divide. Theoretically, a single E. coli cell, which has a reproduction time of about 30 minutes, produces 351,843,724,088,831 offspring in 24 hours if it is able to enter the urinary bladder.

Bacteria often are classified according to their shape, their staining characteristics, and the environmental conditions in which they thrive. Both shape and staining characteristics are direct results of the cell wall composition. Three types of cell wall structures are found among pathogenic bacteria: gram positive, gram negative, and acid fast. These designations are based on reactions in specialized stains used to see the bacteria under the microscope. The bacterial cell wall is composed of peptidoglycan (PG), a molecule composed of carbohydrate and protein. The gram-positive cell has a thick layer of PG with no lipid layer surrounding it; the gram-negative cell has a thin layer of PG with a lipid layer surrounding it; and the acid-fast cell has a thin layer of PG surrounded by a thick layer of waxylike lipids. Acid-fast bacteria do not stain well with Gram stain, and gram-positive and gram-negative bacteria both stain negative with the acid-fast stain. With Gram stain, gram-positive bacteria stain purple, and gram-negative bacteria stain pink. With the acid-fast stain, acid-fast-positive bacteria (AFB) stain pink and acid-fast-negative bacteria stain blue.

Bacterial Shapes

Pathogenic bacteria assume three different morphologic shapes. Spheric bacteria are called cocci (singular, coccus), rod-shaped bacteria are bacilli (singular, bacillus), and spiral bacteria are spirilla (singular, spirillum). Tightly coiled spirilla are called spirochetes. Certain arrangements are also seen in different genera and species. When bacteria are in a chain formation, the prefix strepto- is used. When bacteria are found in pairs, the prefix diplo- is used, and when they are found in grapelike clusters, the prefix staphylo- is used. Cocci in packets of four are called tetrad and in packets of eight or 16 are called sarcinae (Figure 55-4).

Critical Thinking Application 55-3

Anna knows that impetigo is caused by Staphylococcus aureus. Without using a microscope, she knows what the organism looks like. How does she know?
### TABLE 55-3 Common Diseases Caused by Bacilli

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>ORGANISM</th>
<th>DESCRIPTION</th>
<th>TRANSMISSION</th>
<th>SYMPTOMS</th>
<th>TESTS AND SPECIMENS</th>
<th>PREVENTION AND IMMUNIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Acid-fast branching bacilli</td>
<td>Inhalation</td>
<td>Pulmonary—cough, hemoptysis, sweats, weight loss; may affect other systems</td>
<td>Sputum for culture; x-ray; skin tests</td>
<td>BCG vaccine (not routinely given in the United States)</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td><em>Escherichia coli</em>, <em>Proteus spp.</em>, <em>Klebsiella spp.</em>, <em>Pseudomonas aeruginosa</em></td>
<td>Gram-negative bacilli; many flagellated</td>
<td>Ascends urethra; catheterization</td>
<td>Cystitis—frequency, burning bloody urine Pyelonephritis—flank pain, fever</td>
<td>Clean catch urine for culture and analysis</td>
<td>Good personal hygiene; always wipe from front to back</td>
</tr>
<tr>
<td>Legionnaires disease</td>
<td><em>Legionella pneumophila</em></td>
<td>Gram-negative bacillus (stains poorly with usual methods)</td>
<td>Grows freely in water (air conditioning systems)</td>
<td>Pneumonia-like symptoms</td>
<td>Sputum; blood</td>
<td>Avoid smoking</td>
</tr>
<tr>
<td>Tetanus (lockjaw)</td>
<td><em>Clostridium tetani</em></td>
<td>Gram-positive spore-forming bacilli, anaerobic</td>
<td>Open wounds, fractures, punctures</td>
<td>Toxin affects motor nerves; muscle spasms, convulsions, rigidity</td>
<td>Blood</td>
<td>DTaP in childhood; T orTd every 10 yr</td>
</tr>
<tr>
<td>Gas gangrene</td>
<td><em>Clostridium perfringens</em></td>
<td>Gram-positive spore-forming bacilli, anaerobic</td>
<td>Wounds</td>
<td>Gas and watery exudate in infected wound</td>
<td>Swab, aspirate of wound for culture</td>
<td>Proper wound care</td>
</tr>
<tr>
<td>Botulism</td>
<td><em>Clostridium botulinum</em></td>
<td>Gram-positive spore-forming bacilli, anaerobic</td>
<td>Improperly cooked canned foods</td>
<td>Neurotoxin affects speech, swallowing, vision; paralysis of respiratory muscles, death</td>
<td>Contaminated food; blood</td>
<td>Botulinus antitoxin; boil canned goods 20 min before tasting or eating</td>
</tr>
<tr>
<td>Diphtheria respiratory secretions</td>
<td><em>Corynebacterium diphtheriae</em></td>
<td>Gram-positive bacilli, club shaped</td>
<td></td>
<td>Sore throat, fever, headache, gray membrane in throat</td>
<td>Swabs; Gram stain, culture; Schick test for immunity</td>
<td>DTaP in childhood</td>
</tr>
<tr>
<td>Whooping cough</td>
<td><em>Bordetella pertussis</em></td>
<td>Gram-negative bacilli</td>
<td>Respiratory secretions</td>
<td>Upper respiratory tract symptoms; high-pitched, crowing whoop</td>
<td>Swabs for culture</td>
<td>DTaP in childhood</td>
</tr>
<tr>
<td>Plague</td>
<td><em>Yersinia pestis</em></td>
<td>Gram-negative bacilli</td>
<td>Flea bite from infected rodents</td>
<td>Fever and chills, delirium, enlarged, painful lymph nodes</td>
<td>Sputum for culture; blood</td>
<td>Vaccine available; rodent control</td>
</tr>
</tbody>
</table>

* Courtesy Kathleen Moody.

*BCG, Bacille Calmette-Guérin vaccine; DTaP, diphtheria-tetanusacellular pertussis vaccine; T, tetanus (toxoid); Td, tetanus and diphtheria (toxoids).*

**pneumoniae** is nonpathogenic if it is not producing a capsule; however, it is the most common cause of pneumonia in older adults when it is encapsulated. The Pneumovax vaccine, which is given to older patients and those at high risk for respiratory complications (e.g., patients with asthma), is composed of highly purified capsular polysaccharides from 23 strains of *S. pneumoniae*. Certain bacteria are able to form intracellular structures called endospores that allow the cell to remain viable when environmental conditions are not favorable. *Bacillus anthracis* produces such spores, as does *Clostridium tetani*. If spores of *C. tetani* enter a wound and germinate, they cause the disease known as tetanus. Tables 55-3 to 55-5 list some important infectious diseases caused by typical pathogenic bacteria.

**Unusual Pathogenic Bacteria: Chlamydiae, Mycoplasmas, and Rickettsiae**

In the small scale used to measure microorganisms, viruses range from 10 to 100 nanometers (nm). Typical pathogenic bacteria measure 1,000 to 5,000 nm. Chlamydiae, mycoplasmas, and rickettsiae are tiny, unusual bacteria that fall between the size ranges of viruses and typical pathogenic bacteria.

The rickettsiae are tiny gram-negative bacteria that are transmitted by blood-sucking arthropods. They cannot multiply outside a host cell, and once inside the host cell, they are able to perform only some of the life-sustaining metabolic reactions on their own. Chlamydiae also are tiny bacteria that require
### TABLE 55-4 Common Diseases Caused by Cocci

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>ORGANISM</th>
<th>DESCRIPTION</th>
<th>TRANSMISSION</th>
<th>SYMPTOMS</th>
<th>SPECIMENS</th>
<th>TESTS</th>
<th>PREVENTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td><em>Streptococcus</em></td>
<td>Gram-positive encapsulated cocci</td>
<td>Direct contact, droplets</td>
<td>Productive cough, fever, chest pain</td>
<td>Sputum; bronchoscopy secretions</td>
<td>Culture, Gram stain</td>
<td>Vaccine</td>
</tr>
<tr>
<td></td>
<td><em>pneumoniae</em></td>
<td>in pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strept throat</td>
<td><em>Streptococcus</em></td>
<td>Gram-positive cocci in chains</td>
<td>Direct contact, droplets, fomites</td>
<td>Severe sore throat, fever, malaise</td>
<td>Direct swab</td>
<td>Rapid strep test, fritot culture</td>
<td>Good personal hygiene</td>
</tr>
<tr>
<td></td>
<td><em>pyogenes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(group A strep)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound infection, abscesses, boils</td>
<td><em>Staphylococcus aureus</em></td>
<td>Gram-positive cocci in clusters</td>
<td>Direct contact, fomites, carriers; poor hand washing</td>
<td>Area red, warm, swollen; pus; pain; ulceration or sinus formation</td>
<td>Swab</td>
<td>Rapid swab, aspirate of drainage</td>
<td>Culture and sensitivity (anaerobic); Control of source of infection</td>
</tr>
<tr>
<td>Staphylococcal food poisoning</td>
<td><em>Staphylococcus aureus</em></td>
<td>Gram-positive cocci in clusters</td>
<td>Direct contact, fomites, carriers; poor hand washing</td>
<td>Area red, warm, swollen; pus; pain; ulceration or sinus formation</td>
<td>Swab</td>
<td>Rapid swab, aspirate of drainage</td>
<td>Culture and sensitivity (anaerobic); Control of source of infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxic shock</td>
<td><em>Staphylococcus</em></td>
<td>Gram-positive cocci in clusters</td>
<td>Use of absorbent packing materials (e.g., tampons, nasal packs)</td>
<td>Fever, headache, nausea, vomiting, delirium, low blood pressure</td>
<td>Swab, blood</td>
<td>Culture and serology</td>
<td>Change tampon, packing material often</td>
</tr>
<tr>
<td></td>
<td><em>aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonorrhea</td>
<td><em>Neisseria</em></td>
<td>Gram-negative cocci in pairs; intracellular in white blood cells</td>
<td>Sexually transmitted</td>
<td>Females: Pelvic pain, discharge; may be asymptomatic; Males: Urethral drip, pain on urination</td>
<td>Swab of cervix, urethra; rectal and pharyngeal swabs in homosexual men</td>
<td>Gram stain, culture</td>
<td>Avoid unprotected sex</td>
</tr>
<tr>
<td></td>
<td><em>gonorrhoeae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meningococcal meningitis</td>
<td><em>Neisseria</em></td>
<td>Gram-negative diplococci</td>
<td>Respiratory tract secretions</td>
<td>High fever, headache, projectile vomiting, delirium, neck and back rigidity, convulsions, petechial rash</td>
<td>Nasopharyngeal swabs, cerebrospinal fluid, blood</td>
<td>Gram stain, culture; cell counts and chemistries</td>
<td>Vaccine; prophylactic antibiotics</td>
</tr>
<tr>
<td></td>
<td><em>meningitidis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Courtesy Kathleen Moody.*

host cells for growth and once were considered viruses. Unlike rickettsiae, chlamydiae are not transmitted by arthropod vectors.

Mycoplasma are unusual in that they have no PG in the cell wall, but they are not obligate parasites as are rickettsiae and chlamydiae. Rickettsiae and chlamydiae do not grow on artificial media in the laboratory; tissue culture or serologic testing is required to identify them. Mycoplasmas can be cultivated from a patient specimen in the laboratory (Table 55-6).

### Fungi

Mycology is the study of fungi and the diseases they cause. Fungi (singular, *fungus*) are eukaryotes that are larger than bacteria; they include unicellular yeasts and multicellular molds. Fungi are present in the soil, air, and water, but only a few species cause disease. They are transmitted by direct contact with infected persons, by prolonged exposure to a moist environment, and by inhalation of contaminated dust or soil. Fungal infections may be superficial, affecting only the skin, hair, or nails. However, some fungi can penetrate the tissues of the internal body structures and produce serious diseases of the mucous membranes, heart, lungs, and other organs. Fungal infections are resistant to the antibiotics used in the treatment of bacterial infections, and fungi must be treated with drugs active against their unusual cell walls.

A superficial fungal infection often is referred to as a *tinea* (Latin for "ringworm"). Tinea pedis, for example, is athlete’s foot; tinea barbae is a fungal infection of the facial hair follicles. The term *ringworm* arose because the infected area is often circular.
and appears wrinkled in the center as a result of the healing process. Diagnosis of fungal infections usually is based on culturing or microscopic observation of skin scrapings, hair samples, or samples of sputum or mucous membranes. Usually the samples are treated with potassium hydroxide before microscopic observation to dissolve away nonfungal material, making the fungal elements easier to observe (Table 55-7).

### Parasites

Parasitology includes the study of all parasitic organisms that live on or in the human body. In parasitic relationships the host is harmed as the parasite thrives. Parasites are transmitted by ingestion during the infective stage, direct penetration of the skin by infective larvae, and inoculation by an arthropod vector. A parasite cannot be identified accurately on the basis of a single test or specimen. Most parasites are identified in urine, sputum, tissue fluids, or tissue biopsy samples (Table 55-8).

### Helminths

Helminths are eukaryote parasites called worms. Helminths live on or within another living organism and nourish themselves at the expense of the host organism. They can live in animals or humans and usually are transmitted through the soil, by infected clothing or fingernails, or through contact with infected persons or contaminated food or water. Helminths go through the same life cycle as other worms. The adult worm lays eggs (ova). The ova develop into larvae. Larvae grow into adult worms, which lay eggs, and the cycle begins again. Diagnosis usually is based on microscopic examination of feces for ova and parasites and on the patient's signs and symptoms (Figure 55-5).

### Protozoa

Protozoa (singular, protozoon) are single-celled parasitic eukaryotes that range in size from microscopic to macroscopic (visible to the naked eye). They are present in moist environments and
TABLE 55-7 Common Diseases Caused by Fungi

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>ORGANISM</th>
<th>PREDISPONING CONDITIONS AND TRANSMISSION</th>
<th>SYMPTOMS</th>
<th>TESTS AND SPECIMENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrush (oral yeast), vulvovaginal candidiasis, or monilia (vaginal yeast)</td>
<td>Candida spp. (yeast)</td>
<td>Oral: During birth, after antibiotic therapy; oral birth control, severe diabetes</td>
<td>White, cheesy growth</td>
<td>Swab for KOH prep, culture</td>
</tr>
<tr>
<td>Athlete’s foot, jock itch, ringworm (tinea)</td>
<td>Trichophyton spp., Microsporum spp., and others (skin fungi)</td>
<td>Opportunist; direct contact; clothing; prolonged exposure to moist environment</td>
<td>Hair loss, thickening of skin, nails; itching; red, scaly patches</td>
<td>Skin scraping for KOH prep; skin, hair for culture</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>Histoplasma capsulatum</td>
<td>Inhalation of dust contaminated with bird or bat droppings</td>
<td>Mild, flu-like to systemic</td>
<td>Serologic; culture of biopsy material</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>Cryptococcus neoformans</td>
<td>Contact with poultry droppings</td>
<td>Cough, fever, malaise; can become systemic</td>
<td>Sputum culture; cerebrospinal fluid culture, India ink direct examination</td>
</tr>
<tr>
<td>Sporotrichosis</td>
<td>Sporothrix schenckii</td>
<td>Farmers, florists, people exposed to soil</td>
<td>Skin lesions that spread along lymphatics; can become systemic</td>
<td>Skin scraping for KOH prep; serologic</td>
</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td>Pneumocystis carinii</td>
<td>Widely prevalent in animals; occurs in debilitated or immunosuppressed individuals; common in patients with AIDS</td>
<td>Pneumonia-like</td>
<td>Biopsy of lung tissue with microscopic examination</td>
</tr>
</tbody>
</table>

Courtesy Kathleen Moody.
AIDS, Acquired immunodeficiency syndrome; KOH, potassium hydroxide.


in bodies of water such as lakes and ponds. Protozoa are transmitted through contaminated feces, food, and drink. Some pathogenic protozoa inhabit the bloodstream, whereas others inhabit the intestines and genitai tract. Diagnosis usually is based on the patient’s signs and symptoms and on the microscopic examination of stool and blood (Table 55-8).

Stool specimens commonly are examined for parasitic protozoa and helminths. The stool specimen is collected and placed into two vials, each with a preservative. Most commonly, sodium acetate acetic acid formalin (SAF) and polyvinyl alcohol (PVA) are used. From these preparations, a wet mount is made to observe motile organisms, a stained smear is made to provide contrast to the existing debris in the stool, and the specimen is concentrated either by sedimentation or flotation to allow recovery of protozoal cysts and helminth eggs. The medical assistant should always consult the procedure manual provided by the referral laboratory when an ova and parasites stool examination (O&P) is ordered to ensure proper collection and transport of the specimen (Procedure 55-1).

**MICROBIOLOGY LABORATORY**

The equipment and supplies in a microbiology laboratory vary with the size of the facility. Most laboratories have a refrigerator, an autoclave, a safety cabinet, a microscope, and an incubator (discussed in Chapter 51). In addition, you are likely to find the following equipment and supplies.

**Inoculating Equipment**

Cultivation and identification of microbes require the use of certain tools. Inoculating loops and needles (Figure 55-6) are needed to transfer samples or microbes to growth media or to
**TABLE 55-8 Common Diseases Caused by Protozoa and Parasites**

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>ORGANISM</th>
<th>TRANSMISSION</th>
<th>SYMPTOMS</th>
<th>TESTS AND SPECIMENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td><em>Plasmodium spp.</em> (protozoa)</td>
<td>Bite of the Anopheles mosquito</td>
<td>Chills, fever (cyclic)</td>
<td>Blood: examination of stained blood for parasites</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td><em>Toxoplasma gondii</em> (protozoa)</td>
<td>Fecal contamination (cat litter); congenital</td>
<td>Febrile illness, rash; congenital: jaundice, enlarged liver and spleen, brain abnormalities</td>
<td>Skin test for screening blood, fluid, or tissue for confirmation</td>
</tr>
<tr>
<td>Amebic dysentery</td>
<td><em>Entamoeba histolytica</em> (protozoan)</td>
<td>Fecal contamination of food and water</td>
<td>Bloody diarrhea, cramping, fever</td>
<td>Stool for O&amp;P</td>
</tr>
<tr>
<td>Giardiasis</td>
<td><em>Giardia lamblia</em> (protozoan)</td>
<td>Common in intestinal tract, opportunists; contaminated surface water</td>
<td>Asymptomatic to severe diarrhea and abdominal discomfort</td>
<td>Stool for O&amp;P; intestinal biopsy</td>
</tr>
<tr>
<td>Trichinosis</td>
<td><em>Trichiella spiralis</em> (roundworm)</td>
<td>Ingestion of undercooked pork, bear meat</td>
<td>Nausea, fever, diarrhea, muscle pain and swelling, edema of face</td>
<td>Biopsy; blood tests</td>
</tr>
<tr>
<td>Tapeworm</td>
<td><em>Toenia spp.</em></td>
<td>Undercooked meat (beef and pork)</td>
<td>Abdominal discomfort, diarrhea, weight loss</td>
<td>Stool for O&amp;P</td>
</tr>
<tr>
<td>Diphylobothrium latum</td>
<td></td>
<td>Undercooked fish; common among Norwegians, Japanese</td>
<td>As above; may become anemic</td>
<td>Stool for O&amp;P</td>
</tr>
<tr>
<td>Pinworm</td>
<td><em>Enterobius vermicularis</em> (roundworm)</td>
<td>Fecaloral</td>
<td>Severe rectal itching, restlessness, insomnia</td>
<td>Scotch tape applied to perianal region for ova</td>
</tr>
<tr>
<td>Scabies</td>
<td><em>Sarcoptes scabiei</em>—itch mite</td>
<td>Direct contact; clothing, bedding</td>
<td>Nocturnal itching; skin burrows</td>
<td>Skin scrapings for parasites</td>
</tr>
<tr>
<td>Lice</td>
<td><em>Pediculus humanus</em>; <em>Phthirius pubis</em> (crabs)</td>
<td>Direct contact; clothing, bedding, furniture (can transmit other diseases via bite)</td>
<td>Intense itching; skin lesions</td>
<td>Finding adult lice or eggs (nits) on body or hair</td>
</tr>
</tbody>
</table>

Courtesy Kathleen Moody.

*O&P, Ova and parasites.*

Slides for staining. Loops and needles may be disposable and presterilized, or they may be made of wire and can be heat sterilized before and after use. An inoculating loop is shaped like a thin, solid rod with a small, thin filament of liquid adhering to the loop. The amount of fluid held by the loop can be calibrated; for a urine culture, a 1-ml sample must be applied to the culture medium, and special loops are available that deliver this amount. An inoculating needle is thin and pointed and is ideal for sampling single colonies.

### Incineration Equipment

Incineration is the fastest way to sterilize reusable equipment (e.g., wire loops, needles, and metal forceps) that must be sterilized before and after use. Some laboratories use a Bunsen burner connected to a natural gas supply, but most use an electric incinerator because of the reduced fire hazard (Figure 55-7). An incinerator can also be used to heat-fix smears for a bacterial stain.

### Culture Media

Once a specimen has been properly collected, it must be inoculated onto an appropriate medium (Figure 55-8). Under the proper incubation conditions, the bacteria or fungi in the sample metabolize and reproduce using the nutrients in the medium and become visible as colonies. Media can be solid, liquid, or semi-solid. A liquid medium is called a broth. The addition of a powdered extract of seaweed called agar to a boiling liquid medium allows it to solidify and remain solid at 37°C (98.6°F). The molten medium can be poured into Petri dishes (a plastic dish with a lid) or into tubes. Four types of media typically are used in a microbiology laboratory:

- **All-purpose or nutritive media.** All-purpose media are used to support the growth of a wide variety of bacteria; however, they do not support the growth of fastidious bacteria.
- **Selective media.** Selective media support the growth of one type of organism while inhibiting the growth of others through the addition of a salt, dye, antibiotic, or chemical. For example, phenyl ethanol agar contains alcohol, which inhibits the growth of gram-negative bacteria and permits gram-positive bacteria to flourish.
- **Differential media.** Differential media contain chemicals or dyes that alter the appearance of certain types of bacteria. Many differential media are also selective. For example, mannitol salt agar contains a higher level (7.5%) of sodium chloride (salt), which selects for staphylococci. It also contains mannitol, a carbohydrate that can be fermented to an acid end-product by *Staphylococcus aureus* but not by *Staphylococcus epidermidis*. The medium contains a pH...
PROCEDURE 55-1

Instruct Patients According to Their Needs: Instruct Patients in the Collection of Fecal Specimens to Be Tested for Ova and Parasites

GOAL: To instruct a patient in the proper collection of stool for an ova and parasite microscopic examination.

EQUIPMENT and SUPPLIES

- Clean, dry container for stool collection
- Parasitology collection vials*
- Plastic biohazard zipper-lock bag

PROCEDURAL STEPS

1. Instruct the patient not to take any antacids, laxatives, or stool softeners before collecting the specimen.
   PURPOSE: Laxatives increase fecal transit time and may result in a false-negative test result.
2. Instruct the patient to urinate before collecting the specimen.
   PURPOSE: This eliminates the possibility of contaminating the stool with urine.
3. The patient then collects the specimen.
   a. From adults: Instruct the patient to defecate into the container. Stool cannot be retrieved from the toilet bowl.
   b. From children: Loosely drape the toilet rim with plastic wrap and lower the seat. The child should have a bowel movement into the toilet, onto the wrap. Remove the stool using a disposable plastic spoon.
      PURPOSE: The stool cannot be contaminated by or diluted with water.
   c. From infants: Fasten a “diaper” made of plastic wrap over the child using tape. Remove the plastic wrap immediately after a bowel movement and remove the stool using a plastic spoon. Never leave the child unattended with the plastic wrap in place, as it could cause suffocation should it be removed.
      PURPOSE: Stool cannot be collected in a diaper.
4. Instruct the patient to add stool to the collection container.
   a. If the stool is formed, use the scoop on the lid of the container to add a large, jelly bean-sized piece of stool to the liquid in the containers (Figure 1).
   b. If the stool is liquid, pour it into the container until the preservative in the vial reaches the indicated level on the containers.
5. Instruct the patient to tighten the caps completely and wipe the outside of the vials with rubbing alcohol or to wash carefully with soap and water.
   PURPOSE: To ensure infection control.
6. The vials should be labeled, placed in a biohazard bag with a zipper closure, and transported to the laboratory immediately if possible. Do not refrigerate the vials.
7. Instruct the patient to wash his or her hands after the procedure.
   PURPOSE: To ensure infection control.

* Several types of preservatives are available. Check with the referral laboratory to make sure the patient is given the proper vials for collection. Preservatives include low-viscosity polyvinyl alcohol (LVP), zinc sulfate polyvinyl alcohol (ZnP), sodium acetate acetic acid formalin (SAF), and 10% neutral buffered formalin.

Inoculation of Media

Once the specimen has been collected, it must be "plated," or inoculated onto the appropriate medium. If the specimen was collected with a swab, the swab is rolled onto a portion of the agar medium in a Petri dish, and a sterile inoculating loop is used to spread the sample. If the sample is liquid, such as sputum or urine, a sterile inoculating loop is used to spread it.

Several techniques can be used to spread the sample. For the quadrant streak, a loop is used to spread the sample thinly over the agar medium in several directions. This effectively separates the bacteria so that they can grow in individual colonies. The lawn, or spread, streak is used when an antibiotic's effectiveness must be assessed or colonies counted. This involves using a swab or loop to spread the sample continuously over the entire plate.
Once the sample has been inoculated onto the plates, the plates are incubated in an inverted position so that any condensation that accumulates on the underside of the lid does not fall down onto the growth. The temperature and conditions of incubation depend on the source of the specimen and the suspected pathogens. Most cultures are incubated in an aerobic atmosphere enriched with 5% carbon dioxide at 37°C (98.6°F). Cultures for fungi are incubated both at room temperature and at 37°C (98.6°F) to promote dimorphism, a characteristic of some fungi in which they appear as a budding yeast at 37°C (98.6°F) and as a filamentous mold at room temperature.

Cultures for anaerobes must be incubated in an atmosphere devoid of oxygen. Special jars called anaerobe jars chemically remove oxygen from the environment and are small enough to place in an incubator (Figure 55-9).

**Assessing a Culture**

When the original (primary) culture has incubated at the appropriate temperature for 18 to 24 hours, it is examined for evidence of pathogens. Because normal floras often are present in samples in addition to pathogens, a trained eye is required to spot the organisms that might be causing an infection. Suspicous colonies are subcultured onto the appropriate medium to isolate them in pure culture. When the organism is in pure culture, staining and
additional biochemical testing can be done to identify it at the genus and species level. Throat and urine cultures may be performed in POLs that have been certified to perform moderately complex testing.

**Throat Culture**

*Streptococcus pyogenes*, also known as group A beta-hemolytic *streptococcus*, causes septic sore throat ("strep throat"). If not diagnosed and treated promptly, this organism can cause severe complications, including scarlet fever, rheumatic fever, and glomerulonephritis. A swab of the throat is streaked on a sheep's blood agar plate, and then a differentiation disk is placed on the most heavily streaked first quadrant. This disk contains an antibiotic (bacitracin), which inhibits the growth of *S. pyogenes* and is used for differential diagnosis. Complete clearing of the agar around the colonies indicates beta hemolysis as a result of a toxin produced by the organism; the toxin lysys the sheep red blood cells in the agar, hence the name *beta-hemolytic strep* (Figure 55-10 and Procedure 55-2). The presence of beta-hemolytic colonies and a zone of no growth around the disk indicates that the patient has strep throat. Additional testing may be needed to confirm the identity of the organism.

**Urine Cultures**

With urine cultures, the number of bacterial colonies present in a sample are counted. Most laboratories use a variety of differential and selective media, along with an all-purpose medium to which 1 ml (1000 mL) of urine is applied. By the standards established by the Clinical Laboratory Improvement Amendments (CLIA), this is a moderately complex test. A calibrated inoculating loop is dipped into a well-mixed urine sample that has been collected by the CCMS method (described in Chapter 52) or by catheterization. The urine from the loop is spread on the medium and incubated for 18 to 24 hours at 37°C (98.6°F). Each colony that grows on the plate represents 1,000 colony-forming units (cfu) per milliliter. A system of numeric values has been devised to assess the possibility of a urinary tract infection (Procedure 55-3).

Several self-contained, convenient culture systems for urine are available. These systems are ideal for the smaller laboratory that does not want to buy plate media and that has been certified to perform moderately complex tests. Examples of these systems include the Dialslide (Diatech Diagnostics, Boston, Massachusetts), Bacturcul (Carter-Wallace, Wampole Division, Cranbury, New Jersey), and Uri-Kit (Culture Kits, Norwich, New York). These devices contain culture media either on a paddle or on the walls of a container. The paddle is dipped into the urine, or the urine is poured into the container, swirled, and discarded. The device then is incubated (often at room temperature), and the number of colonies that forms on the media is compared with a colony density chart to determine the level of bacteria (Procedure 55-4).

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**Critical Thinking Application 55-4**

The technician from the referral laboratory indicated that Ms. Walker had a urinary tract infection. Anna recorded the test results as ">100,000 cfu/mL" in the patient's record. What does this number mean?

---

**Choosing an Appropriate Antimicrobial Agent**

The appropriate antimicrobial agent meets the following criteria:

- Demonstrates the most activity against the infectious agent
- Has the least toxicity to the patient
- Has the least impact on the normal microbiota of the body
- Has the desired pharmacologic characteristics
- Is the most economic
PROCEDURE 55-2

Inoculate a Blood Agar Plate to Culture *Streptococcus pyogenes*

**GOAL:** To inoculate a blood agar plate to detect the etiologic agent for strep throat.

**EQUIPMENT and SUPPLIES**

- Blood agar plate
- Bacitracin disk or strep A disk
- Incinerator
- Inoculating loop
- Permanent marker
- Swab from patient’s throat (see Procedure 37-8)
- Forceps
- BacT-Cinerator
- Disposable gloves
- Face protection

**PROCEDURAL STEPS**

1. Sanitize your hands. Put on face protection and gloves.
   **PURPOSE:** To ensure infection control.

2. Remove the swab from the transport device. Grasp the plate by the bottom (media side) and lift the base from the cover, or lift the cover while the plate is on the table.
   **PURPOSE:** To make handling the plate easier and to prevent contamination of the plate.

3. Roll the swab down the middle of the top half of the plate, then use the swab to streak back and forth on the same half of the plate. Dispose of the swab properly (Figure 1).
   **PURPOSE:** Rolling the swab ensures contact with the surface of the agar.

4. Sterilize the loop in the BacT-Cinerator and allow it to cool (Figure 2).
   **PURPOSE:** Loops must be sterilized before and after use to prevent cross-contamination of specimens.

5. Use the loop to streak for isolation of colonies in the second, third, and fourth quadrants. Pull the loop over the surface of the agar, pulling some of the inoculum into the uninoculated portion of the plate, and spread it around. Flame the loop again and pull some of the inoculum from the second area into the third area, and so on (Figure 3).

6. Use the loop to make three slices approximately 1 cm long in the agar in the heavy inoculum—swabbed area. Sterilize the loop (see Figure 2).
   **PURPOSE:** Isolated colonies are needed for observation of colony morphology. The agar is sliced to allow for detection of subsurface hemolysis.

---

**FIGURE 1** (From Slep LA, Woods WA: Laboratory procedures for medical office personnel, Philadelphia, 1998, Saunders.)

**FIGURE 2**

**FIGURE 3**

**FIGURE 4**
**PROCEDURE 55-2—cont’d**

7. Sterilize the forceps and remove one disk from the vial. Place the disk on the agar in the first quadrant. Sterilize the forceps.
   
   **PURPOSE:** Group A beta-hemolytic streptococci are presumptively identified by their sensitivity to the bacitracin.

8. With permanent marker, label the agar side of the plate with the patient's name and identification number and the date.
   
   **PURPOSE:** Labeling the agar side of dish rather than the lid prevents mixing up of specimens.

9. Place the plate in the incubator in an inverted position.
   
   **PURPOSE:** Placing the plate with the agar side up prevents the accumulation of moisture on the surface of the agar.

10. Incubate for 24 hours. Beta-hemolytic colonies are shown in Figure 4.

11. Incubate negative cultures for an additional 24 hours.
   
   **PURPOSE:** Some hemolysis patterns are not well defined after 24 hours of growth.

12. Clean the work area and properly dispose of all biohazardous waste.

13. Remove your gloves and sanitize your hands.

**PROCEDURE 55-3**

**Perform a Urine Culture**

**GOAL:** To inoculate three plates with 1 μL of urine to quantitate the number of bacteria and aid in the diagnosis of a urinary tract infection.

**EQUIPMENT and SUPPLIES**

- Urine specimen, collected clean catch midstream (CCMS) in a sterile container
- Bacti-Cinerator
- 1-μL calibrated inoculating loop
- Blood agar plate, MacConkey agar plate, and Columbia nutrient agar plate (or an appropriate selection of all-purpose, differential, and selective media)
- Disposable gloves
- Face protection

**PROCEDURAL STEPS**

1. Sanitize your hands. Put on face protection and gloves.
   
   **PURPOSE:** To ensure infection control.

2. With the screw-cap lid in place, mix the urine specimen thoroughly by swirling.
   
   **PURPOSE:** When a specimen is allowed to stand, microorganisms settle to the bottom.

3. Sterilize the calibrated loop, cool it, and dip the tip into the specimen.
   
   **PURPOSE:** The loop must be allowed to cool, or the heat will destroy the microorganisms as the loop comes in contact with the urine specimen, resulting in falsely low colony counts on the culture. Urine on the shaft of the loop will run down the shaft and increase the size of the specimen deposited on the plate, resulting in a falsely elevated colony count on the culture.

4. Spread the urine on the plate by “painting” the specimen down the center of the plate and then streaking thoroughly at right angles (Figure 1).
   
   **PURPOSE:** Careful streaking of the plates is necessary for an accurate count of the organisms present.

5. Inoculate the second and third plates in the same manner.

6. Label the bottom of the plates with the patient's name and identification number and the date.
   
   **PURPOSE:** Labeling the bottom of the plates prevents mixing up of the specimens.

7. Place the plates in the incubator with the agar sides facing up.

8. Incubate for 24 hours and then count the colonies on the all-purpose medium.

9. The results will be interpreted by a physician or medical technologist as follows:
   
   - >100 colonies = >100,000 colony-forming units (cfu/mL) of urine; indicates a urinary tract infection.
   - 10 to 100 colonies = 10,000 to 100,000 cfu/mL of urine; indicates suspicion. The urine may have been allowed to stand at room temperature, which facilitated overgrowth of bacteria, or the patient may have a subclinical infection. Recollection of the specimen is recommended.
   - <10 colonies = 10,000 cfu/mL of urine; indicates normal urethral microbiota.

   **PURPOSE:** Because the urine was collected by passing through the urethra, some normal bacteria should be present. This system of quantitation accounts for the presence of normal flora.

10. Clean the work area, dispose of all biohazardous waste, remove your gloves, and sanitize your hands.

**FIGURE 1**

Original inoculum
PROCEDURE 55-4

Perform Microbiologic Testing: Perform a Screening Urine Culture Test

GOAL: To assess the level of bacteriuria using a dip and count method so as to aid in the diagnosis of urinary tract infections.

EQUIPMENT and SUPPLIES
- Clean catch midstream (CCMS) urine specimen
- Uricult test kit
- Incubator
- Biohazardous waste container
- Disposable gloves
- Patient’s record

PROCEDURAL STEPS
1. Sanitize your hands, assemble the equipment and the specimen, and put on gloves. Check the expiration date on the test kit. Label the vial with the patient information (Figure 1).
   PURPOSE: An expired test kit may yield inaccurate test results.
2. Remove the slide from the test kit. Do not touch the slide or lay it down.
   PURPOSE: Touching the slide or laying it down contaminates the slide.
3. Dip the slide into the urine specimen, tipping the cup carefully if necessary. Alternatively, the urine may be poured over the slide and caught in another container (Figure 2).
   PURPOSE: The entire slide must be covered with urine for accurate results.
4. Allow excess urine to drain and then replace the slide in the protective vial. Screw the cap on loosely.
   PURPOSE: The cap must be loose to allow gas exchange in the tube.
5. Incubate the vial upright in an incubator at 35° to 37° C (90° to 98.6° F) for 18 to 24 hours.
   PURPOSE: Incubation for less or more time may produce erroneous results. Disease-causing bacteria grow best at body temperature, which is 35° to 37° C (90° to 98.6° F).

6. After incubation, the test results are interpreted by removing the slide from its protective vial, assessing the bacterial colony density, and comparing the density on the slide with the density chart provided. No actual colony counting is necessary (Figure 3).
7. The results are interpreted as follows:
   - Normal: <10,000 colony-forming units (cfu)/mL of urine; no urinary tract infection (UTI) is present.
   - Borderline: 10,000 to 100,000 cfu/mL of urine; a chronic or relapsing infection may be present, and the test should be repeated.
   - Positive: >100,000 cfu/mL of urine; a UTI is likely.


PROCEDURE 55-4—cont’d

8. Return the vial to the protective case and replace the cap.
9. Dispose of the test in a biohazardous waste container (Figure 4).
   PURPOSE: The slide is contaminated. Alternatively, the protective case may be filled with a disinfectant, such as 1:10 chlorine bleach, before the slide is reinserted.
10. Remove your gloves and sanitize your hands.
    PURPOSE: To ensure infection control.
11. Record the results in the patient’s medical record.
    PURPOSE: A procedure is not considered done until it is recorded.

FIGURE 4 (From Bonewit-West K: Clinical procedures for medical assistants, ed 5, Philadelphia, 2000, Saunders.)

FIGURE 55-11 Gram stain. A, Red blood cells (RBCs) and gram-positive cocci. B, RBCs with gram-negative bacilli. (From De la Maza LM, Pezzlo MT, Baron JS: Color atlas of diagnostic microbiology, St Louis, 1997, Mosby.)

Staining

Pathogenic microorganisms generally are colorless, and a microscope is needed to see them. Special differential stains, such as the Gram stain and the acid-fast stain, often are used to differentiate bacteria based on biochemical differences. As discussed previously, the Gram stain differentiates bacteria into two categories according to cell wall thickness, and the acid-fast stain differentiates bacteria into two categories based on the presence or absence of a waxy lipid in the cell wall.

Before staining can be done, the bacteria must be applied to a labeled slide. A direct smear from a swab can be made, or a culture can be stained. Individual colonies growing on the culture medium can be spread into a drop of sterile saline on a glass slide, or material directly from the site of infection can be spread on the slide from the swab used to collect it. The slide then is air dried and fixed. Either heat or methanol can be used to fix the slide, which results in the material adhering to the slide. Both heat (e.g., from a Bunsen burner or an incubator) and methanol cause protein in the sample to denature and stick to the slide, much as egg white sticks to a hot frying pan (Procedure 55-5).

Gram Stain

The Gram stain, developed by Dr. Hans Christian Gram more than 100 years ago, is still the most commonly used stain in the microbiology laboratory. It involves applying a sequence of primary dye, mordant, decolorizer, and counterstain to the slide. The dyes are taken up differently according to the chemical composition of the cell walls. Bacteria react best in the Gram stain when they are 24 hours old or less. Gram-positive bacteria stain purple, and gram-negative bacteria stain pink or red (Figure 55-11). Although Gram staining is considered a CLIA moderately complex test, it is useful for the medical assistant to understand the procedures and the microscopic results obtained.

Acid-Fast Stain

The acid-fast stain is used in the identification protocol for Mycobacterium species. M. tuberculosis and M. avium complex (MAC) are two important species of mycobacteria. The former causes tuberculosis and can be isolated from sputum or tissue samples from infected patients; the latter is a common soil organism that enters through the respiratory tract and disseminates throughout
**Procedure 55-5**

**Prepare a Direct Smear or Culture Smear for Staining**

**Goal:** To prepare a smear for staining from a clinical specimen or from a culture medium.

**Equipment and Supplies**
- Clean glass slides
- Permanent marker
- Incinerator
- Normal saline solution
- Specimen collected on a smear
- 24-hour culture on agar
- Biohazardous waste container
- Disposable gloves
- Face protection

**Procedural Steps**

**Direct Smear**
1. Sanitize your hands. Put on face protection and gloves.
2. Label the slide with a permanent marking pen.
   **Purpose:** Other labels are destroyed in the staining process.
3. Prepare a thin smear by rolling the swab on the slide. Make sure all areas of the swab touch the slide (Figure 1).
   **Purpose:** Rolling the swab ensures that all parts of the swab come in contact with the slide so that the organisms collected are deposited on the slide. Thin smears are needed for evaluation.
4. Allow the smear to air dry. Do not wave it or heat dry it.
   **Purpose:** Waving the slide spreads pathogens. Overheating organisms distorts them.
5. Hold the slide with the smear up. Heat-fix the slide using an incinerator.
   **Purpose:** Check the heating process by touching the slide to the back of the gloved hand (Figure 2). The slide should feel warm, not hot. Check it often by touching the back of the slide to the back of the gloved hand. Cool the slide.
   **Purpose:** Heat-fixing causes materials to adhere to the slide.

**Culture Smear**
1. Sanitize your hands. Put on face protection and gloves.
2. Identify the colonies to be stained by circling them on the back of the plate and numbering them with a permanent marker. Label the slide accordingly.
   **Purpose:** This allows accurate identification of colonies.
3. Using a loop, apply a small drop of saline solution to the slide.
   **Purpose:** Liquid is needed to emulsify the colony. Large drops require a longer drying time.
4. Using a sterile loop, touch only the top of the colony chosen. Transfer the material picked up to the appropriate area of the slide and spread it in a circular motion to the size of a dime. Repeat for each colony chosen using a separate slide.
   **Purpose:** Only a small amount of colony is needed for staining.
5. Allow the smear to air dry.
6. Heat-fix the smear as described for a direct smear.
7. Properly dispose of all biohazardous materials and clean the work area.
8. Remove your gloves and sanitize your hands.

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**Biochemical Testing**

Once a suspected pathogen has been isolated and is in pure culture, biochemical testing must be performed to identify the genus and species. Some of these tests, such as a catalase rapid enzyme test or an oxidase test, take only a few minutes. Others, such as fermentation testing using various carbohydrates, take up to 24 hours. Hundreds of tests are available to identify an organism biochemically, and manufacturers have developed miniaturized multitest systems that speed inoculation and identification (Figure 55-13).
highly sensitive. This means that if the test results are positive, there is a high degree of confidence that \textit{S. pyogenes} is in the sample; if the test results are negative, the organism may not have been present in sufficient numbers to be detected.

\section*{Influenza A and B Testing}
Influenza virus causes influenza, or "the flu," a highly contagious, acute viral infection of the respiratory tract. The infection is highly communicable through the respiratory route, and outbreaks typically are seen in the fall and winter. Type A viruses usually are more prevalent than type B viruses; type A viruses typically are associated with epidemics, and type B viruses cause a milder infection. Rapid diagnosis of influenza can assist with decisions to administer antiviral medications, which must be given early in the course of the infection if they are to be effective. CLIA-waived rapid immunochromatographic assays detect both influenza A and influenza B antigens from nasopharyngeal swabs or nasal washes. If a swab is used, the sample is removed from the swab using saline, a transport medium, or a solution provided by the manufacturer. Nasal washings can be used directly in the test kit.

\section*{Respiratory Syncytial Virus Testing}
RSV is a major cause of upper and lower respiratory tract infections and the major cause of bronchiolitis and pneumonia in children and infants. Outbreaks typically occur yearly in the fall, winter, and spring and can be severe for very young children. The CLIA-waived rapid immunochromatographic assay for RSV uses a nasopharyngeal swab specimen or nasal washings to detect a protein the virus uses to fuse to human cells. Because antiviral agents are available to treat RSV infection, rapid diagnosis can lead to shorter hospital stays, a reduced need for antibiotic therapy to treat secondary bacterial infection, and a lower cost for hospital care. The tests are intended for children under age 5.

\section*{AMICROBIAL SUSCEPTIBILITY TESTING}
Isolating the infectious agent from a patient is only the first step in successful treatment. When a physician wants to determine the appropriate antibiotic through laboratory testing, he or she orders a culture and sensitivity (C&S) test. "Culture" refers to cultivating the organisms, and "sensitivity" refers to a test to determine the organism's susceptibility to certain antibiotics. Most bacteria show resistance to \textit{antimicrobial agents}, and because these patterns of resistance are continuously changing, they cannot be predicted. Shifting patterns of resistance require testing of individual bacteria against the appropriate antimicrobial agent.

The clinical microbiology laboratory can recommend antimicrobial agents based only on their \textit{in vitro} activity. The healthcare practitioner must decide which medication to order based on test results and a physical examination.

Whenever specimens are inoculated, asepsis must be strictly observed to ensure safety and good results. The organism being tested must also be isolated in pure culture before the test. The test, often referred to as the \textit{Kirby-Bauer Antimicrobial Susceptibility Test}, is performed by inoculating sterile water with the pure
PROCEDURE 55-6

Screen Test Results: Perform a Rapid Strep Test

GOAL: To perform a rapid strep screening test to assist in the diagnosis of strep throat and to follow up negative results by performing a throat culture collection.

EQUIPMENT and SUPPLIES

- Directigen Strep A test kit
- Timer or wristwatch with sweep second hand
- Throat swab specimen (see Procedure 36-8)
- Biohazardous waste container
- Disposable gloves
- Face protection
- Patient’s record

PROCEDURAL STEPS

1. Collect all necessary supplies and equipment. Bring all reagents and reaction disks to room temperature (minimum of 30 minutes).
2. Sanitize your hands. Put on gloves and face protection.
   PURPOSE: To ensure infection control.
3. Position all bottles vertically and dispense reagents slowly as free-falling drops. Avoid reagent contact with your eyes, because the reagent is an irritant (Figure 1).
4. Add three drops of reagent 1 to an extraction tube. This solution is pink.
5. Add three drops of reagent 2 to the same tube. The solution should turn yellow.
6. Place the specimen swab in the tube, twirling the swab in the mix.
7. Let stand for exactly 1 minute.
8. Add three drops of reagent 3 to the same tube, again twirling the swab in the tube to mix. This solution should be pink.
9. Express the liquid from the swab by squeezing the tube with the thumb and forefinger and rotating the swab as it is withdrawn. The liquid must be thoroughly removed from the swab. Best results are achieved when the liquid reaches or exceeds the line on the tube.
10. Discard the swab in a biohazardous waste container.
11. Remove the reaction disk from the pouch and place it on a dry, flat surface.
12. Pour the entire contents of the tube into the reaction disk.

13. Read the test results when the entire end of the assay window turns red (5 to 10 minutes).
14. Properly dispose of all contaminated waste.
   PURPOSE: Items that come in contact with samples are considered potentially infectious.
15. Clean the work area, remove your gloves, and sanitize your hands.
   PURPOSE: To ensure infection control.
16. Record the test results in the patient's medical record.
   PURPOSE: A procedure is considered not done until it is properly recorded.
17. If the test results are negative, a second throat swab should be obtained and a throat culture should be performed. Often two swabs are used simultaneously when the sample is collected from the throat to prevent the need to recollect a specimen.
   PURPOSE: Negative rapid strep test results should be confirmed with a throat culture.

FIGURE 1

culture of bacteria to a specified degree of turbidity. This suspension is spread with a swab in a lawn pattern on the surface of the appropriate agar medium. Disks that each contain an antimicrobial agent, such as penicillin or tetracycline, are placed on the agar with forceps or an automatic dispenser. After incubation, the zone of inhibition (area of no growth) around each disk is measured in millimeters and compared with values provided by the manufacturer of the disks (Figure 55-14). Three determinations are possible: S, R, or I. S means that the pathogen is "susceptible," or that the antibiotic is effective against the organism in that particular concentration in vitro; R means that the organism is "resistant" to the antibiotic; I means "intermediate." That is, additional testing must be performed to determine the dosage of antimicrobial necessary for therapeutic treatment.

CRITICAL THINKING APPLICATION 55-5

Anna has recorded the results of Ms. Walker’s urine culture. She notes that 10 antimicrobial agents had been tested, but the Escherichia coli was susceptible to only five of them. How will Dr. Ling determine which of these five antibiotics would be best for Ms. Walker?
In children, specimens are best collected late at night or early in the morning before a bowel movement, urination, or bathing. Paraffin swabs impregnated with petroleum jelly or cellulose tape may be used to collect the eggs deposited by the adult worm during the night. The diagnosis is based on laboratory detection of the eggs in fecal smears. If the parent does not feel comfortable about obtaining the needed specimen, instruct the parent to bring the child to the office as soon as he or she awakens in the morning. Instruct the parent not to change the child’s clothing or the child’s diaper before coming to the office, but to bring the child immediately on waking. When the child arrives, have all the needed supplies ready to use and perform the procedure immediately (Procedure 55-7).

**Immunologic Testing**

Immunologic testing provides information about past or present infections with bacteria or viruses and also is done to detect certain types of cancers. Testing done in the immunology laboratory is designed to demonstrate the reaction between antigen and antibody. Antibodies are formed when the body encounters a foreign agent. In the acute phase of a disease, the antibody level is high; during the convalescent stage, the antibody level declines. Once an antigen has been recognized by the immune system and antibodies have been made, the level of antibody to that particular antigen remains at a low but detectable level indefinitely. The amount of antibody at any given time can be measured with serologic testing and is referred to as the titre.

The reaction between antigen and antibody is demonstrated in vitro through several means, most commonly agglutination,
7. To obtain the perianal sample, first peel back the tape on the slide by gripping the label (Figure 2). With the tape looped (adhesive side outward) over a wooden tongue depressor that is held against the slide and extended about 1 inch beyond it, press the tape firmly against the right and left anal folds (Figure 3).
8. Spread the tape back on the slide, adhesive side down (Figure 4).
9. Smooth the tape using a cotton ball or gauze square (Figure 5).
10. Write the patient's name and date on the slide label.
11. Advise the parent that the child can be dressed or assist with dressing the child if needed.

**Testing the Sample**

1. Lift one side of the tape and apply one drop of toluene before pressing the tape back down on the glass slide.

*Purpose:* This clears the specimen so that any eggs are visible.

2. Place the prepared slide under the microscope's low-power objective for examination by a physician or medical technologist during low illumination (Figure 6).

3. Record in patient record.
4. Dispose of all biohazardous waste, clean the work area, remove your gloves, and sanitize your hands.

precipitation, and immunochromatographic assay. In agglutina-
tion and precipitation reactions, latex beads or RBCs from an
animal such as a rabbit are needed. The antigen or antibody is
chemically bound to the bead or cell by the manufacturer.
When the corresponding antibody or antigen molecule comes
into contact with the bead or cell, they link and clump (agglu-
tinate). If this reaction occurs in a test tube, the clumps pre-
cipitate to the bottom of the tube. If the reaction occurs on a
glass or paper surface, such as a slide, the clumps are visible to
the naked eye. Antigen and antibody must be present in
roughly equal proportions for clumping to occur, because the
linking is much like latticework. If markedly more antigen
than antibody is present, or vice versa, the lattice cannot form
properly; this is a false-negative reaction and is called the
prozone reaction.

Immunochromatographic assays are replacing many of the
precipitation and agglutination tests in the serology laboratory
because of their enhanced specificity and sensitivity. Solid-phase
immunoassay, described in Chapter 52, involves the immobilization (attachment) of antigens or antibodies on solid surfaces such as beads, wells in plastic dishes, or plastic cartridges. Generally, when an antigen-antibody reaction occurs, a color change is visible. With some tests, the more intense the color, the higher the concentration of the antibody being measured.

Most serologic testing performed in the physician's office is done with individual testing kits. When a serologic test is performed, the first step is to review the package insert provided by the manufacturer. This review provides valuable information about the test, the principle on which the test is based, the reagents and equipment required, proper specimen collection techniques, preparation requirements, test procedures, and any precautions or warnings that pertain to the procedure. In addition, the inserts provide information about quality control, interpretation of results, limitations of the procedure performance characteristics, and references.

CLIA-waived tests that can be performed by a medical assistant to detect antibody to a pathogen include those for infectious mononucleosis, *Helicobacter pylori*, human immunodeficiency virus (HIV), and Lyme disease.

### Infectious Mononucleosis Testing

Infectious mononucleosis, also called "mono" or the "kissing disease," is an acute infectious disease caused by the Epstein-Barr virus (EBV). EBV is one of the most common human viruses. The virus occurs worldwide; it is especially common in teenagers and occasionally in adults, but it is found most frequently in people between the ages of 10 and 25. Most people are infected with EBV at sometime during their lives. In the United States, as many as 95% of adults between 35 and 40 years of age have already been infected.

In children the infection may pass unrecognized or result in a mild illness lasting only a few days, with sore throat, fever, swollen tonsils, and enlarged lymph nodes in the neck. These signs and symptoms can be indistinguishable from those of other mild illnesses of childhood. In young people, some of the most common complications include the abrupt onset of fatigue, headaches, aching muscles, faint rash, fever, very swollen tonsils, enlarged lymph glands, and loss of appetite often associated with nausea. There may be a short or prolonged period (days or weeks) after the initial illness when the fatigue continues. Occasionally, complications occur, including the development of a swollen spleen or liver. Heart problems or any involvement of the central nervous system (CNS) is rare, and infectious mononucleosis is almost never fatal.

Testing for mononucleosis involves a complete blood count (CBC) and serologic tests. The CBC reveals an increased number of lymphocytes that appear atypical on the differential examination. The infected lymphocytes undergo a cellular transformation, causing them to take on an appearance similar to a monocyte (hence the name mononucleosis). Most patients exposed to EBV develop a nonspecific antibody response to the virus. The antibodies react with surface antigens of horse erythrocytes, causing agglutination (clumping) that is visible on the test slide (Procedure 55-8). Solid-phase immunochromatographic assay tests may also be used to diagnose EBV.

### H. pylori Antibody Testing

*H. pylori* is a spiral-shaped bacterium that can infect the gastric mucous layer or adhere to the epithelial lining of the stomach. *H. pylori* causes more than 90% of duodenal ulcers and more than 80% of gastric ulcers (see Chapter 39). Several methods can be used to diagnose *H. pylori* infection. Serologic tests that measure specific *H. pylori* IgG antibodies can determine whether a person has been infected. CLIA-waived rapid qualitative immunochromatographic assay tests use whole blood applied to a well in a test cartridge. After the blood migrates through the cartridge, lines appear in the window, indicating the presence of antibodies to the pathogen.

### Human Immunodeficiency Virus Antibody Testing

The OraQuick Advance Rapid HIV-1/2 Antibody Test (OraSure Laboratories, Bethlehem, Pennsylvania) is a single-use, qualitative, two-step immunochromatographic assay to detect antibodies to HIV type 1 (HIV-1) and type 2 (HIV-2) in oral fluid and whole blood. It is a CLIA-waived screening test for HIV-1, the virus that causes AIDS (see Chapter 40). However, the test does not detect HIV-1 infection in people who contracted the virus within 3 months (approximately) before taking the test, because it can take that long for detectable antibodies to HIV-1 to appear in the blood. Because this is a screening test, the results must be confirmed by additional, more specific tests. All individuals taking this test must receive the "Subject Information" pamphlet before specimen collection and counseling after receiving their test results.

The test kit (Figure 55-15) includes a testing device with a flat pad that is rubbed once over the upper and lower gums. It then is inserted into the test vial, which is placed in a plastic stand that holds the device at the proper angle. The test results are read in 20 minutes. If whole blood is used, it can be obtained from a
**PROCEDURE 55-8**

**Perform Immunologic Testing: Perform the Mono-Test for Infectious Mononucleosis**

**GOAL:** To perform and interpret a slide test for infectious mononucleosis.

**EQUIPMENT and SUPPLIES**
- Mono-Test kit
- Blood specimen (serum or plasma)
- Timer or wristwatch with sweep second hand
- Biohazardous waste container
- Disposable gloves
- Face protection
- Patient’s record

**PROCEDURAL STEPS**

1. Remove the test kit from the refrigerator and allow the reagents to warm to room temperature. Check the expiration date of the kit.  
   **PURPOSE:** Outdated or cold reagents do not react as expected.

2. Sanitize your hands. Put on face protection and gloves.  
   **PURPOSE:** To ensure infection control.

3. Fill a disposable capillary tube to the calibration mark with serum or plasma (see Chapter 53 for collection of blood). Using the rubber bulb included in the kit, deposit the specimen in the first circle of the clean glass or paper slide also provided in the kit (Figure 1).  
   **PURPOSE:** The capillary tube measures the exact amount of sample for accurate testing.

4. Place one drop of negative control in the second circle and one drop of positive control in the third circle (Figure 2).  
   **PURPOSE:** Known controls ensure that reagents are functioning properly.

5. Thoroughly mix the Mono-Test reagent by rolling the bottle gently between the palms of the hands. Squeeze the enclosed dropper to mix all the contents of the bottle.  
   **PURPOSE:** Reagent RBCs settle on standing and must be mixed before use.

6. Hold the dropper in a vertical position and add one drop of Mono-Test reagent to each area of the slide. Do not touch the dropper to the slide.  
   **PURPOSE:** Holding a dropper vertically ensures delivery of the same size drop. If the dropper touches other materials, it becomes contaminated, and the results will be inaccurate.

7. Using separate stirrers, quickly and thoroughly mix each area, spreading each area out to 1 inch in diameter.

8. Rock the slide gently for exactly 2 minutes; observe immediately for agglutination. A dark background is best for viewing.  
   **PURPOSE:** Timing is always important.

9. Interpret the test results and record them. Agglutination is positive, and no agglutination is negative.

10. Clean the work area, remove your gloves, and sanitize your hands.

11. Record the test results in the patient’s medical record.  
   **PURPOSE:** A procedure is not considered done until it is properly recorded.

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**FIGURE 1**

**FIGURE 2**

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**Lyme Disease Antibody Testing**

Lyme disease is the most common insect-borne infectious disease in North America, and it is a significant public health concern. The spirochete bacterium, *Borrelia burgdorferi*, is the causative agent in Lyme disease.

The disease is contracted from the bite of a tick with saliva that contains the bacteria. These ticks typically are found on deer, mice, dogs, horses, and birds. Infection occurs after the bacteria enter the wound, and a characteristic bull’s-eye rash, known as *erythema migrans* (EM), may develop at the bite site in 60% to 80% of patients. Lyme disease progresses in three stages, which have unclear transition and overlapping symptoms. As the disease progresses, the spirochete bacterium invades...
the skin, joints, CNS, heart, eyes, bones, spleen, and kidneys. Arthritic or CNS syndromes often accompany late-stage disease and may be the only clinical, symptomatic indications of infection.

Early detection of Lyme disease can be accomplished using a CLIA-waived test such as the Wampole PreVue B. burgdorferi test (MedPointe Co, Princeton, New Jersey). This immunochromatographic assay tests for IgG and IgM antibodies in whole blood. A sample of blood is applied to a test cartridge, a diluent is added, and the results are read in 20 minutes. Positive results should be verified by confirmatory testing.

## Closing Comments

### Patient Education

Microorganisms such as bacteria, viruses, fungi, and parasites are responsible for most human diseases. Patient education plays an important role in helping the patient and family control the spread of infection. The following list of teaching topics can help you educate a patient in infection control:

- An explanation of the patient’s type of infection—bacterial, viral, fungal, or parasitic
- How infection spreads
- Normal barriers to infection
- Risk factors for infection
- Patient preparation for cultures and serologic, hematologic, and imaging tests, as necessary
- The patient’s role in specimen collection
- Hand sanitation, proper storage and cleaning of personal items, and disposal of contaminated supplies

Explain to the patient that infection does not always occur at the entry site; for example, measles can be transmitted through the respiratory tract. Reinforce the need for strict adherence to the prescribed antimicrobial therapy by pointing out the possible complications of noncompliance, such as relapse or systemic involvement. Explain to the patient that inadequate drug therapy (not taking the medication as prescribed) may cause the infection to worsen and spread.

Above all, always listen to the patient; be sure to answer all questions. However, do not try to answer a question if you are unsure of the answer. Notify the physician of the patient’s concerns so that he or she can provide further detail before the patient leaves the facility.

### Critical Thinking Application

Aaron’s culture was confirmed as *Staphylococcus aureus*. What information can Anna give Aaron and his mother about the contagiousness of this infection?

### Legal and Ethical Issues

Maintaining a laboratory in the office increases the physician’s liability. By testing patients’ specimens in the office, the physician assumes responsibility for the interpretation and accuracy of the results. As the person in the office who runs the tests and notes the results in the patient’s record, you are responsible for maintain optimum accuracy in the testing results. A quality assurance (QA) program for POLs may reduce the risks involved and still allow the patient to benefit from the convenience of office testing. Strict confidentiality is essential. Never release information to anyone other than the patient or legal guardian; however, certain infectious diseases must be reported to the Centers for Disease Control and Prevention (CDC) or local board of health. Each state legislature determines how the data is to be reported and what diseases must be reported. The data for nationally notifiable diseases is published weekly by the CDC in the *Morbidity and Mortality Weekly Report* (MMR). An annual report is available on the Internet at www.cdc.gov. The 2009 summary lists the following conditions as notifiable:

- Acquired immunodeficiency syndrome (AIDS)
- Anthrax
- Arboviral neuroinvasive and non-neuroinvasive diseases
- Botulism
- Chancroid
- *Chlamydia trachomatis*, genital infections
- Cholera
- Coccidioidomycosis
- Cryptosporidiosis
- Cyclosporiasis
- Diphtheria
- Ehrlichiosis/anaplasmosis
- Giardiasis
- Gonorrhea
- *Haemophilus influenzae*, invasive disease
- Hansen’s disease (leprosy)
- Hantavirus pulmonary syndrome
- Hemolytic uremic syndrome, postdiarrheal
- Hepatitis, viral, acute (A, B, C)
- Hepatitis, viral, chronic
- HIV infection
- Influenza-associated pediatric mortality
- Legionellosis
- Listeriosis
- Lyme disease
- Malaria
- Measles
- Meningococcal disease
- Mumps
- Novel influenza A virus infections
- Pertussis
- Plague
- Poliomyelitis, paralytic
- Poliovirus infection, nonparalytic
- Psittacosis
- Q fever
- Rabies
- Rocky Mountain spotted fever
- Rubella
- Rubella, congenital syndrome
- Salmonellosis
- Severe acute respiratory syndrome (SARS)
- Shiga toxin–producing *Escherichia coli* (STEC)
- Shigellosis
- Smallpox
- Streptococcal disease, invasive, Group A
- Streptococcal toxic shock syndrome
- *Streptococcus pneumoniae* infection
- Syphilis, congenital
- Tetanus
- Toxic shock syndrome (other than streptococcal)
- Trichinellosis (Trichinosis)
- Tuberculosis
- Tularemia
- Typhoid fever
- Varicella (morbidty)
- Varicella (deaths only)
- Vibriosis
- Yellow fever

**SUMMARY OF SCENARIO**

It seems to Anna that she sees something new about harmful bacteria on the television or in the newspaper every day. Outbreaks on cruise ships and bio-warfare have become common topics, yet Anna knows that most bacteria are harmless. People can protect themselves from infection by using a few simple techniques, such as frequently sanitizing the hands and keeping the hands away from the face. Anna makes a point of explaining this to the patients she sees at the family clinic. Prevention, she knows, is the key to controlling infection.

Anna realizes that the POL can play a vital role in the diagnosis and treatment of infectious diseases. She knows that proper specimen collection is of the utmost importance in microbiology testing and that contamination could mean vital time lost in identifying pathogens. Rapid testing allows for quick diagnosis, which is important when dealing with infectious organisms that reproduce quickly. Anna has learned that certain microbiology tests are CLIA waived, whereas others can be performed in a POL that has obtained a certificate for CLIA moderate-complexity testing. As a CMA (AAMA) she is qualified to perform waived tests, and with the appropriate documented training, she may perform moderately complex tests. Testing, she has discovered, may not always involve detection of the pathogen itself. Culturing on artificial media may be performed on urine, wound, or throat specimens, and sometimes the pathogen is detected by demonstrating its presence using a rapid identification test, such as the rapid strep test. At other times, however, antibodies made in response to the pathogen must be detected to diagnose the disease, such as with the mononucleosis test.

Anna knows that the technology for rapid testing is evolving quickly, and she is aware that she can easily check the FDA’s Web site for new tests that could be performed in the POL.

**SUMMARY OF LEARNING OBJECTIVES**

1. **Define, spell, and pronounce the terms listed in the vocabulary.**
   Spelling and pronouncing medical terms correctly bolster the medical assistant’s credibility. Knowing the definitions of these terms promotes confidence in communication with patients and co-workers.

2. **Apply critical thinking skills in performing the patient assessment and patient care.**
   Completing the Critical Thinking Application exercises throughout the chapter can help the student medical assistant become more adept at critical analysis of real-life situations.

3. **Cite the protocols for the collection, transport, and processing of specimens.**
   Specimens for the microbiology laboratory must be collected in sterile containers. Transport systems are available if the specimen cannot be plated immediately. These systems often contain a transport medium that keeps the organisms alive but does not let them multiply (see Table 55-1).

4. **Identify the elements needed for microbial growth.**
   All microbes require nutrients and water to stay alive. Aerobes require oxygen; anaerobes die in the presence of oxygen. Most pathogens prefer an incubation temperature of 37° C (98.6° F) and a pH of 7.

5. **Compare bacteria with viruses.**
   Viruses differ from bacteria in that they are not cells. Viruses have a core of nucleic acid surrounded by a protein coat. Unlike bacteria, they do not metabolize, and they cannot replicate on their own.

6. **Describe the characteristics of common viral diseases.**
   Refer to Table 55-2.

7. **Describe the bacterial structures used in identification.**
   Some bacteria have flagella protruding from the cell wall. These structures aid in propulsion. Some bacteria produce gelatinous capsules that enhance their virulence. Bacteria in the genera Bacillus and Clostridium produce endospores that allow them to survive harsh conditions.

8. **Compare bacteria with fungi, parasites, and protozoa.**
   Bacteria are prokaryotic; fungi, protozoa, and parasites are eukaryotic. Bacteria, fungi, and protozoa must be observed microscopically; helminths, or worms, can be seen with the naked eye (see Tables 55-3 thru 55-8).

9. **Describe various bacterial morphologies.**
   Identification of bacteria begins with the observation of their morphology. Cocci are spherical organisms; bacilli are rod-shaped organisms; and spirilla are spiral-shaped organisms. Staphylococci are cocci in clusters; streptococci and streptobacilli are organisms arranged in chains, and diplococci and diplobacilli are organisms arranged in pairs.

10. **Explain the characteristics of common diseases caused by bacteria.**
    Refer to Tables 55-3 thru 55-5.

11. **Describe the unusual characteristics of *Chlamydia, Rickettsia*, and *Mycoplasma* organisms.**
Chlamydia and Rickettsia organisms are tiny bacteria, but unlike most bacteria, they require a host cell for replication. Rickettsia organisms are transmitted by arthropods. Mycoplasma organisms are bacteria without cell walls (see Table 55-6).

12. Identify the characteristics of common diseases caused by fungi, protozoa, and parasites.
Refer to Tables 55-7 and 55-8.

13. Perform patient education on the collection of a stool specimen for ova and parasite testing.
Stool is collected in special transport devices that contain preservatives and fixatives that will assist the microscopic examination of the specimen. Explicit instructions must be given to the patient to ensure proper collection (see Procedure 55-1).

14. Describe the equipment needed in a microbiology laboratory.
Cultivation equipment includes inoculating loops and needles, Petri dishes with agar media, and incubators. Viewing equipment includes slides, stains, and microscopes. Sterilizing equipment includes incinerators and autoclaves.

15. List the different growth media used for cultivating.
Growth media consists of nutrients selected for certain species. Media can be liquid or can be made solid by the addition of agar. Solid media can be prepared as Petri plates or as tube media. Media can be all purpose and support the growth of many species. It also can be selective, permitting only a certain type of microbe to grow. Media can be differential, allowing differentiation of species based on color changes caused by different biochemical reactions. Enriched media support the growth of fastidious bacteria.

Smears can be prepared directly from swabs or from growth on solid media. If a smear is made from a bacterial colony, a small amount of water or saline must be placed on the slide first. Smears must be air dried and heat fixed before staining.

17. Perform the procedure for inactivating a blood agar plate.
Refer to Procedure 55-2.

18. Perform a urine culture.
Refer to Procedure 55-3.

19. Perform a screening urine culture test.
Refer to Procedure 55-4.

20. Prepare a direct smear or culture smear for staining.
Refer to Procedure 55-5.

21. Compare and contrast the throat culture for Streptococcus pyogenes with the rapid strep test.
The throat culture involves obtaining a swabbing of the throat and culturing it on a sheep blood agar plate. The throat culture is observed for beta hemolysis and susceptibility to bacitracin, both of which indicate the presence of group A beta-hemolytic streptococci.
The rapid strep test does not involve culturing but is an immunochromatographic assay that assesses the presence of Streptococcus antigen in the sample. A negative rapid strep test should be confirmed with a throat culture.

22. Perform a rapid strep test.
Refer to Procedure 55-6.

23. Describe three microbiologic tests that use a rapid identification technique.
The rapid strep test detects S. pyogenes and is used in the diagnosis of streptococcal pharyngitis. The influenza A and B rapid tests detect surface antigens of the virus that causes influenza. The RSV rapid test detects antigens from RSV, which causes pneumonia and bronchiolitis in young children.

24. Describe the method used for antimicrobial susceptibility testing.
Antimicrobial susceptibility testing uses discs impregnated with antimicrobial agents dropped onto the surface of an agar plate inoculated with a pathogen. The pathogen displays susceptibility, resistance, or an intermediate reaction to the antimicrobial agent. These determinations are made by measuring the zone of inhibition around each disk and comparing them with a chart provided by the manufacturer.

25. Explain how pinworm testing is done and when it must be performed.
Pinworm testing detects the eggs of the pinworm, E. vermicularis. The worm deposits eggs in the anal folds at night. The eggs can be retrieved by using a sticky collection device either late in the evening or in the morning before a bowel movement. The diagnosis is made if the eggs are found microscopically.

26. Perform a cellulose tape collection for pinworms.
Refer to Procedure 55-7.

27. Discuss the purpose of immunologic testing.
Often cultivation of a pathogen is difficult, so demonstrating the presence of the pathogen with antigen testing is difficult. Immunologic testing detects antibodies to a pathogen.

28. Describe three rapid immunologic tests that could be done in the physician’s office laboratory.
Mononucleosis testing detects the heterophile antibodies made in reaction to infection with the Epstein-Barr virus. Serum, plasma, or whole blood can be used, depending on the test. H. pylori testing detects antibodies to the bacterium, which is a common cause of stomach ulcers. Whole blood is used for the test. Rapid HIV testing detects the two viruses, HIV-1 and HIV-2, which cause AIDS. Either oral swabblings or whole blood can be used.

29. Perform the Mono-test for mononucleosis.
Refer to Procedure 55-8.

30. Discuss legal and ethical issues involved in laboratory testing.
The medical assistant must be aware that patient confidentiality is of utmost importance; however, certain infections must be reported to the CDC and to the local board of health.
CONNECTIONS

Study Guide Connection: Go to the Chapter 55 Study Guide. Read and complete the activities.

Evolve Connection: Go to the Chapter 55 link at evolve.elsevier.com/kinn to complete the Chapter Review and Chapter Quiz. Peruse other resources listed for this chapter to increase your knowledge of Assisting in Microbiology and Immunology.