Laboratory Tests to Direct Antimicrobial Pharmacotherapy

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KEY CONCEPTS

1. Familiarity with normal host flora and typical pathogens will help to determine whether a patient is truly infected or merely colonized.
2. Direct examination of tissue and body fluids by Gram stain provides simple and rapid information about the causative pathogen.
3. Isolation of the offending organism by culture assists in the diagnosis of infection and allows for more definitive directed treatment.
4. The development of molecular testing systems has improved our ability to diagnose infection and determine the antimicrobial susceptibilities for numerous fastidious or slow growing pathogens, such as mycobacteria and viruses.
5. Although highly standardized, in vitro antimicrobial susceptibility testing has limitations and often cannot truly mimic the conditions found at the site of an infection. This can cause discordance between in vitro susceptibility results and in vivo response to therapy.
6. The laboratory evaluation of antimicrobial activity is an important component of the pharmacotherapeutic management of infectious diseases.
7. When used appropriately, rapid automated susceptibility test systems appear to improve therapeutic outcomes of patients with infection, especially when they are linked with other clinical information systems.
8. Laboratory tests such as minimum bactericidal concentration tests, time-kill tests, post-antibiotic effect tests, and antimicrobial combination testing are important for the clinician to understand because they help to determine antimicrobial pharmacodynamic properties.
9. Routine monitoring of serum concentrations is currently used for a select few antimicrobials (e.g., aminoglycosides, chloramphenicol, and vancomycin) in an attempt to minimize toxicity and maximize efficacy.
10. Appropriate timing for the collection of serum samples when measuring antimicrobial serum concentrations is crucial to ensure that proper data are generated on the pharmacokinetics of antimicrobials.
11. The monitoring of aminoglycoside serum concentrations and the use of extended-interval doses can help to maximize the probability of therapeutic success and minimize the probability of aminoglycoside-related toxicity.
12. Vancomycin and aminoglycoside serum concentration monitoring should be routinely done to ensure adequate serum concentrations, minimize toxicity, and avoid the potential for resistance.
13. Antimicrobial pharmacodynamics have become a crucial consideration for the selection of both empirical and pathogen-directed therapy in the current era of antimicrobial resistance.
14. Optimization of antimicrobial pharmacodynamic parameters such as the ratio of the peak serum concentration to minimum inhibitory concentration (MIC) or the time that the serum concentration remains above the MIC and area above the curve over MIC can improve infection treatment outcomes.

Selection of an appropriate antimicrobial therapeutic regimen for a given infectious disease requires knowledge of the infecting pathogen, host characteristics, and the drug’s expected activity against the pathogen. The most fundamental aspect of therapy starts with an appropriate diagnosis. A vast array of laboratory tests including rapid diagnostic technology is available to assist in verifying the presence of infection and for monitoring the response to therapy. Although useful, these tests are subject to interpretation and cannot be substituted for sound clinical judgment. Organism susceptibility to the administered antimicrobials is key to determining the outcome from therapy. Host characteristics, however, such as immune status, infection site location, and body organ function, play a significant role in selecting the most appropriate antimicrobial for a given individual. This chapter reviews the routine laboratory tests that are used to assist in the diagnosis and treatment of infection.

In order to optimize antimicrobial treatment outcomes for patients with infectious diseases, the clinician should always attempt to determine the infecting pathogen(s), consider host characteristics, and select an antimicrobial drug with the best expected activity against the pathogen(s). A vast array of laboratory tests are available to assist the clinician with this crucial but difficult clinical activity. Although rigorous standardization of these tests is desirable, many of the tests may be difficult to interpret correctly and therefore, often they should be considered complementary to sound clinical judgment. This chapter reviews the wide array of laboratory tests that are routinely used by the clinician for the diagnosis and treatment of infection.

LABORATORY TESTS CONFIRMING THE PRESENCE OF INFECTION

Non-specific Tests

Many tests can be conducted to determine whether a patient has an infection. Often, no single test can prove that a patient is infected, but when used in combination with other tests and clinical findings,
the clinician can reliably make a definitive diagnosis of infection. Because many tests are nonspecific, there are factors other than infection that can cause a test to be reported as positive when no infection exists. Therefore, the importance of careful interpretation and sound clinical judgment cannot be overemphasized.

**White Blood Cell Count and Differential**

Understanding the role of the white blood cell (WBC) in fighting infection is important in the diagnosis of infection, the selection of drug therapy, and the monitoring of patient progress. The major role of the WBC is to defend the body against invading organisms such as bacteria, viruses, and fungi. The typical normal range of the WBC is 4,500 to 11,000 cells/mm$^3$ (4.5 × 10$^3$ to 11 × 10$^3$/L). This range will vary between laboratories and patients, as it is dependent on patient age, gender, comorbidity status (WBC, especially neutrophils, increase naturally during pregnancy). WBCs usually are elevated in response to infection, but many other noninfectious conditions can increase the WBC, including stress, inflammatory conditions such as rheumatoid arthritis, and leukemia or in response to certain drugs (e.g., corticosteroids).

WBCs are divided into two groups: the granulocytes, which have prominent cytoplasmic granules, and the agranulocytes, which lack granules. Polymorphonuclear (PMN) granulocytes are made up of neutrophils, basophils, and eosinophils. The two other classes of WBCs are the monocytes and lymphocytes. Neutrophils are the most common type of WBC in the blood, comprising approximately 70% of the total WBC count. In response to infection, they leave the bloodstream and enter the tissue to interact with and phagocytize offending pathogens. Mature neutrophils sometimes are referred to as segs because of their segmented nucleus, which usually consists of two to five lobes. Immature neutrophils lack this segmented feature and are referred to as bands. During an acute infection, immature neutrophils, such as bands (single-lobed nucleus), are released from the bone marrow into the bloodstream at an increased rate, and the percentage of bands (usually 5%) can increase in relationship to mature cells. The change in the ratio of mature to immature cells is often referred to as a “shift to the left” because of the way the cells were counted by hand with a microscope and charted from immature to mature cells.

Leukocytosis, an increase in WBCs, is a normal host response to infection. Unfortunately, bacterial infection is a common complication of neutropenia from cancer chemotherapy. Neutropenia occurs when the bone marrow does not produce enough WBCs to fight infection. Patients who are neutropenic are incapable of increasing their WBCs in response to infection. In fact, susceptibility to infection in these patients is highly dependent on their WBC status. Patients with absolute neutrophil counts of less than 500 cells/mm$^3$ (0.5 × 10$^3$/L) are at high risk for the development of bacterial or fungal infections. The absence of leukocytosis also frequently can occur in the elderly and in severe cases of sepsis.$^2,3$

Lymphocytes comprise 15% to 40% of all WBCs and are of central importance to the immune system. Two functional types of lymphocytes are the T cell, which is involved in cell-mediated immunity, and the B cell, which produces antibodies involved in humoral immunity. Lymphocytosis is frequently associated with acute viral infections such as Epstein–Barr virus infection (mononucleosis) and Cytomegalovirus (CMV) infection and rarely with unusual bacterial infections (i.e., *Brucella* species infections).

T lymphocytes are characterized on the basis of function (i.e., T-helper cells, T$_{H1}$ and T$_{H2}$) and on the basis of surface protein. Most type 1 and type 2 T cells carry a T4 (CD4) marker that recognizes class II major histocompatibility complex (MHC) antigens, and most cytotoxic T cells carry a T8 (CD8) marker that recognizes class I MHC antigens. A severe deficiency of CD4 cells is associated with human immunodeficiency virus (HIV) infection and opportunistic infections. Malignancies also can adversely affect cellular immunity. Patients with Hodgkin’s disease and other types of lymphoma exhibit defective cell-mediated immunity that predisposes them to a variety of infections, notably fungal diseases and infections by the Listeria species. Drug treatment with cytotoxic chemotherapy and corticosteroids also can have profound deleterious effects on cell-mediated immunity. Defects in cell-mediated immune function can be demonstrated by a variety of simple laboratory tests, including quantification of lymphocytes on a routine complete blood cell count and skin testing for anergy. A more detailed investigation includes quantitative measurements of CD4$^+$ and CD8$^+$ cells. Monocytosis is correlated less frequently with acute bacterial infection, although its presence has been associated with the response of certain infections (e.g., tuberculosis) to chemotherapy. Eosinophilia can result from parasitic infection. eFigure 25-1 describes a number of cell types and their biologic function.

**Other Tests**

Some nonspecific laboratory tests are useful to support the diagnosis of infection. The inflammatory process initiated by an infection sets up a complex host response that includes. Activation of the nuclear factor-$\kappa_B$ (NF-$\kappa_B$) transcription factors plays an important role in the regulation of the immune system. NF-$\kappa_B$ is activated by bacterial and viral antigens, which eventually leads to the production of proinflammatory cytokines and chemokines. The rapid detection of activated NF-$\kappa_B$ can be measured by transcription

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**eFIGURE 25-1** Various cell types and their biologic functions.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cellular function</th>
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<tbody>
<tr>
<td>Macrophage/monocyte</td>
<td>Antigen presenting cell</td>
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<tr>
<td></td>
<td>Surveillance of foreign antigens</td>
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<tr>
<td>Neutrophils</td>
<td>Defense against bacteria and fungus</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Defense against parasites</td>
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<td></td>
<td>Response against allergic reactions</td>
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<tr>
<td>Basophil</td>
<td>Allergic response</td>
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<td>B lymphocyte</td>
<td>Antibody production</td>
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<td></td>
<td>Antigen presenting cell</td>
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<tr>
<td>T lymphocytes</td>
<td>Cellular immunity against virus and tumors</td>
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<td>Regulation of the immune system</td>
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factor enzyme-linked immunoassay (TF-ELISA) during a systemic inflammatory response syndrome (SIRS) and is considered to be crucial for the treatment of patients with septicemia. Acute-phase reactants, such as the erythrocyte sedimentation rate (ESR) and the C-reactive protein concentration, are elevated in the presence of an inflammatory process but do not confirm the presence of infection because they are often elevated in noninfectious conditions, such as collagen-vascular diseases and arthritis. However, large elevations in ESR are associated with infections such as endocarditis, osteomyelitis, and intraabdominal infections.7,8

Procalcitonin (PCT) is another acute-phase reactant that is released in response to various cytokines. PCT appears to be a more specific marker for bacterial infections than either C-reactive protein (CRP) or ESR. Controlled clinical trials have show that it can be a valuable tool for the clinician to help assess mortality risks of patients with infections and also help to determine when to initiate antibacterial therapy in respiratory tract infections.9

Changes in endothelial membranes and the presence of a foreign pathogen and its endotoxins cause inflammatory cytokines, such as interleukin 1 (IL-1), IL-6, and IL-8 and tumor necrosis factor-α (TNF-α), to be produced by macrophages or lymphocytes. Fluctuations in cytokine levels occur during the course of an infection, which can be useful in staging and monitoring the response to therapy. Although abnormally high levels of TNF have been associated with a variety of noninfectious causes, spiked elevations in TNF are found in patients with serious infections, such as sepsis. Studies of the relationship of circulating mediators to patient outcome have determined the value of endotoxin and cytokine measurements in patients with sepsis. Although the combination of elevations in endotoxin and individual cytokines has correlated well with the mortality rate, measurement of IL-6 was by far the best individual cytokine that predicted patient outcome.7 Understanding the balance between these proinflammatory and antiinflammatory processes likely will lead to interventions that can have a direct impact on the outcome of patients with sepsis.10

**LABORATORY IDENTIFICATION OF PATHOGENS**

**Colonization Versus Infection**

Pathogens are organisms that are capable of damaging host tissues and that elicit specific host responses and symptoms that are consistent with an infectious process. These organisms are transferred from patient to patient, vector to patient (animals, insects, and so on), environment to patient (e.g., hospital settings) or are derived from the patient’s own flora. Conversely, the human body contains a vast variety of microorganisms that colonize body systems and make up the so-called normal flora. These organisms occur naturally in the tissues of the host and provide some benefits, including defense by occupying space, competing for essential nutrients, stimulating cross-protective antibodies, and suppressing the growth of potentially pathogenic bacteria and fungi (eTable 25-1).

Organisms that comprise the normal flora can become pathogenic when host defenses become impaired or if they are translocated to other body sites during trauma. The identification of an organism that is considered to be normal flora in a wound or otherwise sterile body cavity or fluid often becomes a dilemma for the clinician in deciding whether or not a patient is infected and whether or not the patient requires treatment. Such is the case with *Staphylococcus epidermidis* when it is identified in the blood of a hospitalized patient. *S. epidermidis* is considered normal skin flora and commonly colonizes IV catheters. In these conditions, identification of the organism must be taken in light of the patient circumstances (signs and symptoms, laboratory indices supporting infection) and the probability of the organism being responsible for the infection. Often the simple removal of the catheter can eliminate the organism from the bloodstream, thereby preventing misdiagnosis and unnecessary application of antimicrobials.11

**Direct Examination**

Direct examination of tissue samples or body fluids believed to be infected can provide simple, rapid information to the clinician. Microscopic examination of wet-mount specimen preparations can provide valuable information regarding potential pathogens. Applications of this procedure with or without staining preparations include direct examination of sputum, bronchial aspirates, scrapings of mucosal lesions, and urinary sediment. The Gram stain is one of the first identification tests run on a specimen brought to the laboratory. For this procedure, crystal violet is applied as the primary step in the procedure. Gram-negative cells are decolorized by the addition of alcohol, and they take in a red color when counterstained by safranin. Gram-positive cells are not decolorized by alcohol and retain the crystal violet color and appear purple. Gram staining in conjunction with microscopic examination can provide a presumptive diagnosis and some indication of the organism’s morphologic characteristics (gram-positive, gram-negative, gram-variable, bacillus, or cocci). This is extremely useful information for the selection of empirical antibiotic therapy.

Gram stains are performed routinely on cerebrospinal fluid (CSF) in cases of suspected meningitis, on urethral smears for venereal diseases, and on abscess or effusion specimens. They are helpful in identifying organisms that may not grow on culture and which otherwise would be missed. Although Gram stains of sputum are performed routinely when respiratory tract infections are suspected, there is controversy regarding the usefulness of this test because the sputum is often contaminated with mixed or normal flora. The predominance of one particular organism, the overall number of organisms present, the amount of PMN granulocyte present, and the presence or absence of a significant amount of squamous epithelial
cells (<10 per low-power field) can improve the significance of the sputum Gram stain specimen. **eFigure 25-2** lists some common infecting pathogens grouped according to Gram stain and other characteristics.

Other staining techniques are used to identify pathogens such as those that are best identified microscopically because of their poor growth characteristics in the laboratory setting. The best examples of these are the Ziehl–Neelsen stain for acid-fast bacilli, which is used for the identification of mycobacteria species, and the India ink, potassium hydroxide (KOH), and Giemsa stains, which are useful for detecting certain fungi.12

**Cultures**

Isolation of the etiologic agent by culture is the most definitive method available for the diagnosis and eventual treatment of infection. Although suspicion of a specific pathogen or group of pathogens is helpful to the laboratory for the selection of a specific cultivating medium, the more common procedure for the laboratory is to screen for the presence of any potential pathogen. After receipt of a clinical specimen, the laboratory will inoculate the specimen in a variety of artificial media. Some culture media are designed to differentiate various organisms on the basis of biochemical characteristics or to select specific organisms on the basis of resistance to certain antimicrobials. Other media are employed commonly for the isolation of more fastidious organisms, such as *Listeria, Legionella, Mycobacterium,* or *Chlamydia.* Cultures for viruses are more difficult to perform and are undertaken primarily by larger institutions or outside laboratories because of the technical expense and time involved in processing samples.

When a culture is obtained, careful attention must be paid to ensuring that specimens are collected and transported appropriately to the laboratory. Every effort should be made to avoid contamination with normal flora and to ensure that the specimen is placed in the appropriate transport medium. Culture specimens should be transported to the laboratory as soon as possible because organisms can perish from prolonged exposure to air or drying. This is especially important for swab specimen preparations. Transport media may not be ideal for all organisms. Specimens that contain fastidious organisms or anaerobes require special transport media.
and should be forwarded immediately to the laboratory for processing. Finally, the source of the specimen should be clearly recorded and forwarded along with the culture to the laboratory. This process will aid the laboratory in differentiating true pathogens from the expected normal flora, and it will help in the selection of the appropriate culture media. Detection of microorganisms in the bloodstream by standard culturing techniques is difficult because of the inherently low yield of organisms diluted by blood, hemolysis, or other factors with bacteriolytic activity, and the potential of antimicrobial pre-treatment affecting organism growth. Most blood collection bottles dilute the blood specimen 1:10 with growth medium to neutralize the bacteriolytic properties of blood and antimicrobials. The addition of a polyanionic anticoagulant abolishes the effect of complement and antiphagocytic activity in the specimen. Some laboratories also add β-lactamase to their blood collection bottles to inactivate antibiotics such as penicillin or cephalosporins.

The initial identity of the organism can be determined by a variety of testing procedures. General schemes differentiate organisms into primary groups, such as gram-positive and gram-negative bacteria. This can be accomplished by simple Gram staining, as described previously, by evaluating organism growth patterns on selective media, and by testing for the presence or absence of specific enzymes and chemical characteristics, such as hemolysis and fermentation properties. For example, non–lactose-fermenting gram-negative bacilli that are oxidase-positive can suggest Pseudomonas aeruginosa as opposed to a variety of other potential gram-negative organisms. This preliminary information, which is readily obtainable from the laboratory, can greatly assist the clinician in choosing the appropriate empirical therapy. Definitive identification of organisms requires more complex testing procedures and devices that can further differentiate the organism on the basis of specific fermentation and biochemical reactive properties.

A method that provides a positive microbiological sample in a few hours, as opposed to days (culture method), is the use of automated culturing systems. One commonly used system is the BACTEC (Becton Dickinson Diagnostic Instruments, Sparks, MD) system, which uses bottles of growth medium containing a fluorescent sensor that monitors culture bottles every 10 minutes for the presence of carbon dioxide (CO2) as a by-product of microorganism growth. Computers monitoring the system alert laboratory personnel of positive culture results by both audible and visual alarms. Once detected, a battery of testing can be performed rapidly that shortens the reporting time and that enables clinicians to obtain preliminary information about the organism. Commercially available automated systems can inoculate the test organism into a series of panels containing a variety of test media, sugars, and other reagents. The system can then photometrically determine the results and compare the findings to a library of organism characteristics to produce a definitive identification.

Viral agents can be detected by direct observation of inoculated culture cells for cytopathic effects or by detection of antigens after incubation by immunofluorescent methods. The culture method is most useful for organisms such as CMV or herpes simplex virus because these viral agents are rapidly propagated in culture cells, making them easily detected.

**Rapid Diagnostic Technologies**

There has been a recent explosion of FDA-approved rapid diagnostic testing methodologies for infectious diseases. In the current era of managed care and antimicrobial stewardship programs the need for rapid diagnostic tests (RDTs) is critical. In addition, RDT is further highlighted by the emergence of multidrug resistant bacteria, and increased pathogenic virulence (i.e., toxin production). A major focus of RDT is on pathogens associated with increased morbidity and mortality, which include influenza virus, methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus spp. (VRE), Clostridium difficile, extended-spectrum β-lactamase (ESBL)-producing Klebsiella spp., and Mycobacterium tuberculosis.

The benefit of rapid diagnostic technology is to quickly identify and/or rule out infectious pathogens, streamline antimicrobial therapy, and improve infection control measures such as isolation. Utilization of rapid diagnostic testing significantly reduces the time required to identifying the infecting pathogen, thus improving clinician’s ability to more rapidly diagnose and treat infections. Pathogen RDT also prompts de-escalation of antibiotic therapy and if the test is negative, discontinuation of therapy which decreases the potential for antimicrobial resistance.

The process of RDT often requires the evaluation of a clinical specimen such as blood (i.e., serum or plasma), stool, or bodily fluids (i.e., as saliva, urine). These specimens are processed in a qualitative or quantitative manner, to provide a result that available within 15 minutes to a few hours (depending on the technology). This is in contrast to traditional culture methodologies discussed above which may take 4 to 6 days (i.e., for Staphylococcus in the blood) or up to 6 weeks (i.e., mycobacterium).

The most current RDT involves genomic testing methodologies, which include immunologic, molecular technologies, and mass spectrometry.

**DIAGNOSIS OF INFECTION USING IMMUNOLOGIC ASSAYS**

1. The use of immunologic methods for the diagnosis and monitoring of human host immune response (i.e., antibody and antigen detection) to infection has become an indispensable laboratory tool. The primary immunologic methods involve the detection and quantification of antibodies directed against a specific pathogen or its components. These methods have the advantage of a rapid turnaround time and an acceptable level of sensitivity and specificity. Some rapid antigen detection tests (e.g., identification of group A streptococci and the Rapid Influenza diagnostic tests) are simple to use, can be performed conveniently in the physician’s office, and can often be used to decide whether antimicrobials should be administered for a suspected infection. Limitations with these tests exist as antigens will still exist even if the pathogen is not longer alive, hence allowing for a false-positive test. In addition, the positive test result indicating the presence of the pathogen does not assist in determining if the patient was infected or simply colonized with the pathogen.

Antibody or antigen detection can be accomplished by a variety of techniques, including immunofluorescence, which has been used routinely for the detection of CMV, respiratory syncytial virus, varicella-zoster virus, Treponema pallidum (syphilis), Borrelia burgdorferi (Lyme disease), and Chlamydia trachomatis. Latex agglutination is useful for detecting meningococcal capsular antigens in CSF of patients suspected of having bacterial meningitis and as an aid in the diagnosis of Legionella pneumophila. Enzyme-linked immunosorbent assay (ELISA) is a commonly employed method for detecting HIV, herpes simplex virus, respiratory syncytial virus, pneumococcal serum antibody, Neisseria gonorrhoeae, and Haemophilus pylori.

**Molecular Techniques for the Detection of Microorganisms**

**Hybridization DNA Probes**

1. The traditional and more labor intensive means for gene detection involves the use of separation of the organism DNA into specific
fragments (gel electrophoresis), transfer and fixation of the mixture to specialized paper or nylon membranes (Southern or Northern blotting), the mixing of the DNA fragments with the labeled probe (hybridization), and transfer to radiographic or photographic film for processing. These techniques have been used for many years and are fairly standardized methods for the detection of a variety of organisms. However, with increased technology, highly sensitive and specific molecular methods are commonly being used for a more rapid detection and identification of a variety of microorganisms.

The use of hybridization probes is particularly helpful for the detection of pathogenic bacteria, and for slow-growing organisms such as Mycobacterium tuberculosis, N. gonorrhoeae, and certain species of fungi such as Candida species.

One of the most widely used FDA-approved hybridization probe technology is the peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) assay. This technology uses fluorescent-labeled probes (peptide nucleic acid molecules) to target ribosomal RNA sequences. These rRNA sequences are specific to different species of microorganisms.

Peptide nucleic acid molecules contain the same nucleotide bases found in DNA, but they are noncharged, which allows for more specific hybridization to target nucleic acids in ribosomal RNA of bacteria and yeast. With this technology, samples are taken from positive blood culture vials after a Gram stain is performed, and results can be obtained within 90 minutes as opposed to the 1 to 5 days it can take for traditional laboratory methods. When viewed under a fluorescence microscope, different colors make it easy to decipher results in regard to microorganism speciation.

Currently, FDA-approved PNA-FISH assays are available for S. aureus, coagulase-negative staphylococci, E. coli, P. aeruginosa, E. faecalis, E. faecium, C. albicans, C. glabrata, C. tropicalis, and C. parapsilosis. Limitations of the PNA-FISH procedure include its sole application to positive blood cultures as well as the requirement of isolation on solid media in order to differentiate organisms.

The principle of these probe-amplification schemes is to boost the probe’s signal-emitting molecule to make it more easily detected. A more advanced signal-amplification system available is the branched DNA (bDNA) probe system (Chiron Corp., Emeryville, CA) that is often used to identify retroviruses such as HIV. This system uses multiple probes and multiple signal-emitting molecules (reporters). The target-binding probe contains two hybridization regions. One region is complementary to the target, and the other region is capable of binding with the bDNA amplification multimer. Branched DNA probe systems are being developed for rapid detection of hepatitis B and C, HIV-1, and CMV. Because of the system’s high specificity and quantitative ability, bDNA probe assays can be useful for therapeutic monitoring, such as in the case of monitoring the response to antiretroviral therapy in acquired immunodeficiency syndrome (AIDS).

**Nucleic Acid Amplification Methods**

Nucleic acid amplification methods are now considered a standard laboratory tool. They have had a tremendous impact on the diagnosis and treatment of infectious diseases. These highly sensitive methods have the capability to detect and quantitate minute amounts of target nucleic acid in a rapid manner. The polymerase chain reaction (PCR) is based on the capability of a DNA polymerase to copy and elongate a targeted strand of DNA. Each cycle doubles the amount of DNA originally present at the start of the cycle, thereby exponentially increasing the overall number of DNA copies. In theory, more than 1 million copies of the original DNA can be generated from as few as 20 cycles. Although this amplification technique is very sensitive and has tremendous application potential, it is not without problems. The powerful amplification procedure can yield false-positive results when samples are contaminated by nucleic acid left over from previously amplified DNA or by dead pathogens that exist in the sample.

Several modifications to the original PCR technology have been made over the years to improve the sensitivity and application potential for PCR, including the use of multiple sets of amplification primers, multiplex PCR, PCR amplification of RNA by converting targeted RNA with reverse transcriptase to complementary DNA templates, and real-time quantitative PCR. The cost–benefit ratio of PCR as compared with traditional microbiologic methods must be evaluated.

Molecular amplification schemes such as PCR have become routine in situations in which rapid turnaround time is essential to improve patient diagnosis and outcome, for example, real-time universal screening for acute HIV infection and routine testing and monitoring of patients receiving treatment for HIV infection, and the isolation and detection of fastidious or slow-growing organisms such as M. tuberculosis, B. burgdorferi, and Helicobacter pylori.

Rapid polymerase chain reaction (rPCR) technology has been used with increasing excitement. rPCR is a multistep process that involves the amplification of specific DNA sequences for rapid and specific detection of microorganisms. Contrasted to conventional PCR, both steps of rPCR amplification and detection occur within the same closed system. As a result, rapid PCR methods are less labor-intensive, yield a faster turnaround time, and reduce the likelihood of contamination. Other benefits to this technology include the ability to detect slow-growing or nonfastidious organisms as well as being limited to only a positive blood culture as the source of specimen.

Probe-based methods require the extraction of DNA or RNA from a clinical specimen (i.e., body fluid, tissue, or WBC) or directly from a microorganism culture. The extract is then tested for the presence of pathogen DNA or RNA using a probe that contains a specific oligonucleic acid-based sequence for the organism. For example, a probe with a sequence of ACTGTT would bind to the complementary organism nucleic acid sequence of TGACAA. Because the probe is labeled with a signal-emitting molecule (i.e., radiolabeled, colorimetric, or chemoluminescent), a match would be detected.

**Mass Spectrometry to Detect Microorganisms**

Mass spectrometry may be used to identify microorganisms and create a rapid technology for their identification. However, the technologies to create small economical models had yet to be employed. Today, a promising technology, the matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry is under evaluation to identify selected preprogrammed bacteria in clinical situations. This technology measures molecular masses of proteins and other bacteria components using a low bacterial load (approximately 1,000 colony forming unit per mL [10^6 CFU/L]). At time of writing this chapter, the FDA has not approved this technology for use in clinical laboratories.

**EVALUATION OF ANTIMICROBIAL ACTIVITY AND DETERMINATION OF ANTIMICROBIAL PHARMACODYNAMICS**

The laboratory evaluation of antimicrobial activity is an important component of the pharmacotherapeutic management of infectious diseases. The integration of this activity with various pharmacokinetic properties of the antimicrobial agent determines
Most antimicrobial susceptibility testing methods that are used in the clinical laboratory are well characterized and have been standardized by the Clinical and Laboratory Standards Institute (CLSI). However, controversies exist about which test methods provide the most useful information, how to best report these results to clinicians, and how to apply them to the treatment of patients. CLSI provides guidelines regarding MIC testing including the acceptable range for MIC results to a given pathogen. Nevertheless, there are many investigations that show that the general antimicrobial susceptibility or resistance profile of an infecting organism correlates with clinical and/or microbiologic responses to therapy.

Most of the standardized and well-accepted test methods evaluate the susceptibility of aerobic, nonfastidious bacteria. However, substantial progress has been made to develop sensitive, specific, reproducible, and clinically useful susceptibility tests for anaerobic bacteria, yeasts, mycobacteria, and viruses. Continued advances in technology should further improve test methods and the rapidity with which the results can be applied to the management of patients. Although these newer systems are often expensive, the increased quality and decreased overall costs of patient care can determine their cost-effectiveness.

QUANTITATIVE ANTIMICROBIAL SUSCEPTIBILITY TESTING

Minimum Inhibitory Concentrations

The minimum inhibitory concentration (MIC) is defined as the lowest antimicrobial concentration that prevents visible growth of an organism after approximately 24 hours of incubation in a specified growth medium. The MIC quantitatively determines in vitro antibacterial activity. Classically, MICs were determined through the macrotube dilution method, which uses liquid growth medium (broth), doubling serial dilutions of antimicrobials in test tubes, and a standard inoculum of bacteria (approximately $10^8$ colony-forming units [CFU/mL]). The tubes (up to 10 mL) were incubated at approximately 35 to 37°C (95 to 98.6°F) for 18 to 24 hours and then examined for visible bacterial growth (eFig. 25-3).

The macrodilution MIC testing is laborious and supply intensive, it is not used often in the contemporary clinical microbiology laboratory. However, one advantage of the macrodilution method is that it tests a large inoculum of bacteria—a factor that can improve the detection of small numbers of resistant subpopulations or document the presence of inducible resistance.

The use of 96-well microtiter plates substantially reduces the amount of growth medium and preparation time needed for broth-dilution MIC testing in the clinical laboratory. Volumes of 100 to 200 microliters ($\mu$L) or less of medium are used, and multichannel pipets and/or automated systems allow efficient preparation of numerous tests (eFig. 25-4). The microdilution MIC test method is currently the most commonly used and automated susceptibility test method in the clinical microbiology laboratory. Although microdilution MIC testing is a vast improvement over macrodilution MIC testing, it still has important shortcomings. These include both limitations in the numbers and various types of antimicrobials to use in the test (especially with premade or premanufactured trays) and a limited ability to detect some forms of antimicrobial resistance (e.g., $\beta$-lactamases in gram-negative bacteria).

Limitations and Problems with MIC Testing

Some of the limitations and problems of MIC testing are academic in nature, whereas others can have important implications for
the management of patients with serious infections. For example, because the MIC only represents the concentration of antimicrobial that is needed to inhibit visual growth of the most resistant cells within the tested bacterial population, there can be a small percentage of bacteria present within the large numbers at the site of infection that are more antimicrobial-resistant than the MIC would indicate. Therefore, the bacterial density used to perform the susceptibility testing (e.g., 10^5 CFU/mL [10^8 CFU/L]) may over represent the antimicrobial’s activity at the site of infection. Use of the antimicrobial then could select these more resistant subpopulations, resulting in poor clinical response. This phenomenon can be observed with intermediate vancomycin resistance in S. aureus, as well as in strains of gram-negative bacteria such as the Enterobacteriaceae species that produce both plasmid-borne and chromosomal β-lactamases.22

Many other factors also can influence the in vitro MIC value obtained and its subsequent application to the in vivo situation. The bacterial growth medium used and cation content can affect the activity of many drugs significantly. For example, aminoglycosides are more active against P. aeruginosa in a medium supplemented with physiologic concentrations of magnesium and calcium (CLSI standardized method) than in a medium without these cations. MIC values of antibiotics that are highly bound to plasma proteins are significantly higher when the test medium contains human serum. As testing of these drugs in a serum-supplemented medium has not gained widespread acceptance, their in vivo activity can be overestimated by in vitro MIC test results. Fortunately, the standardized guidelines for testing and quality assurance procedures proposed by the CLSI attempt to minimize the impact of these problems and are followed by most clinical and research laboratories.23 However, when a patient infected with an apparently susceptible organism fails therapy, it is important for the clinician to consider these potential confounding factors as possibly being related to the observed failure. In such situations, consideration of antimicrobial pharmacokinetics and pharmacodynamics also often can help to better predict therapeutic response as compared with organism susceptibility alone.

Clinical Controversy...

Some clinicians believe that the MICs for all antimicrobials for which susceptibility testing was performed should be reported to allow for the most appropriate antimicrobial selection for the patient. However, others believe that only selective antimicrobial susceptibility should be reported to avoid overprescribing of more costly broad-spectrum antimicrobials.

QUALITATIVE ANTIMICROBIAL SUSCEPTIBILITY TEST METHODS

Disk Diffusion Assay

The disk diffusion assay method for susceptibility testing (Kirby–Bauer method) was developed in the 1960s by Bauer and coworkers as a way to reduce the labor needed for tube dilution susceptibility testing.21 It still is used in many clinical microbiology laboratories owing to its high degree of standardization, reliability, flexibility, low cost, and simplicity of test interpretation. Up to 12 user-selected antibiotic-impregnated paper disks are placed on an agar plate previously streaked with a standard suspension of bacteria (1 to 2 × 10^6 CFU/mL [1 to 2 × 10^4 CFU/L]). The drug contained in the disk diffuses in a concentration gradient out into the agar. The plate is incubated (18 to 24 hours at 35°C), and visual bacterial growth occurs only in areas in which the drug concentrations are below those required for growth inhibition. The diameters of the zones of inhibition are measured via calipers or automated scanners and are compared with standard zone size ranges that determine susceptibility, intermediate susceptibility, or resistance to the antimicrobials that were tested (eFig. 25-5). Although factors such as agar composition, incubation temperature, bacterial inoculum, and antibiotic paper disk composition can influence results, the standards for testing conditions and interpretive zone sizes are well defined by the CLSI.

Qualitative Versus Quantitative Susceptibility Testing of Microorganisms

Quantitative MIC data often are reported to the clinician qualitatively by deeming an organism “susceptible” reported as (“S”), “intermediate or indeterminate” reported as (“I”), or “resistant” reported as (“R”) to a given antimicrobial agent. Many factors are considered to determine these qualitative susceptibility classifications (also referred to as “breakpoints” for the antibiotic). These include pharmacokinetic properties, the distribution of MICs for the organisms, and the clinical and bacteriologic responses observed for the antimicrobial against strains of bacteria with various MIC values. This simplification makes the susceptibility data easily interpretable by noninfectious disease clinicians. Pathogens classified as susceptible to an antibiotic are those with the lowest MICs, and they are the most likely to be eradicated during therapy of infections using typical drug doses. Conversely, resistant organisms are bacteria with significantly higher MICs that, when treated with the antimicrobial, will result in a less-than-optimal clinical response, even at the highest doses. The indeterminate classification exists when the number of strains with MICs in the given range is too small to derive robust conclusions on susceptibility or resistance to the antimicrobial. Responses to therapy for organisms that are moderately susceptible/intermediately susceptible/indeterminate can be variable. These organisms can respond to treatment with maximal doses of the antimicrobial or can respond when the drug is known to be concentrated at the site of infection (e.g., urinary tract infections treated by drugs excreted by the kidneys).
There are concerns that the “user friendly” susceptible/resistant classification system can oversimplify the decision-making process for treating infections. For example, a critically ill patient may not respond to the antimicrobial therapy of a susceptible organism at the usual doses. If serum concentrations or concentrations at the site of infection could be assayed (not practically done), one might discover suboptimal concentrations as a result of inadequate tissue perfusion. Likewise, a patient with severe vascular insufficiency and a diabetic foot infection may fail a course of therapy with normal doses of an antimicrobial and a susceptible organism because of inadequate drug delivery. Additionally, some investigators have shown that different outcomes can be achieved for “susceptible” organisms with different MIC values and also that substantial (although not clinically acceptable) clinical and/or microbiologic cure rates can occur for infections that are caused by resistant organisms. These reports emphasize that in vitro susceptibility does not correlate unequivocally with clinical success and that resistant organisms do not always equate with impending clinical failure.

Similarities in the spectrum of activity for classes of antibiotics have led to the concept of class testing. Thus cephalothin susceptibility results are extrapolated to other first-generation cephalosporins, such as cephalexin or cefazolin. Likewise, susceptibility to an antibiotic that typically has minimal activity usually ensures that other more potent agents in its class will have activity as well. However, many gram-negative organisms have now developed ESBLs that often have different activity against members of the same drug class. These developments significantly limit the utility of class testing to reduce susceptibility testing workload.

### OTHER SUSCEPTIBILITY TESTS

#### Epsilometer Test

The Epsilometer test (Etest; AB bioMérieux, Nouvelle, France) combines the benefits of quantitative MIC test methods with the ease of agar diffusion testing. The Etest is a plastic strip impregnated with a known, prefixed concentration gradient of antibiotic that is placed on an agar plate streaked with a suspension of known bacterial inoculum. The drug instantly diffuses from the plastic strip to form an effective concentration gradient within the agar. After overnight incubation, elliptical zones of inhibition are formed; the point where the bottom of the ellipse crosses the plastic strip is correlated with an MIC value printed on the strip (Fig. 25-6). A similar product (M.I.C. Evaluator; Oxoid Limited, Hampshire, U.K.) is also available. Many investigators have analyzed the Etest’s correlation with standard susceptibility data. They can produce chartable patient data reports, software that allow for the storage and rapid retrieval of historical information, and data management systems to determine antibiotic resistance/susceptibility. Output of data is via computer or video display.

#### AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY TESTING

Various degrees of automation have been applied to susceptibility testing. Early advances included automated preparation of microtiter trays, instrument-assisted readers, and computer-assisted result databases. Rapid automated susceptibility tests became available in the 1980s, and their use has increased substantially in the three subsequent decades. These systems often incorporate microprocessors, robotics, and microcomputers to rapidly identify organisms and produce susceptibility test results in as few as 3 hours.

There are three rapid automated susceptibility test systems in common use in clinical microbiology laboratories. The Vitek system (bioMerieux, Durham, NC) uses small plastic reagent “cards” that contain 64 microwells for the testing of various antimicrobials or indicator chemicals. Bacterial test suspensions enter the wells by capillary diffusion, and growth is monitored automatically via photometric assessment of turbidity every hour for up to 15 hours. When the growth control reaches a specified turbidity level, growth curves for all wells are calculated and compared with the growth control curve for slope normalization. Computerized linear regression and the use of best-fit line coefficients produce an algorithm-derived MIC. The clinical laboratory can control the output that is generated (qualitative susceptibility, quantitative susceptibility, or both).

The Microscan WalkAway system (Siemens Healthcare Diagnostics, Deerfield, IL) is a rapid test system that uses fluorogenic substrate hydrolysis as an indicator of bacterial growth. This system uses standard microdilution test trays and a computer-controlled incubator and reader unit that can perform robotic manipulations, such as reagent addition and tray rotation, to allow for spectrophotometric or fluorometric growth assessments. As with the Vitek system, growth curves are generated, and algorithms applied for the determination of MICs; output is via computer or video display.

The final system is the BD Phoenix Automated Microbiology System (BD Diagnostics, Sparks, MD). This system utilizes an oxidation–reduction detector and a turbidometric growth detection system to determine antibiotic resistance/susceptibility. Output of data is similar to the other two automated systems previously described.

The results obtained from all of these three systems generally are comparable. However, there have been documented differences in the ability of these systems to accurately detect emerging resistance mechanisms such as vancomycin resistance in Staphylococci and carbapenem resistance in gram-negative pathogens. Importantly, all of the systems contain information management software that allow for the storage and rapid retrieval of historical susceptibility data. They can produce chartable patient data reports,
ADVANCES IN SUSCEPTIBILITY TESTING FOR MYCOBACTERIA, FUNGI, AND VIRUSES

Impressive advances have been made in the last decade in the areas of mycobacterial, fungal, and viral susceptibility testing. The use of radiometric techniques, such as the BACTEC TB460 system (Becton Dickinson Biosciences, Sparks, MD), has revolutionized the analysis of antimicrobial susceptibility for M. tuberculosis and other slow-growing mycobacteria. Radiometric susceptibility testing involves the incubation of M. tuberculosis in liquid medium containing carbon-14 (14C)-labeled growth substrate. As organisms grow, respiration causes the release of 14C, which is then detected. The growth indices for antimicrobial-containing bottles are compared with those of a control bottle with the calculation of an MIC. Use of this method, when coupled with the rapid processing of samples, can reduce the time to susceptibility result generation to approximately 1 week. A newer mycobacterial susceptibility testing method (the BACTEC Mycobacteria Growth Indicator Tube [MGIT 960]; Becton Dickinson Diagnostic Instruments, Sparks, MD) that is fully automated and that employs detection of fluorescence related to growth also has been developed. It produces results in a similar time frame and with similar reliability as the radiometric method. Primary advantages of this system are its automation, the elimination of radioactivity, and the elimination of needle use. Although the slower agar proportion susceptibility method (generating results in approximately 1 month) is still considered the reference standard for mycobacterial susceptibility testing by the CLSI, the group now recommends the use of a rapid susceptibility testing method to ensure that the Centers for Disease Control and Prevention (CDC) guidelines for reporting susceptibility results for M. tuberculosis infections within 28 days of specimen receipt in the laboratory can be met. In the future, the use of molecular probes for mycobacterial resistance genes most likely will become a more important component of mycobacterial susceptibility determinations, especially in light of the increasing problems with antimicrobial resistance.

There has been a substantial increase in the prevalence of fungal infections in the last two decades and an increase in the development and use of antifungal agents has followed. Historically, antifungal susceptibility testing was imprecise and fraught with many inconsistencies. However, pioneering research in the last decade has resulted in the development of CLSI guidelines for the antifungal susceptibility testing methods of both yeasts and filamentous fungi (molds). Use of these techniques can result in greater than 90% inter- and intralaboratory reproducibility. Although routine antifungal testing of every isolate is not generally necessary for most clinical microbiology laboratories, periodic batch testing for the development of antibiograms and for the surveillance of resistance and/or antifungal testing of patients with such infections as cryptococcal meningitis or oropharyngeal candidiasis refractory to therapy are warranted.

DETECTION OF RESISTANCE FACTORS

There are a number of methods in use that directly detect the production of antimicrobial resistance in pathogens. β-Lactamase production can be detected rapidly and easily in the clinical laboratory with the use of nitrocefin disks. Nitrocefin is a chromogenic cephalosporin derivative that changes color on hydrolysis by β-lactamase. Colonies from a growing bacterial culture can be touched to a disk, with β-lactamase production noted within a few minutes. Although rapid and reliable, this method is limited to the assessment of strains of staphylococci, enterococci, H. influenzae, Moraxella catarrhalis, and N. gonorrhoeae. The nitrocefin disk cannot detect β-lactam resistance caused by altered penicillin-binding proteins or by some of the newer ESBLs.

PCR has now become a standard method to quantify the replication of the HIV and hepatitis viruses in infected patients (the viral load, described as copies per milliliter). Similar methods are used to determine the presence of genetic mutations in the HIV that are associated with increased resistance to one or more of the many antiretroviral medications available for clinical use. The use of these genotyping methods as an aid to select an optimized antiretroviral regimen has been correlated with an improved clinical response to therapy, as well as with a more potent reduction in the viral load.

The detection of methicillin resistance in Staphylococcus (MRSA) is crucial to ensure appropriate therapy. Methicillin resistance is the result of the mecA gene, which encodes for an altered penicillin-binding protein (penicillin-binding protein 2a) that has a low binding affinity for β-lactams. It is particularly difficult to detect this resistance, although, because of the heterogeneous expression of the phenotype—it is common for only 1 in 10^4–6 tested bacterial cells to express methicillin resistance (even though all cells may have the genetic ability to do so). Screening via oxacillin disks or by oxacillin-containing agar (6 mcg/mL [mg/L]) was once considered the gold standard for resistance detection prior to the development of PCR and DNA probes that were specific for mecA. The mecA PCR test is available for clinical use, is 99% sensitive and specific, and allows for the rapid (within 6 hours) determination of the presence of methicillin resistance. Although the mecA PCR test has been available for many years, many laboratories do not use it commonly because of its high cost relative to other screening methods with acceptable sensitivity/specificity. For example, the presence of MRSA in a nasal swab or a blood culture sample can now be determined directly and rapidly within 24 hours using chromogenic technology (CHROMagar MRSA). This technology uses chromogenic substrates and a cephalosporin; MRSA strains will grow in the presence of cephalosporins such as cefoxitin and will produce mauve-colored colonies resulting from hydrolysis of the chromogenic substrates. The sensitivity and specificity for this test is as high as 97% and 99%, respectively.

The detection of decreased vancomycin susceptibility in gram-positive organisms has become more important with the increased prevalence of both VRE and vancomycin-intermediate-resistant and vancomycin-resistant S. aureus (VISA and VRSA). The vancomycin agar screening method (Brain-Heart Infusion agar containing 6 mg/L of vancomycin) is an inexpensive and reliable way to detect vancomycin resistance. With this test, the growth of any colonies from a sample of the test organism (10^3 to 10^5 CFU) after 24 hours of incubation would indicate the presence of decreased vancomycin susceptibility (VISA) or vancomycin resistance (VRE, VRSA) within the test strain. This screening method appears to work well for VRE, but is less reliable for detecting VISA or VRSA strains when compared to the CLSI broth microdilution reference method. It is important to note that most of the MIC testing methods currently used in the clinical microbiology laboratory do appear to reliably detect these VISA and VRSA strains.

The detection of decreased vancomycin susceptibility in gram-positive organisms has become more important with the increased prevalence of both VRE and vancomycin-intermediate-resistant and vancomycin-resistant S. aureus (VISA and VRSA). It is important to note that most of the MIC testing methods currently used in the clinical microbiology laboratory do appear to reliably detect these VISA and VRSA strains. The vancomycin agar screening method (Brain-Heart Infusion agar containing 6 mg/L of vancomycin) is an inexpensive and reliable way to detect vancomycin resistance in Enterococci. With
this test, the growth of any colonies from a sample of the test organism (10^7 to 10^8 CFU) after 24 hours of incubation would indicate the presence of VRE. Although this screening method appears to work well for VRE, it appears to be less reliable for detecting VISA or VRSA strains when compared to the CLSI broth microdilution reference method. However, simply reducing the vancomycin concentration in the agar from 6 mg/L to 3–4 mg/L results in improved sensitivity and specificity (both >90%) for detecting these VISA strains.37

### SPECIAL IN VITRO TESTS OF ANTIMICROBIAL ACTIVITY

#### Minimum Bactericidal Concentration

In certain infections (e.g., gram-positive bacterial meningitis and endocarditis), the bactericidal (killing) activity may be more predictive of a favorable infection outcome than the MIC.38 The minimum bactericidal concentration (MBC) can be performed in conjunction with the broth microtiter MIC test by taking aliquots of broth from microtiter wells that demonstrate no visible growth and plating the samples onto antibiotic-free agar plates for subsequent incubation. The MBC is defined as the lowest concentration of drug that kills 99.9% of the total initially viable cells (representing a 3 log_{10} CFU/mL or greater reduction in the starting inoculum).

For certain antibiotic classes such as the aminoglycosides and the quinolones, the MIC often approximates the MBC. However, for β-lactam antibiotics and glycopeptides, the MBC can exceed the MIC substantially, resulting in an overestimation of in vivo bactericidal activity. When the MBC exceeds the MIC by 32-fold or more, an organism is said to be tolerant to the antimicrobial’s killing activity. Although the phenomenon of tolerance has been documented for β-lactams and glycopeptides against certain staphylococci, streptococci, and enterococci, its impact on the outcome of infections caused by organisms other than those just mentioned appears to be limited.

#### Timed-Kill Curve Tests

Timed-kill curve tests are not performed routinely in the clinical laboratory but can provide important additional data on the effects of an antimicrobial on bacteria. For timed-kill curve tests, a standard inoculum of bacteria (10^8 CFU/mL [10^9 CFU/L]) is placed in a test tube containing liquid growth medium with or without desired test concentrations of antimicrobial. Samples are removed periodically to determine the number of living cells at the given time points. The viable cell counts are plotted versus time to construct the timed-kill profile of the antimicrobial. The tested concentration of antimicrobial is considered to be bactericidal if it causes at least a 3 log_{10} CFU/mL reduction in viable inoculum. Comparisons of the relative rates of bacterial killing also can be performed in timed-kill curve experiments. Additionally, the presence of concentration-dependent killing activity (where killing increases with increasing drug concentrations above the MIC) versus concentration-independent killing activity can be determined from a timed-kill curve experiment. An example of results from a timed-kill curve experiment is depicted in eFigure 25-7. These data can help to predict the best way to administer an antimicrobial to maximize activity. For example, lower-dose, more frequent (or continuous) infusions would be preferable for concentration-independent antibiotics, while higher-dose intermittent administrations would maximize activity for concentration-dependent antibiotics.

#### Postantibiotic Effect

The postantibiotic effect (PAE) is defined as the persistent suppression of an organism’s growth after a brief exposure to an antibiotic.42 A PAE experiment is performed by exposing a fixed inoculum of organism to a set concentration of antibiotic (typically some multiple of the MIC) (eFig. 25-8). The antibiotic is then removed either by inactivation (e.g., inactivation by a β-lactamase or binding the antibiotic to a resin) or by filtration/centrifugation of the mixture. The cells are resuspended in antibiotic-free growth medium, and samples are removed frequently (every 0.5 to 2 hours) to determine resumption of normal growth. The PAE is quantified as the difference in time that it takes the organism exposed to the antibiotic to demonstrate a 10-fold increase in viable cells per milliliter as compared with a separate culture of organism not subjected to the antibiotic. A PAE equal to or greater than 1 hour has been demonstrated...
for most antibiotics against gram-positive bacteria (\(\beta\)-lactams, vancomycin, daptomycin, linezolid, and telavancin). As a general rule, antibiotics that inhibit DNA or protein synthesis (e.g., quinolones and aminoglycosides) demonstrate significant PAEs against gram-negative organisms. An exception to this rule are the carbapenem cell wall synthesis inhibitors (e.g., doripenem, ertapenem, imipenem, and meropenem), which demonstrate PAEs against selected strains of gram-negative organisms. The primary clinical application of the PAE is to allow for less frequent administration of antimicrobials while still maintaining adequate antibacterial activity (e.g., extended-interval aminoglycoside administration).39

**Antimicrobial Combination Effect Test**

Antimicrobial combination therapy is used frequently to treat serious infections. Combination therapy can be used prior to knowing the pathogen or antibiotic susceptibility for the treatment of infections in neutropenic patients and in patients with enterococcal endocarditis or bacteremia, sepsis, or pneumonia caused by *P. aeruginosa*. In these cases, it is important to know whether the combination will have beneficial (or detrimental) effects on the overall antibacterial activity of the regimen. For example, the combination can result in activity that is significantly greater than the sum of activity of either agent alone (i.e., synergy). Conversely, the combination can result in activity that is worse than either agent alone (i.e., antagonism). Combination activity that is neither synergistic nor antagonistic is said to be indifferent or additive.40

Two methods are used to determine the expected effects of combination antibiotic therapy. For the most part, both methods are not used commonly in the clinical microbiology laboratory owing to the substantial labor involved with these tests and the lack of strong correlation with clinical outcome in the majority of infections. The first method is the microtiter fractional inhibitory concentration (FIC, or “checkerboard” method). The FIC is performed in a similar manner to the microtiter broth MIC except that two antibiotics are tested in the same microtiter plate. Twofold serial dilutions of one antibiotic are made in one direction on the plate (e.g., from right to left), whereas dilutions of the second antibiotic are made from the other direction on the same plate (e.g., from top to bottom). This method produces all possible combinations of twofold concentrations for the two drugs being tested.

The second most common method to determine the effects of antibiotic combinations is an adaptation of timed-kill curve tests. Two antibiotics are added to the same test tube at fixed concentrations of the MIC for each drug, and killing is quantified. With this method, synergism is defined as a 100-fold decrease in viable organisms at 24 hours for the combination as compared with the most potent antibiotic tested alone. Antagonism is defined as a 100-fold or greater increase in viable organism count (efig. 25-9).41 It is important to note that although antagonism has been demonstrated for several combinations in vitro (e.g., penicillin plus tetracycline, chloramphenicol and an aminoglycoside, fluoroquinolones and rifampin), antagonism in vivo has been demonstrated only infrequently.

Although the methods for testing the effects of antimicrobial combinations are well described, the results from these tests have not been adequately studied in the context of many infection outcomes. There is little debate that the combination of a \(\beta\)-lactam antibiotic and an aminoglycoside is required for successful treatment of enterococcal endocarditis. For enterococci, susceptibility to high concentrations of aminoglycosides (e.g., gentamicin, 500 mg/mL [g/L]) is evaluated in the clinical laboratory because it correlates closely with synergy when the drug is combined with \(\beta\)-lactam antibiotics.

The concept of combination therapy is not universally accepted for the treatment of other infections. There is ongoing debate as to whether the combination of a broad-spectrum \(\beta\)-lactam and an aminoglycoside is needed (vs. the \(\beta\)-lactam alone) for the therapy of such infections as gram-negative bloodstream infections or infections in neutropenic patients. In individual studies, combination therapy has resulted in improved outcomes in patients with severe illness and in patients with *P. aeruginosa* bloodstream infections, but pooled meta-analyses have disputed these results.41

**Laboratory Monitoring of Antimicrobial Therapy**

Monitoring serum concentrations is the most common method used to attempt to maximize efficacy and minimize toxicity of antimicrobials. Because most antimicrobials are well tolerated at their usual doses, only a select few agents (e.g., aminoglycosides, chloramphenicol, and vancomycin) are monitored routinely in the current clinical environment. Direct and indirect methods can be used to quantify the concentration of antimicrobial in an experimental sample, including radioimmunoassay, high-pressure liquid chromatography, and microbiologic assay, and fluorescence-polarization immunoassay (FPIA).

FPIA is the only method that is commonly used in the clinical management of patients with infections, and assays are available for the determination of aminoglycoside and vancomycin serum concentrations. The FPIA technique involves the application of the principles of fluorescence when molecules are exposed to light. A fluorescein-labeled drug and antibody that is directed against the drug are added in constant amounts to samples with known drug concentrations and to concentration standards. When the fluorescein-labeled drug complexes with the antibody, a quantifiable change in the fluorescence polarization occurs. When a sample containing non–fluorescein-labeled drug (i.e., a patient’s serum sample) is mixed with the standard mixture, competition for antibody binding occurs. Comparison of the change caused by the patient’s sample to the changes caused by standard concentrations determines the specific drug concentration in the patient sample.

**Timing of Collection of Serum Samples**

Peak and/or trough concentrations are monitored routinely for only a select few antimicrobials (e.g., aminoglycosides and vancomycin) during the contemporary management of infections. It is crucial for the healthcare team to ensure that the antimicrobial’s administration time and serum sample time(s) are meticulously recorded because even small errors in recording these values (e.g.,
1 hour) can have a substantial impact on the calculation of the pharmacokinetics for antibiotics such as the aminoglycosides, which have relatively short elimination half-lives.

10 Samples ideally should be obtained after steady state is achieved (usually defined as the passage of at least three to four anticipated half-lives), but in certain situations, this may not be possible (e.g., critically ill patients with fluctuations in drug elimination owing to fluctuating hemodynamics, kidney function, and/or liver function). Generally, the timing of the peak serum sample collection is usually more critical than the trough concentration because adequate time must elapse to allow for completion of the distribution phase and to avoid underestimating the drug’s volume of distribution.

Monitoring of Specific Agents

Aminoglycosides

11 The aminoglycosides (i.e., amikacin, gentamicin, and tobramycin) and vancomycin remain the most common agents for which serum concentrations are monitored. There are many studies that have linked serum aminoglycoside concentrations with clinical response and with the occurrence of nephrotoxicity. One of the classic investigations into the relationship between serum aminoglycoside activity and clinical outcome revealed that peak serum concentrations of at least 5 mcg/mL (mg/L; ~11 μmol/L) for gentamicin and tobramycin and at least 20 mcg/mL (mg/L; 34 μmol/L) for amikacin were associated with a lower prevalence of clinical failure rates during the treatment of gram-negative bacteremia.42

Although earlier studies suggested that trough concentrations exceeding 2 to 4 mcg/mL (mg/L; ~4 to 8 μmol/L) for gentamicin and tobramycin and 10 mcg/mL (mg/L; 17 μmol/L) for amikacin predisposed patients to nephrotoxicity, other investigations indicated that the development of aminoglycoside-related ototoxicity and nephrotoxicity is more complex and also is associated with the total exposure to the aminoglycoside (as measured by the area under the curve [AUC]) and/or the total duration of aminoglycoside therapy.43 The specific recommended serum peak and trough concentrations for the various aminoglycosides are described in eTable 25-2.

Newer regimens of high dose once-daily or extended-interval aminoglycoside administration have gained widespread acceptance for use in the clinical setting. These regimens exploit the pharmacodynamic properties of these agents (i.e., concentration-dependent bacterial killing and a substantial PAE) to maximize activity while also attempting to minimize drug nephrotoxicity by reducing the total aminoglycoside exposure time for the patient’s kidneys. The doses employed for extended-interval treatment typically range from 5 to 7 mg/kg of lean body weight (administered every 24 to 48 hours), with the dose and/or interval adjusted based on renal function or observed mid-dose serum concentrations.44 Many prospective studies have been performed to evaluate the safety and efficacy of once-daily aminoglycoside dosing, and most have revealed similar rates of efficacy and toxicity, or trends toward improved efficacy and reduced toxicity for once-daily dosage regimens as compared with traditional (thrice daily) regimens.

Clinical Controversy...

Some clinicians believe that there is sufficient clinical data to support widespread use of once-daily aminoglycoside dosing without determination of individual patient pharmacokinetics. However, there are some clinicians who believe that the data are incomplete and that patients should receive individualized pharmacokinetic assessments and dosage adjustments.

Traditional methods of aminoglycoside serum concentration monitoring (evaluating peak and trough serum concentrations) cannot be applied to extended-interval dosing because the serum concentrations 24 hours after a dose ideally should be undetectable. A midinterval serum sample can be taken approximately 6 to 12 hours after the dose to allow for use of first-order pharmacokinetic equations or nomograms for interval adjustments.45

Vancomycin

Although IV vancomycin has been associated with ototoxicity in humans, most of these reports occurred with older, impure formulations of the drug, with extremely high concentrations...
Organ-Specific Function Tests and Drug-Induced Diseases

SECTION as demonstrated by both animal and human data.\textsuperscript{45} Clinical stud-

MIC ratio (AUC/MIC) is the parameter that best predicts efficacy

(\textsuperscript{S. pneumoniae})\textsuperscript{ginosa}

P. aeru-

is chosen that achieves an area under the curve (AUC)-to-MIC ratio

microbiologic infection cure can be maximized if a fluoroquinolone

concentration to the organism MIC (peak-to-MIC ratio) is greater

aminoglycosides can be maximized if the ratio of the peak serum

activity of antimicrobials such as the fluoroquinolones and the

that the probability of resistance is minimized. For example, the

bacteremia.\textsuperscript{50} The clinician also should recognize

Staphylococcus aureus

These recommendations have been validated in publications that

improved outcomes of patients with

Staphylococcus aureus

≥\textsuperscript{12} improved outcomes of patients with

Serious infections such as complicated bacteremia endocarditis,

μ\textsuperscript{15} to 20 mg/L (10 to 14

the emergence of resistance, while serum trough concentrations of

trations should be maintained above 10 mg/L (7 μmol/L).

To date, most of the data on optimization of antimicrobial

pharmacodynamics have been generated in in vitro models of infect-

in animal models of infection, within the context of controlled clinical trials, or through mathematical modeling of small data sets.

However, research continues to emerge on the best ways to apply these valuable data to the everyday management of patients in the clinical setting. The recognition of the importance of antimicrobial pharmacodynamics already has resulted in such therapeutic innovations such as (a) the expansion of serum concentration monitoring for select antimicrobials (e.g., antiretroviral agents, antifungal agents), (b) suggested revisions of breakpoint values that define antimicrobial susceptibility and/or resistance, (c) development of nomograms or computer programs that can suggest optimal drugs and doses for a given infection, (d) novel administration methods such as prolonged infusion times for antibiotics such as β-lactams with time-dependent activity, and (e) the development of newer antimicrobial agents with minimized risks of suboptimal pharmacodynamics. These developments present exciting opportunities for healthcare providers to improve the outcomes of patients with infections in a variety of different healthcare settings.

\begin{center}
\textbf{ABBREVIATIONS}
\end{center}

\begin{tabular}{ll}
AIDS & acquired immunodeficiency syndrome \\
AUC & area under the curve \\
bDNA & branched DNA \\
CDC & Centers for Disease Control and Prevention \\
CLSI & Clinical and Laboratory Standards Institute \\
CFU & colony-forming unit \\
CMV & cytomegalovirus \\
CSF & cerebrospinal fluid \\
ELISA & enzyme-linked immunosorbent assay \\
\end{tabular}

Clinical Controversy... Some clinicians believe that vancomycin should no longer be considered as the drug of first-choice for treatment of serious staphylococcal infections—even when it is administered to target the new, higher trough range of 15 to 20 mg/L (10 to 14 μmol/L). The desired target attainment of an AUC/MIC of ≥240 may not be achieved with conventional doses of vancomycin with \textit{S. aureus} exhibiting a vancomycin MIC of ≥2 mg/L.

**USING PHARMACODYNAMICS TO IMPROVE ANTIMICROBIAL THERAPY**

Antimicrobial regimens should be selected and/or designed to maximize the probability that bacterial killing is optimized and that the probability of resistance is minimized. For example, the activity of antimicrobials such as the fluoroquinolones and the aminoglycosides can be maximized if the ratio of the peak serum concentration to the organism MIC (peak-to-MIC ratio) is greater than or equal to 10.\textsuperscript{45, 49} Similarly, the probability of clinical and/or microbiologic infection cure can be maximized if a fluoroquinolone is chosen that achieves an area under the curve (AUC)-to-MIC ratio of 100 to 125 or greater for gram-negative bacteria (e.g., \textit{P. aeruginosa}) and 30 to 40 or greater for gram-positive bacteria (e.g., \textit{S. pneumoniae}) (eFig. 25-10).

![Illustration of the concept of peak concentration to the minimum inhibitory concentration (MIC) ratio for aminoglycosides. The MIC for the given organism to gentamicin is 2 mg/L, whereas the tobramycin MIC is 0.5 mg/L. Administration of gentamicin would result in a suboptimal peak:MIC ratio (<10), which could increase the chances for development of resistance or an inadequate response. Administration of tobramycin would result in a peak:MIC ratio of 12, which should improve efficacy. Note that modification of the gentamicin regimen to produce peak serum concentrations of 20 mg/L (42 μmol/L) or more (as commonly done with once-daily administration) also would result in a peak:MIC ratio of ≥10.](image-url)
REFERENCES


