Chapter e126: Laboratory Tests to Direct Antimicrobial Pharmacotherapy

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KEY CONCEPTS

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1. Understanding the difference between normal host microbiota and typical pathogens will help determine whether a patient is truly infected or merely colonized.

2. Direct examination of tissue and body fluids by Gram stain provides rapid information about the causative pathogen.

3. Isolation of the offending organism by culture or rapid diagnostic testing assists in the diagnosis of infection and allows for more definitive directed treatment.

4. Development of molecular testing systems (or rapid diagnostic testing) has improved our ability to diagnose infections and determine the antimicrobial susceptibilities for numerous pathogens, including fastidious or slow-growing mycobacteria and viruses.

5. *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 antimicrobial therapy.

6. 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. Understanding the fundamentals of antimicrobial pharmacodynamic properties will help the clinician make drug selection and dosing decisions in situations where robust clinical data are lacking.

9. Routine monitoring of serum concentrations is currently used for a select few antimicrobials, eg, aminoglycosides 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 valid pharmacokinetic data are generated.

11. Monitoring of aminoglycoside serum concentrations and the use of extended-interval dosing can help maximize the probability of therapeutic success and minimize the probability of aminoglycoside-related toxicity for certain infections.

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 has 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 antibiotic serum concentration remains above the MIC or the ratio of the area under the concentration-time curve to the MIC can improve infection treatment outcomes.

BEYOND THE BOOK

The goal of this learning activity is to begin thinking about the appropriate interpretation of various tests used to direct antimicrobial therapy. As you read through the chapter, work to complete this chart (**Fig. e126-1**) by indicating in each box whether the descriptors in each column apply to the test in each row.

FIGURE e126-1

Preclass learning activity.

Preclass Learning Activity	Direct or Indirect Tests		Identity vs Susceptibility		Data Type		Test Speed		Methodologic Basis for Test Result				
Test	Physiologic Indicator of Infection	Microbiologic Indicator of Infection	Test Determines Antimicrobial Susceptibility	Test Speciates Micro-organism	Quantitative	Qualitative	Rapid Test	Conventional Test	Immunologic	Molecular	Mass Spectrometry	Fluorescent	Visual
Broth microdilution													
C-reactive protein (CRP)													
Erythrocyte sedimentation rate (ESR)													
Etest®													
Gram stain													
Group A streptococcal antigen test													
Kirby–Bauer disk diffusion													
MALDI-TOF													
Multiplex-PCR													
Next-generation sequencing													
PNA-FISH													
Procalcitonin													
White blood cell count													

Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

INTRODUCTION

Selection of an appropriate antimicrobial regimen is a complex process involving knowledge of the infecting pathogen(s), the physiology of the patient, and the microbiological spectrum and pharmacokinetics and pharmacodynamics (PK/PD) of the anti-infective agent. A fundamental aspect of therapy is an appropriate diagnosis and identification of the pathogen. A vast array of laboratory tests, including rapid diagnostic technology, are available to assist in verifying the presence of infection, confirming the identity and susceptibility of a pathogen, and for monitoring the response to antimicrobial therapy. Organism susceptibility to the administered antimicrobials is key to determining the expected outcome from therapy, but *in vitro* susceptibility must be interpreted in the context of antimicrobial pharmacokinetic principles, since the drug must arrive at the site of infection in high enough concentrations and for long enough to exert its effect on the pathogen. Host characteristics such as immune dysfunction, infection site location, and body organ function all play a significant role in selecting the most appropriate antimicrobial for a given individual.[1] Many of the tests may be difficult to interpret correctly and therefore should be considered complementary to clinical judgment.

LABORATORY TESTS CONFIRMING THE PRESENCE OF INFECTION

Nonspecific Tests

Nonspecific tests of infection measure biomarkers that are typically abnormal in the infected patient but can also be abnormal in patients with noninfectious disorders. These are mostly biomarkers of inflammation, such as white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Since these tests are nonspecific, it is important to interpret these results in the broader clinical context of the possibly infected patient.

White Blood Cell Count and Differential

Understanding the role of the WBC in fighting infection is important for diagnosis of infection, selection of drug therapy, and monitoring of patient progress. The major physiologic role of the WBC is to defend the body against invading organisms such as bacteria, viruses, and fungi. The normal range of the WBC count is 4,500 to 11,000 cells/mm^3 (4.5×10^9 - 11×10^9/L).[2] This range may vary among laboratories and patients, as it is dependent on factors like patient age, gender, or comorbidity status. WBCs usually are elevated in response to infection, but many other noninfectious conditions can increase the WBC count, including stress, rheumatoid arthritis, and leukemia. Pregnancy naturally increases WBC count, especially neutrophils. Certain drugs, eg, corticosteroids, can alter WBC count as well. Neutrophils are the most common type of WBCs 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 2 to 5 lobes. Immature

neutrophils lack this segmented feature and are referred to as bands. During an acute infection, immature neutrophils, or bands, are released from the bone marrow into the bloodstream at an increased rate, and the percentage of bands, which usually is about 5%, can increase relative to mature cells, or segs. The change in the ratio of mature to immature cells is often referred to as a "left shift" because of the way the cells used to be counted by hand with a microscope and charted from immature to mature cells from left to right.

Leukocytosis, an increase in WBCs, is a normal host response to infection. Although there are noninfectious causes of leukocytosis, and those causes must be acknowledged, most patients with bacterial infections will develop an elevated WBC count. This WBC count will almost always return to normal as the infection is treated. Combined with other factors suggesting infection, leukocytosis can be a powerful diagnostic and therapy-guiding tool. Neutropenia is often defined as an absolute neutrophil count of less than 500 cells/mm^3 (0.5×10^9/L) and is a common complication of cancer chemotherapy. Patients who are neutropenic are not only at a high risk for bacterial and fungal infections, but they are also incapable of increasing their WBCs in response to infection. The absence of leukocytosis also frequently can occur in the elderly and in severe cases of sepsis.[2,3]

Other Nonspecific Host Biomarkers of Infection

The inflammatory process initiated by an infection sets up a complex host response. Activation of the nuclear factor kappa B (NF-κB) transcription factors plays an important role in the regulation of the immune system. NF-κB is activated by bacterial and viral antigens, which eventually leads to the production of proinflammatory cytokines and chemokines. The rapid detection of activated NF-κB can be measured by transcription factor enzyme-linked immunosorbent assay (TF-ELISA) during a systemic inflammatory response syndrome (SIRS). This test may have some prognostic value in septic patients but it is uncommonly used clinically.

Acute-phase reactants, such as the ESR and CRP 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 as well, such as collagen-vascular diseases and arthritis. However, large elevations in ESR and CRP are associated with infections such as endocarditis, osteomyelitis, and pyelonephritis.[4,5] ESR can be especially useful in tracking the improvement and resolution of chronic infections such as osteomyelitis, as it has demonstrable value in determining the efficacy of therapy.[6] CRP has a shorter half-life than ESR, normalizing within a week of resolution of tissue damage, and is therefore more useful in assessing response to therapy for acute infections. ESR can be affected not only by red blood cell count and shape, but also by fibrinogen and immunoglobulin production. Consequently, ESR should be interpreted cautiously in the setting of anemia (increased ESR) and polycythemia (decreased ESR), or in patients who receive intravenous immunoglobulin (IVIG; increased ESR).

In healthy individuals, procalcitonin (PCT) is produced by the thyroid during calcitonin synthesis, and only low levels are detected in the serum. However, during bacterial infection, PCT is produced through alternative pathways in diverse tissues including the spleen, kidneys, colon, brain, and lungs in response to inflammatory cytokines. PCT appears to be a more specific marker for bacterial infections than either CRP or ESR, and it can be a valuable tool for the clinician to help assess mortality risks of patients with infections and also can help determine when to initiate antibacterial therapy in respiratory tract infections.[7] PCT levels may help determine whether to discontinue empiric antibiotics in possibly infected patients as well as to determine when antibiotics can be discontinued in patients recovering from infections. In general, PCT levels below 0.25 ng/mL (mcg/L) are associated with low risk of infection and can help justify the discontinuation of antibiotics, while levels >0.5 ng/mL (mcg/L) may indicate that antibiotics should be continued. In patients with pneumonia or sepsis, it may be valuable to recheck a PCT level every 1 to 2 days to monitor resolution. If the PCT reduces by 80% to 90% or falls below 0.25 ng/mL (mcg/L), then it may be reasonable to discontinue antibiotics, assuming they do not continue to show other signs of infection such as hemodynamic instability or respiratory distress.[8]

Other Potentially Useful Biomarkers

Changes in endothelial membranes and the presence of a foreign pathogens and their endotoxins cause inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and IL-10[9] and tumor necrosis factor alpha (TNF-α), to be produced by macrophages or lymphocytes.[10] Fluctuations in cytokine levels occur during an infection, which could 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, and there is a relationship between circulating cytokines and outcomes 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 the most predictive of patient outcome.[10] Understanding the balance between these pro-inflammatory and anti-inflammatory processes likely will lead to interventions that can have a direct impact on the outcome of patients with sepsis.[11] Despite the growing evidence demonstrating the importance of cytokine biomarkers in predicting mortality in invasive infections, these are

not routinely monitored and do not yet have a clear place in directing antimicrobial therapy.[12]

LABORATORY IDENTIFICATION OF PATHOGENS

Colonization Versus Infection

Pathogens are organisms that are capable of damaging host tissues and that elicit inflammatory host responses that produce the signs and symptoms of an infectious process. These organisms can be transferred from patient to patient, animal vector to patient, and environment to patient. They may even be opportunistic pathogens from the patient's own microbiome. Most organisms that make up the human microbiome occur naturally in the tissues of the host and provide myriad benefits, including defense by competing with would-be pathogens for essential nutrients, training and regulating the immune system, and synthesizing vitamins and liberating nutrients from our diets (**Table e126-1**).

TABLE e126-1

Examples of Normal Microbiota

	Gram-Positive		Gram-Negative		*Others*
	Cocci	***Rods***	***Cocci***	***Rods***	
Skin	*Staphylococcus* spp. (eg, *S. epidermidis), Streptococcus* spp.	*Corynebacterium* spp., *Propionibacterium* spp., Cutibacterium		Enteric bacilli (some sites), *Acinetobacter* spp. (Coccobacilli)	
Oropharynx	Streptococci—*Viridans* group *Micrococcus* spp.	*Corynebacterium* spp.	*Neisseria*	*Haemophilus* spp.	Spirochetes (non-pallidum *Treponema* spp.
GI tract	*Enterococcus* spp., *Peptostreptococcus* spp.	*Lactobacillus, Clostridium*		*Bacteroides* spp., Enteric bacilli (*E. coli, Klebsiella* spp.)	
Genital tract	*Streptococcus* spp., *Staphylococcus* spp.	*Lactobacillus, Corynebacterium* spp.		Enterobacteriaceae, *Prevotella* spp., *Candida* spp.	*Mycoplasma*

Organisms that comprise a healthy human microbiome can become pathogenic when host defenses become impaired or if the organisms are translocated to other body sites during trauma. The identification of an organism that is normal microbiota in a wound or otherwise sterile body cavity or fluid often becomes a dilemma for the clinician in deciding whether a patient is infected and requires treatment. Such is the case with *Staphylococcus epidermidis* when it is identified in the blood of a hospitalized patient. *S. epidermidis* is considered part of the normal skin microbiota and commonly colonizes IV catheters. When *S. epidermidis* is identified in the bloodstream, the patient's circumstances, eg, signs, symptoms, and laboratory indices supporting infection, as well as the probability of the organism being responsible for the infection, must be taken into account before determining whether the organism is causing disease. Additionally, the percentage of cultures from sterile sites that are positive for a typically commensal organism can help assess whether it's a true pathogen. For instance, growth of *S. epidermidis* in one of four blood culture samples may indicate contamination, while growth in four of four in combination with clinical symptoms may indicate true infection. Furthermore, the use of quantitative cultures or differential time-to-positivity of peripheral cultures and line cultures can be useful in implicating a line as a source. If the line cultures are positive for bacterial growth ≥2 hours before the peripheral cultures, then the line is likely the source of bacteremia.[13] Often the simple removal of the intravenous catheter can eliminate the organism from the bloodstream, thereby preventing unnecessary antibiotic use.[14]

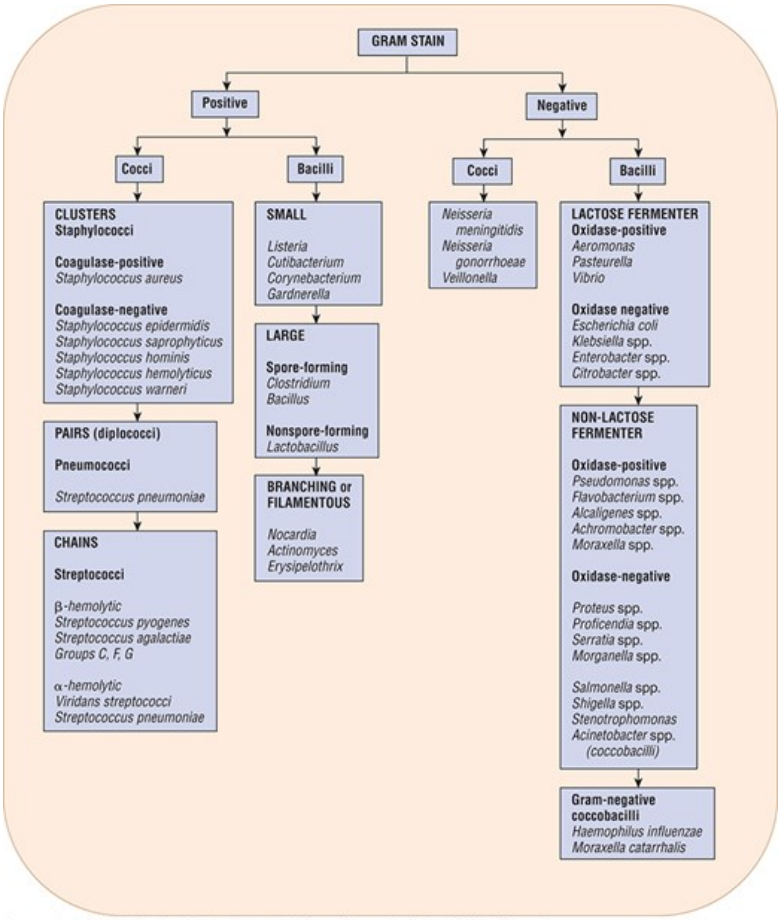
Direct Examination

Direct examination of infected tissue samples or body fluids can provide simple, rapid information to the clinician. The Gram stain is one of the first identification tests run on a specimen brought to the laboratory. Gram-positive cells have a thick peptidoglycan cell wall that retains crystal violet dye, and they appear purple after staining. Gram-negative cells have an additional lipopolysaccharide outer membrane over their peptidoglycan cell wall that retains safranin, and these bacteria appear pink after staining. Gram staining, in conjunction with microscopic examination of morphology, can provide a presumptive diagnosis and some indication of the organism's characteristics (Gram-positive, Gram-negative, Gram-variable, bacilli, or cocci).

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. In addition to being completed quickly, they are helpful in identifying organisms that may not grow easily in 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 or mixed with normal microbiota. If the sputum sample has a predominance of one particular organism in high numbers combined with the presence of many polymorphonuclear leukocyte (PMN), and there are few squamous epithelial cells (<10 per low-power field), the diagnostic utility of the Gram stain is improved. **Figure e126-2** lists some common infecting pathogens grouped according to Gram stain and other characteristics.

FIGURE e126-2

Important bacterial pathogens classified according to Gram stain and morphologic characteristic.



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. Pertinent examples of these include the Ziehl–Neelsen and Kinyoun stains for acid-fast bacilli, which are used for identification of mycobacteria species as well as actinomycetes such as *Nocardia* spp. India ink, useful for identification of *Cryptococcus neoformans*, potassium hydroxide (KOH), useful for identification of *Candida* spp., and Giemsa stains, useful for identification of *Plasmodium falciparum, Treponema pallidum, Histoplasma, Pneumocystis, Toxoplasma*, and *Leishmania*, are other important examples.[15]

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 culture 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 used 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 microbiota 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. Some 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 microbiota, 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, humoral factors with bactericidal activity, and the potential of antimicrobial pretreatment affecting organism growth. Most blood collection bottles dilute the blood specimen 1:10 with growth medium to neutralize the bactericidal properties of blood as well as any circulating 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 penicillins or cephalosporins. Some culture bottles may also contain a suspension of activated charcoal which can help adsorb antimicrobials in the sample, thereby increasing the chances that the organism will grow.

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, and aerobic and anaerobic. This can be accomplished by simple Gram staining, as described previously, by evaluating organism growth patterns on selective media or incubation conditions, and by testing for the presence or absence of specific enzymes and chemical characteristics, such as hemolytic and fermentation properties. This preliminary information, which is readily obtainable from the laboratory, can greatly assist the clinician in choosing the appropriate empirical therapy. Historically, definitive identification of organisms has required multistep testing procedures in order to deduce an organism's identity on the basis of specific biochemical properties. However, increasingly, rapid diagnostic technologies such as matrix-assisted laser/desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and self-contained, direct-from-culture molecular probe and multiplex polymerase chain reaction (PCR) systems (eg, Verigene® Nanosphere, Biofire® Filmarray®) are providing faster and more definitive pathogen identification than traditional biochemical methods. These are described in more detail in the coming sections.

The use of automated culturing systems has reduced the time it takes to identify growth in culture bottles from days to hours. One commonly used system is the BACTEC™ system, which uses bottles of growth medium containing a fluorescent sensor that monitors culture bottles every 10 minutes for the presence of carbon dioxide (CO_2) 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, which shortens the reporting time and enables clinicians to obtain preliminary information about the organism to guide antimicrobial therapy. 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.[15]

Viruses can be detected by direct observation of inoculated culture cells for cytopathic effects or by detection of antigens after incubation by immunofluorescence methods. The culture method is most useful for organisms such as cytomegalovirus (CMV) or *Herpes simplex* virus because these viral agents are rapidly propagated in culture cells, making them easily detected.[16]

Rapid Diagnostic Technologies

There are now many FDA-approved rapid diagnostic testing methodologies for infectious diseases. In the current era of managed care and antimicrobial stewardship programs, rapid diagnostic tests (RDTs) are critical. The need for RDTs is further highlighted by the emergence of multidrug-

resistant bacteria and increased pathogen virulence. A major focus of RDT is pathogens associated with increased morbidity and mortality, which include influenza virus, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE), *Clostridioides difficile*, extended-spectrum β-lactamase (ESBL), and carbapenemase-producing Enterobacteriaceae, 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. Rapid diagnostics can significantly reduce the time to pathogen identification and susceptibility, thus improving the clinician's ability to optimize therapy. Pathogen RDT also prompts de-escalation or potential discontinuation of antimicrobial therapy, which decreases the potential for antimicrobial resistance.

The process of RDT often requires the evaluation of a clinical specimen such as blood, stool, or bodily fluids. These specimens are processed in a qualitative or quantitative manner to provide a result that is available within 15 minutes to a few hours (depending on the technology). This contrasts with traditional culture methodologies discussed above which may take 2 to 4 days, eg, for *Staphylococcus* spp. in the blood, or up to 6 weeks, as in the case of slow-growing *Mycobacterium* spp. The most current RDT modalities are based on immunologic assays, molecular techniques, and mass spectrometry.

DIAGNOSIS OF INFECTION USING IMMUNOLOGIC ASSAYS

4 Immunologic methods for the diagnosis and monitoring of infection have become indispensable laboratory tools. Immunologic assays use reporter-linked antibodies that selectively bind pathogen-specific antigens or resistance determinants in clinical samples in order to identify pathogens and/or susceptibility phenotypes. These methods have the advantage of a rapid turnaround time and a high level of sensitivity and specificity. Some rapid antigen detection tests (eg, identification of group A streptococci and the rapid influenza diagnostic tests) are simple to use, can be performed conveniently in a pharmacy or physician's office, and can be an important part of the decision whether to administer antimicrobials for a suspected infection. One limitation of these assays is that patients may test positive for these antigens even if they have already recovered from an infection. In addition, the positive test result indicating the presence of the potential pathogen does not determine whether the patient was infected or simply colonized with the organism.

Antibody–antigen detection can be accomplished by a variety of reporter systems. Immunofluorescence is 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, in the diagnosis of *Legionella pneumophila*, and to detect penicillin-binding protein 2a, which mediates β-lactam resistance in methicillin-resistant staphylococci. 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 *Helicobacter pylori*.[16]

Molecular Techniques for the Detection of Microorganisms

Nucleic Acid Amplification Methods

Nucleic acid amplification methods are now considered a standard laboratory tool and are increasingly important in situations in which rapid turnaround time is essential to improve patient diagnosis and outcome. Examples include real-time universal screening for acute HIV infection and routine testing and monitoring of patients receiving treatment for HIV infection, as well as the isolation and detection of fastidious or slow-growing organisms such as *M. tuberculosis*, *B. burgdorferi*, and *H. pylori*.[17,18] These highly sensitive methods can detect and quantify minute amounts of target nucleic acid in a rapid manner. PCR is based on the capability of a DNA polymerase to copy and elongate a targeted sequence of DNA. Each cycle doubles the number of copies of the targeted DNA sequence 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 sequence can be generated from a single copy in as few as 20 cycles. Although this amplification technique is sensitive and has wide-reaching applications, 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. Furthermore, it cannot discriminate colonization from infection.

Advancement in molecular genetics techniques has replaced labor-intensive multistep methods with rapid kits capable of identifying a variety of important genetic biomarkers with high sensitivity and specificity and minimal technologist intervention. These advancements include the use of multiple sets of amplification primers, known as multiplex PCR, incorporation of fluorescent reporters for instantaneous detection and quantification

of amplified products, known as real-time PCR, and PCR amplification of RNA by converting target RNA sequences with reverse transcriptase to complementary DNA templates prior to amplification. The integration of nucleic acid extraction, multiplex amplification, and real-time detection into a single closed-system capable of handling minimally processed clinical samples has revolutionized modern laboratory practice. Syndrome-specific multiplex panels, eg, Biofire® Filmarray®, are less labor-intensive, yield a faster turnaround time, reduce the likelihood of contamination, and can be more cost-effective than conventional methods.[19]

Real-time detection of amplified nucleic acid targets in many systems relies on nonspecific fluorescent reporters but DNA microarrays are another option. Microarrays are grids of short sections of DNA that are complementary to a target sequence. Binding of these oligonucleotide probes triggers a colorimetric (based on silver or gold), fluorescence, or chemiluminescence reporter, allowing for quantification of the target sequence. These microarray systems can also be adapted to detect specific proteins. Peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) uses fluorescent-labeled nucleic acid probes to target ribosomal RNA sequences.[20,21] These rRNA sequences are specific to different species of microorganisms. This assay has fallen out of favor and largely been replaced by more efficient hybridization probes, MALDI-TOF MS, and next-generation sequencing.

Mass Spectrometry to Detect Microorganisms

Mass spectrometry is another useful diagnostic technology that can identify microorganisms more rapidly than conventional methods, and it has the added advantage of often being more accurate for species identification as well.[22] This technology vaporizes bacterial samples with a laser, ionizing proteins and other macromolecules. The resulting cloud of ionized analytes is then separated by mass and charge which influence the time of flight to a detector. The resulting spectrum is a sort of fingerprint for an organism that is matched to a database of known species of microorganisms to determine its identity. MALDI-TOF MS has become the primary method for speciating microorganisms in clinical labs that have access to one. The large library of species and the specificity of this system has led to the routine identification of organisms that would not otherwise be speciated by conventional methods. This has led to the recognition of certain pathogens that have been discounted, underappreciated, or completely unrecognized in the past. MALDI-TOF MS systems can identify microorganisms from a single colony of growth in minutes resulting in reliable speciation an average of 1.5 days earlier than conventional methods and can save hundreds of thousands of dollars in reagents and labor in the clinical lab, not to mention the benefits to patient care. When MALDI-TOF MS is integrated into antimicrobial stewardship programs, it is the most cost-effective rapid diagnostic, leading to approximately $30,000 savings per quality-adjusted life-year in patients with bacteremia and preventing 1 out of every 14 deaths.[23] Many experts believe that the full potential of MALDI-TOF MS to direct antimicrobial therapy has not yet been realized. Direct-from-culture bottle methods reduce the time to speciation by 12 to 48 hours, depending on the organism, and there is potential for MALDI-TOF MS spectra to be used to identify certain types of antimicrobial resistance in addition to speciation.[24] Earlier speciation and susceptibility determination can mean fewer days of ineffective or unnecessary antimicrobial therapy.

Next-Generation Sequencing to Identify Microorganisms

Next-generation sequencing (NGS) is a technique that allows for rapid genomic sequencing of microorganisms. This approach does not require targeted primers or prior knowledge of the most likely pathogens to encounter in a sample, can be performed directly from clinical samples, and can identify all organisms present in a sample without prior isolation or culture. Consequently, NGS is the most comprehensive and reliable method for speciation of microorganisms and can be especially useful for fastidious organisms. Many times, clinical laboratories will use this technology to sequence species-specific variable DNA regions such as the 16S-24S rRNA to differentiate organisms, but whole-genome sequencing can also be performed. Consequently, in addition to speciation, NGS can be useful to detect novel resistance variants or to track outbreaks. Similar to other molecular methods, the presence of microorganism DNA in a clinical sample does not differentiate infection from colonization or contamination. Whole genome sequencing has demonstrated excellent concordance with MALDI-TOF in bacterial identification, and it also has demonstrated concordance with standard susceptibility testing methods for determining bacterial antibiotic susceptibilities.[24] However, while sequencing technology has become much faster and more affordable in recent years, cost, data processing skills, and turnaround time for NGS are inferior to other molecular tests and MALDI-TOF, so NGS is generally used as a secondary method when other techniques fail. In the future NGS could become a universal method for rapid speciation and susceptibility determination while providing rich data for tracking clonal transmission.[25,26]

EVALUATION OF ANTIMICROBIAL ACTIVITY AND DETERMINATION OF ANTIMICROBIAL PHARMACODYNAMICS

5 6 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 the drug's pharmacodynamic characteristics. Antimicrobial pharmacodynamics has become a crucial consideration for the clinician in selecting appropriate therapy, formulary decision making, and developing antimicrobial stewardship protocols.

5 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).[27] However, controversies exist about which testing method provides the most useful information, how to best report these results to clinicians, and how to apply them to the treatment of patients.[28]

Most of the standardized and well-accepted susceptibility testing methods evaluate the minimum inhibitory concentration (MIC) of aerobic, non-fastidious bacteria. However, substantial progress has been made to develop sensitive, specific, reproducible, and clinically useful susceptibility tests for anaerobic bacteria, fungi, mycobacteria, and viruses. Continued advances in technology should further improve testing methods and the speed 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 improve their cost-effectiveness. Susceptibility testing methods available in the clinical laboratory can be qualitative (eg, disk diffusion), semi-quantitative (eg, MIC testing), or deductive (eg, molecular, immunologic, or chromogenic screening) in nature.

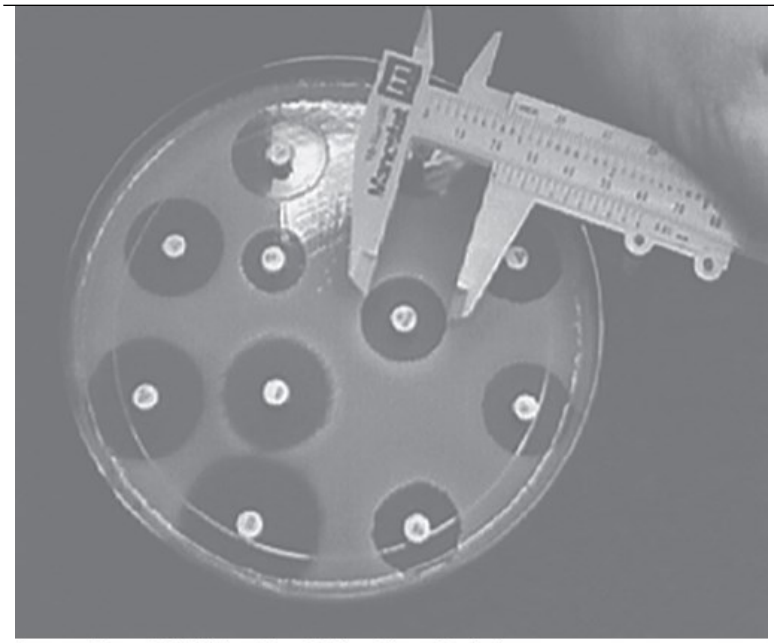
QUALITATIVE ANTIMICROBIAL SUSCEPTIBILITY TEST METHODS

Disk Diffusion Assay

The disk diffusion assay method for susceptibility testing (Kirby–Bauer method) was developed to reduce the labor needed for tube dilution susceptibility testing.[29] 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 (1×10^8 to 2×10^8 CFU/mL [1×10^{11} - 2×10^{11} CFU/L]). The drug contained in the disk diffuses in a concentration gradient out into the agar. The plate is incubated for 16 to 24 hours at 35°C to 37°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 tested (Fig. e126-3).

FIGURE e126-3

Disk diffusion susceptibility test. Antibiotic-impregnated disks are placed on the surface of a plate previously inoculated with the test organism. The plate is incubated for 18 hours, and the subsequent zones of inhibition are measured. The zone size correlates with the sensitivity of the organism. The larger the zone, the more sensitive is the organism to the specific antibiotic. On the basis of predetermined zone breakpoints, organisms can be classified as susceptible, resistant, or intermediately susceptible to the antibiotic. *(Photograph reprinted with permission from the Anti-Infective Research Laboratory, Wayne State University, Detroit, MI.)*



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

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. In some laboratories disk diffusion tests are performed with nonstandard inocula directly from positive culture bottles. The sensitivities may not be fully reported, but they result a day before other methods and can be a useful way to screen for multiple organisms or colony types with different susceptibility patterns for further workup.

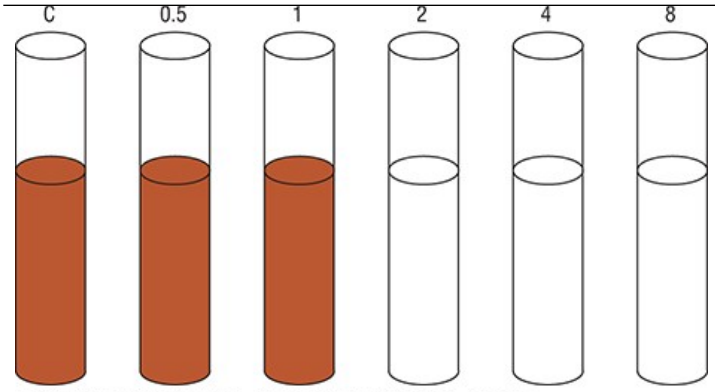
SEMI-QUANTITATIVE ANTIMICROBIAL SUSCEPTIBILITY TESTING

Minimum Inhibitory Concentrations

5 The minimum inhibitory concentration (MIC) is defined as the lowest antimicrobial concentration that prevents visible growth of a standard inoculum of organism after approximately 24 hours of incubation under standardized growth conditions. The MIC semi-quantitatively determines *in vitro* antibacterial activity. The MIC test is "semi-quantitative" because it allows detection of inhibitory concentrations based on doubling of antibiotic, and as such there is no ability to determine an exact concentration required to inhibit bacterial growth. MICs were traditionally determined through the micro or macro dilution methods, which use liquid growth medium (broth), doubling serial dilutions of antimicrobials in test tubes or microtiter wells, and a standard inoculum of bacteria (approximately $10^{5.5}$ to 10^{6} colony-forming units [CFU]/mL [$10^{8.5}$ - 10^{9} CFU/L]). The organism, broth, and antibiotic are incubated at 35°C to 37°C (95°F-98.6°F) for 16 to 24 hours for most organisms and drugs and then examined for visible bacterial growth (**Fig. e122-4**). Using these methods, the MIC can be determined manually by a technician, or by an automated system. The other two methods for MIC determination are the gradient strip method and new morphokinetic cellular analytical systems.

FIGURE e126-4

Macrotube MIC determination. The growth control (*C*), 0.5 mg/L, and 1 mg/L tubes are visibly turbid, indicating bacterial growth. The MIC is read as the first clear test tube (2 mg/L).



Automated Antimicrobial Susceptibility Testing

The microdilution MIC testing method is the most commonly used susceptibility testing method in the clinical microbiology laboratory and the method used by automated systems (**Fig. e122-5**). 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. These systems often incorporate microprocessors, robotics, and microcomputers to rapidly identify organisms and produce susceptibility test results in as few as 3 hours. These systems have limitations in the numbers and 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 (eg, β-lactamases in gram-negative bacteria). There are three rapid automated susceptibility test systems in common use in clinical microbiology laboratories. The Vitek system 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 whether the output that is generated contains qualitative susceptibility, quantitative susceptibility, or both.

FIGURE e126-5

Depiction of a 96-well microtiter plate with MIC assays for antibiotics used commonly against gram-negative pathogens. The shaded wells indicate visible bacterial growth. The MICs (milligrams per liter) for this organism would be 16 for piperacillin, 4 for aztreonam, 2 for ceftazidime and cefepime, 1 for meropenem, 0.5 for ciprofloxacin and gentamicin, and 0.25 for tobramycin. GC is the growth control (no antibiotic added).

	128	64	32	16	8	4	2	1	0.5	0.25	0.125	GC
Aztreonam	○	○	○	○	○	○	●	●	●	●	●	●
Cefepime	○	○	○	○	○	○	○	●	●	●	●	●
Ceftazidime	○	○	○	○	○	○	○	●	●	●	●	●
Ciprofloxacin	○	○	○	○	○	○	○	○	○	●	●	●
Gentamicin	○	○	○	○	○	○	○	○	○	●	●	●
Meropenem	○	○	○	○	○	○	○	○	●	●	●	●
Piperacillin	○	○	○	○	●	●	●	●	●	●	●	●
Tobramycin	○	○	○	○	○	○	○	○	○	○	●	●

The MicroScan WalkAway system is a rapid test system that uses fluorogenic substrate hydrolysis as an indicator of bacterial growth. This system uses

standard micro-dilution 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 are applied for the determination of MICs; output is via computer or video display.

The final system is the BD Phoenix Automated Microbiology System. This system uses an oxidation–reduction detector and a turbidimetric 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 three systems generally are comparable, meaning reproducibly within one doubling dilution of each other. However, there have been documented differences in the ability of these systems to accurately detect emerging resistance mechanisms such as staphylococci with reduced susceptibility to vancomycin and carbapenem resistance in gram-negative pathogens.[30,31] Importantly, all of the systems contain information management software that allow for the storage and rapid retrieval of historical susceptibility data. They can produce patient data reports, antibiograms (summaries of overall resistance for organisms causing infections in a given hospital), and epidemiologic reports. These systems also can be interfaced with other clinical information systems, such as the pharmacy, infection control, or other laboratory data systems, which can help direct antimicrobial stewardship policies and interventions to improve clinical outcomes.[32]

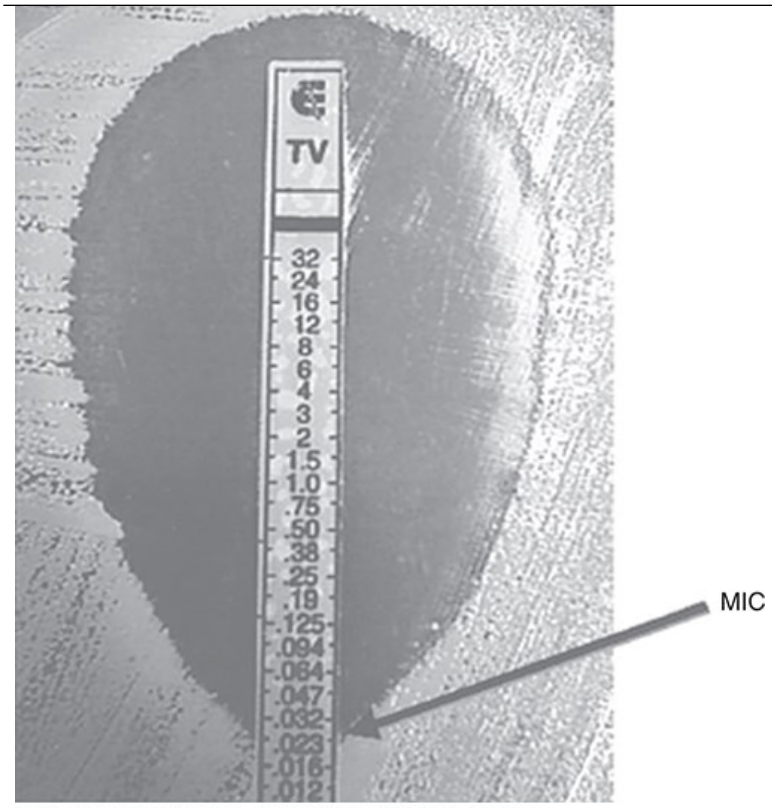
OTHER SUSCEPTIBILITY TESTS

Gradient Strip Tests

The Epsilometer test (Etest) and related gradient strip tests combine 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 rapidly diffuses from the plastic strip to form a 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. e122-6**). Similar products such as the M.I.C. Evaluator™ and the Liofilchem® MIC Test Strips are also available and differential performance characteristics are being evaluated.[33] Many investigators have analyzed the Etest's correlation with standard susceptibility methods and assessed its potential clinical use. In general, values obtained with Etest methods are comparable with or even more consistent and accurate than standard methods.[34] The widespread clinical use of the Etest has been limited primarily by the higher costs of the test strips (nearly 10 times costlier than antibiotic-impregnated disks) in relation to the benefits that may be gained from their use. However, many clinical laboratories use Etests for new antibiotics for which they may lack other means of susceptibility testing or for agents that may not be in their standard panels for automated susceptibility testing. Although their use is not as widespread as other means, they can potentially provide important information for clinical decisions.

FIGURE e126-6

Photograph of Etest susceptibility strip. The MIC is determined from the point where the zone of inhibition intersects with the numerical scale. *(Photograph courtesy of the Anti-Infective Research Laboratory, Wayne State University, Detroit, MI.)*



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

Morphokinetic Cellular Analysis

The newest and fastest method for determining MIC is morphokinetic cellular analysis (MCA). MCA is a microscopy-based platform that allows for automated single-cell growth analysis at 10-minute intervals of cells exposed to different concentrations of antimicrobials. MCA can yield an MIC result in approximately 7 hours directly from a positive culture bottle, much faster than conventional methods. This turnaround time is slower than many current molecular systems but can detect mechanisms of resistance that we cannot yet screen for genetically. Like many other automated systems, MCA susceptibility systems such as the Accelerate PhenoTest™ BC Kit are equipped with a fluorescence-based molecular hybridization system (FISH) for rapid pathogen identification in approximately 90 minutes.[35,36] Similar to MCA, resonant mass measurement-based MIC testing systems, which weigh individual organisms over time, may provide even shorter turnaround time in the future.

Limitations and Problems with MIC Testing

5 Some of the limitations and problems of MIC testing 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 standard bacterial density used to perform the susceptibility testing (eg, $10^{5.5}$ CFU/mL [$10^{8.5}$ CFU/L]) may overestimate the antimicrobial's actual activity at the site of infection, where bacterial densities can be orders of magnitude higher for certain infections. Use of the antimicrobial then could select for 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 that produce inducible β-lactamases.[37]

Many other factors can also influence the *in vitro* MIC value obtained and its subsequent application to a clinical situation. Factors such as the choice of bacterial growth medium, the cation content of the medium, and the presence or absence of protein in the medium can affect the activity of a few drugs significantly. 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 clinical activity may 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.[27] 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 can help better predict therapeutic response as compared with organism susceptibility alone.

Qualitative versus Quantitative Susceptibility Testing of Microorganisms

Quantitative MIC data often are reported to the clinician qualitatively by deeming an organism "susceptible" (S) or "resistant" (R). Organisms can also be labeled "intermediate" (I) or "susceptible-dose-dependent" (S-DD) if those criteria are applicable for the organism and agent. The S-DD label indicates that for some drugs and pathogens successful treatment may be possible with PK-PD optimized regimens. Many factors are considered to determine these qualitative susceptibility classifications, also referred to as "breakpoints" for the antibiotic. These include pharmacokinetic properties of the drug, the epidemiological distribution of MICs for the organism(s), and the clinical and microbiologic responses observed for the antimicrobial against strains of bacteria with various MIC values. The derived breakpoints make the susceptibility data easier to interpret for clinicians. Pathogens classified as susceptible to an antibiotic are those that fall below a breakpoint MIC or zone size, and these breakpoint values have considered clinically achievable, safe serum concentrations in a patient as well as killing activity of the antimicrobial agent. Conversely, resistant organisms are bacteria that tend to have higher MICs and, when treated with the antimicrobial, are likely to result in a suboptimal clinical response, even at the highest doses. The intermediate 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 can be variable. These organisms may 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, eg, urinary tract infections treated by drugs excreted by the kidneys. The S-DD designation was created to reduce ambiguity associated with the intermediate classification. Cefepime activity against Enterobacteriaceae is one such example. It carries the S-DD label against Enterobacteriaceae at MIC values of 4 to 8 mcg/mL. At these values, clinicians are advised to use higher-than-usual doses of up to 2 g every 8 hours. Along with cefepime, the CLSI has established S-DD breakpoints for daptomycin, ceftaroline, and fluconazole against specific pathogens, and the use of this designation is likely to expand in the future.[38]

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 due to pharmacokinetic abnormalities, poor source control, or other host issues. 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 penetration. Additionally, variable outcomes can be achieved for "susceptible" organisms with elevated MIC values, and clinical and/or microbiologic cure rates can sometimes occur for infections that are caused by resistant organisms.[39] 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 reduced activity usually ensures that other more potent agents in its class will have reliable 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.

Deductive Susceptibility Testing by Detection of Resistance Factors

Chromogenic Detection of Resistance Determinants

There are several 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 from yellow to red 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, *Haemophilus 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. The Rapidec® Carba-NP is a newer chromogenic system that detects carbapenemase-producing gram-negative organisms using principles similar to nitrocefin.

The detection of methicillin resistance in *S. aureus* (MRSA) is crucial to ensure appropriate therapy. MRSA is commonly detected within 24 hours using chromogenic agar technology and a variety of such products are available, including chromID® MRSA and BrillianceTM MRSA 2, BBLTM CHROMagar®, MRSA II, and MRSASelectTM. This technology uses chromogenic substrates and a cephalosporin; MRSA strains will grow on an agar plate in the presence of cephalosporins such as cefoxitin and will produce differentially colored colonies resulting from hydrolysis of the chromogenic substrates. The sensitivity and specificity for these tests are often reported as >95% for the detection of mecA-positive strains.[40] chromID® MRSA and BrillianceTM MRSA 2 were significantly more sensitive than other chromogenic agars at detecting methicillin resistance mediated by the *mecC* gene, which is becoming more common in parts of Europe.[41] These tests can be preferable in small labs that don't have access to rapid molecular screening techniques but may be increasingly valuable in regions with increasing prevalence of mecC strains. A variety of chromogenic agars have been developed to detect gram-negative organisms that produce ESBLs or carbapenemases, rapidly identify *Listeria* in neonatal sepsis patients, and polymyxin resistance.[40] However, given the pace of development of new molecular tests it is unclear which of these agar-based methods will continue to be the most useful in the future and which will be replaced. Furthermore, a variety of chromogenic agars have been developed to detect gram-negative organisms that produce ESBLs or carbapenemases.[40]

Molecular Detection of Resistance Determinants

A variety of the molecular identification methods discussed earlier also include targets for drug resistance determinants. The detection of the *mecA* gene in staphylococci and the *vanA* gene in enterococci are some of the most commonly included targets on molecular probe and multiplex PCR panels. The *mecA* gene encodes the mutated penicillin-binding protein 2a in *Staphylococcus* spp., which has low affinity for all traditional β-lactams. Due to the limitations of MIC testing discussed above, such as heterogeneous expression of resistance, molecular detection of the *mecA* gene is the most reliable and fastest way to identify MRSA or methicillin-resistant strains of coagulase-negative staphylococci. Similarly, the *vanA* gene, which encodes for a peptidoglycan variant with low affinity for glycopeptides and mediates high-level vancomycin resistance in enterococci, can be detected molecularly. Early detection of these resistance factor is crucial since most empiric regimens will not include an agent active against most strains of VRE.

Newer molecular panels such as the GeneXpert® Carba-R have targets for increasingly common carbapenemases including, KPC, NDM, VIM, IMP, and OXA-48. Similar to VRE, the rapid detection of carbapenemase-producing pathogens is critical since most patients will not receive empiric therapy that is active against these organisms.

ADVANCES IN SUSCEPTIBILITY TESTING FOR MYCOBACTERIA, FUNGI, AND VIRUSES

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, was an important advancement in the analysis of antimicrobial susceptibility for *M. tuberculosis* and other slow-growing mycobacteria.[42] Use of this method, when coupled with the rapid processing of samples, reduced the time to susceptibility results to approximately 1 week. However, newer FDA-cleared mycobacterial susceptibility testing methods including the VersaTREK™ Myco Susceptibility Kit and the BACTEC Mycobacteria Growth Indicator Tube [MGIT 960] are fully automated and produce results in a similar time frame and with similar reliability as the radiometric method but without radioactive compounds.[43] 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. The use of molecular probes such as the Xpert® for mycobacterial resistance genes has become a more important component of mycobacterial susceptibility determinations, especially considering the increasing problems with antimicrobial resistance.[44]

PCR has now become a standard method to quantify the replication of HIV and hepatitis viruses in infected patients (the viral load described expressed 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.[45]

SPECIAL *IN VITRO* TESTS OF ANTIMICROBIAL ACTIVITY

8 Some pharmacodynamic tests are not routinely performed in the clinical microbiology laboratory but are nevertheless useful for the clinician to understand conceptually when making therapeutic decisions. These tests are performed in research labs and are often reported in the literature.

Minimum Bactericidal Concentration

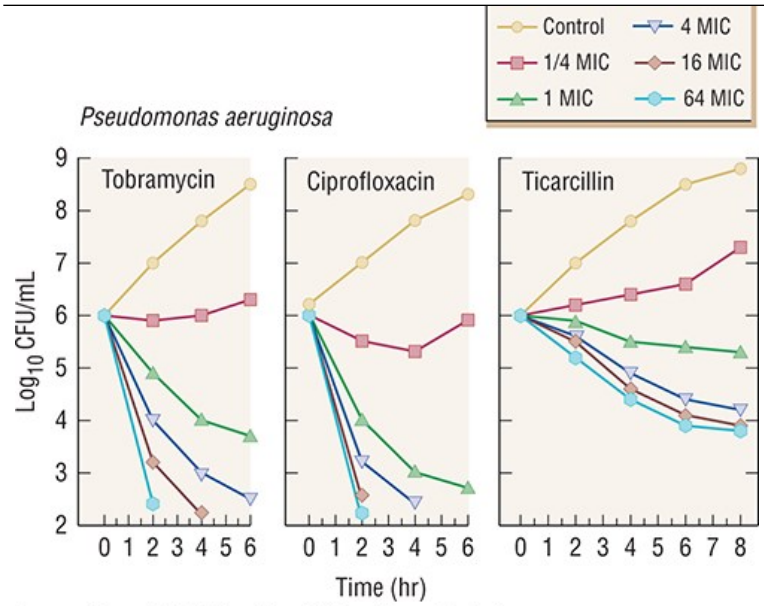
7 The minimum bactericidal concentration (MBC) can be performed in conjunction with the broth microtiter MIC test by taking aliquots of broth from microtiter wells that demonstrate no visible growth and plating the samples onto antibiotic-free agar plates for subsequent incubation. The MBC is defined as the lowest concentration of drug that kills 99.9% of the total initially viable cells (representing a 3 $\log_{10}$ CFU/mL [6 $\log_{10}$ CFU/L] 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 sometimes said to be tolerant to the antimicrobial's killing activity. Although the phenomenon of tolerance has been documented for a variety of drugs and pathogens, there is limited evidence to suggest that it correlates well with clinical failure. Recent systematic reviews of the literature have failed to a differentiate in outcomes of patients treated with bactericidal versus bacteriostatic drugs.[46]

Time-Kill Curve Tests

Time-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 time-kill curve tests, a standard inoculum of bacteria (10^6 CFU/mL [10^9 CFU/L]) is suspended in a test tube containing liquid growth medium with or without desired test concentrations of antimicrobial(s). Samples are removed periodically, usually over a 24-hour period, to determine the number of living cells at the given time points. The viable cell counts are plotted versus time to construct the time-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 (6 $\log_{10}$ CFU/L) reduction in viable inoculum. Comparisons of the relative rates of bacterial killing of different drugs, exposures, or combinations can also be performed in time-kill curve experiments. Additionally, optimal PK/PD indices can be determined from a time-kill curve experiments. An example of results from a time-kill curve experiment is depicted in **Fig. e122-7**. These data can help predict the best way to administer an antimicrobial to maximize activity. For example, lower doses given as more frequent, more frequent (or even continuous) infusions would be preferable for concentration-independent antibiotics (ie, time dependent), while higher doses administered intermittently would maximize activity for concentration-dependent antibiotics.

FIGURE e126-7

Killing curve depicting the effect of concentration on antibiotic bactericidal activity. (CFU, colony-forming unit; MIC, minimum inhibitory concentration [0.25-64 times the MIC; the organism tested was *P. aeruginosa* ATCC 27853]). *(Data from Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: A review. Scand J Infect Dis Suppl 1991;74:63-70.)*

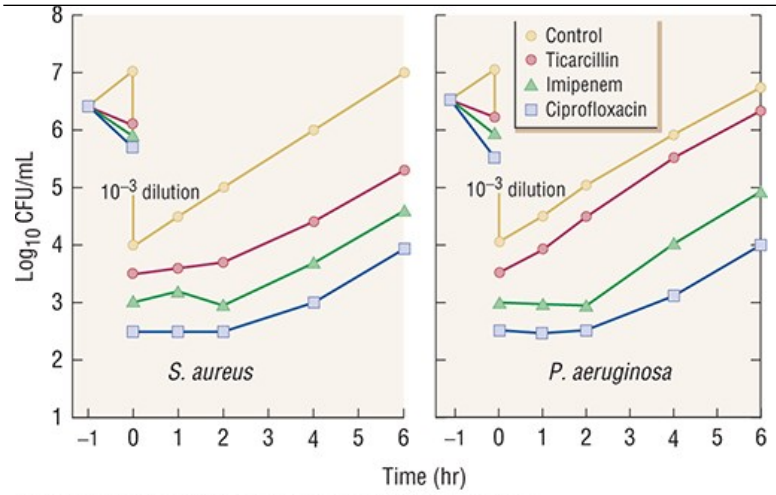


Postantibiotic Effect

The postantibiotic effect (PAE) is defined as the persistent suppression of an organism's growth despite antibiotic concentrations falling below the MIC.[47] A PAE experiment is performed by exposing a fixed inoculum of organism to a set concentration of antibiotic (typically some multiple of the MIC) (**Fig. e122-8**). The antibiotic is then removed by inactivation (eg, inactivation by a β-lactamase or binding the antibiotic to a resin), dilution, or filtration/centrifugation of the mixture. The cells are resuspended in antibiotic-free growth medium, and samples are removed frequently (every 0.5-2 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, including β-lactams, vancomycin, daptomycin, linezolid, and telavancin. Generally, antibiotics that inhibit DNA or protein synthesis (eg, quinolones and aminoglycosides) demonstrate significant PAEs against gram-negative organisms. An exception to this rule is the carbapenems (eg, doripenem, imipenem, and meropenem), which inhibit cell wall synthesis, but demonstrate PAEs against selected strains of gram-negative organisms such as *Pseudomonas aeruginosa*. The primary clinical application of the PAE is to allow for less frequent administration of antimicrobials while still maintaining adequate antibacterial activity (eg, extended-interval aminoglycoside administration).[47]

FIGURE e126-8

Postantibiotic effect (PAE). In this experiment, fixed inocula of *S. aureus* and *Pseudomonas aeruginosa* are exposed to ticarcillin, imipenem, and ciprofloxacin at a set concentration of four times the MIC. The organism and the antibiotic are then diluted 1,000-fold to a point where the antibiotic concentration is far below the MIC of the organism. Growth suppression of *S. aureus* following exposure to the three drugs (PAE) occurs for approximately 2 hours. Growth suppression of *P. aeruginosa*, however, is only demonstrated for imipenem and ciprofloxacin. The β-lactam ticarcillin has no effect on the growth of *P. aeruginosa*. (CFU, colony-forming unit.) *(Data from Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: A review. Scand J Infect Dis Suppl 1991;74:63-70.)*



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

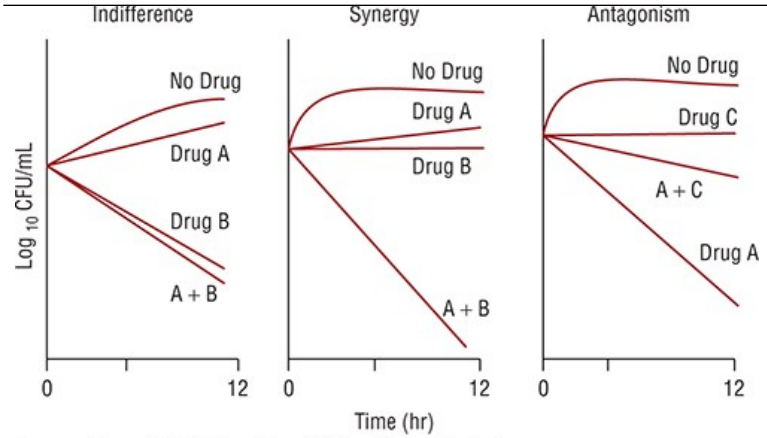
Antimicrobial Combination Effect Test

Antimicrobial combination therapy is used frequently for empiric therapy and to treat serious infections due to multidrug-resistant organisms. Combination therapy can be used prior to knowing the pathogen or antibiotic susceptibility for the treatment of infections in neutropenic patients and as definitive therapy for specific infections such as enterococcal endocarditis or bacteremia, sepsis, or pneumonia caused by *P. aeruginosa,* among other situations. In these cases, it is important to know whether the combination will have beneficial or detrimental effects on the overall antimicrobial activity of the regimen. For example, the combination can result in activity that is far greater than the activity of either agent alone, known as synergy. Conversely, the combination can result in activity that is worse than the most active agent alone, referred to as antagonism. Combination activity that is neither synergistic nor antagonistic is said to be indifferent or sometimes additive.[48]

There are two main research methods used to determine the expected effects of combination antibiotic therapy. The first method is the microtiter fractional inhibitory concentration (FIC, or "checkerboard" method), in which antibiotics are tested in combination in microwell trays, and the MIC of the antibiotic in combination is compared with the MIC of that agent alone. Criteria for synergy, indifference, and antagonism have been established for FIC indices.[49] This method has fallen out of favor in the last several decades due to increased specificity of the time-kill assays. The time-kill assays determine the effects of antibiotic combinations by combining two antibiotics to the same test tube at fixed concentration fractions of the MIC for each drug, and killing is quantified at multiple time points over, usually, 24 hours. With this method, synergy 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 10-fold or greater increase in viable organism count (**Fig. e126-9**).[48] Although antagonism has been demonstrated for several combinations *in vitro* (eg, penicillin plus tetracycline, chloramphenicol plus aminoglycoside, fluoroquinolones and rifampin), theoretically antagonistic combinations do not always result in worse outcomes *in vivo.*

FIGURE e126-9

Time-kill curve illustrating indifference, synergy, and antagonism. (CFU, colony-forming unit.)



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

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 therapy, typically consisting of a β-lactam antibiotic and an aminoglycoside, is required for successful treatment of enterococcal endocarditis. For enterococci, susceptibility to high concentrations of aminoglycosides (eg, gentamicin, 500 mg/mL [g/L]) is evaluated in the clinical laboratory because it correlates closely with synergy when the drug is combined with β-lactam antibiotics. *In vitro* synergy data with two β-lactams, namely ampicillin and ceftriaxone, has led to clinical studies which have shown this regimen to be similarly efficacious and less toxic than the aminoglycoside-based combinations.[50]

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 β-lactam with an aminoglycoside is needed (vs the β-lactam alone) for invasive gram-negative 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 and discourage the use of aminoglycosides in combination with β-lactams in septic patients due to increased rates of nephrotoxicity.[51]

LABORATORY MONITORING OF ANTIMICROBIAL THERAPY

9 Monitoring serum concentrations is the most common method for maximizing efficacy and minimizing toxicity of antimicrobials. Because most antimicrobials are well tolerated at their usual doses, few agents, eg, aminoglycosides, vancomycin, and select antifungals, are monitored routinely in the current clinical environment. However, emerging data suggesting the benefit of monitoring a wider variety of agents has led some centers to begin broader therapeutic drug monitoring programs. The most common analytical methods for measuring antimicrobial concentrations in the clinical lab include fluorescence-polarization immunoassay (FPIA), enzyme immunoassay, and ELISA, but other assays such as radioimmunoassay and high-performance liquid chromatography (HPLC) have been used as well.

TIMING OF COLLECTION OF SERUM SAMPLES

9 Peak and/or trough concentrations are monitored routinely for only a select few antimicrobials (eg, aminoglycosides and vancomycin) during the contemporary management of infections. It is crucial for the healthcare team to ensure that antimicrobial administration time and serum sample time(s) are meticulously recorded because even small errors in recording these values (eg, 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 four to five anticipated half-lives), but in certain situations, this may not be possible, eg, 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 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.[52,53] Although earlier studies suggested that trough concentrations exceeding 2 to 4 mcg/mL (mg/L; ~4-8 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).[52,54] The specific recommended serum peak and trough concentrations for various aminoglycosides are described in **Table e126-2**.

TABLE e126-2

Suggested Therapeutic Serum Concentrations for Selected Antimicrobial Agents

Drug	Sample	Target Concentrations (mg/L [μmol/L])	Comments
Gentamicin, tobramycin (traditional dosage regimens)	Peak (1 hour after start of 15- to 45-minute infusion)	<5 (11 μmol/L)	Urinary tract infections
		>5 (11 μmol/L)	Bacteremia
		>6 (13 μmol/L)	Bacterial pneumonia
		>12 (25 μmol/L)	Endocarditis caused by *P. aeruginosa*
	Trough	<2-3 (4-6 μmol/L)	High trough concentrations are most likely a result of and not a cause of nephrotoxicity.
Amikacin	Peak	>15 (26 μmol/L)	Urinary tract infections
		>20 (34 μmol/L)	Bacteremia
		>24 (41 μmol/L)	Bacterial pneumonia, other serious infections
	Trough	>9-10 (15-17 μmol/L)	See comments regarding trough gentamicin/tobramycin concentrations
Single daily dosage regimens			
Gentamicin	8-hour postdose (*mid-dose*)	1.5-6 (3-13 μmol/L)	Concentrations above this range associated with nephrotoxicity in one study with netilmicin
Netilmicin			
Tobramycin			
Vancomycin (for serious MRSA infections		15-20 (10-14 μmol/L) 400-600 mg × hr/L (276-414 μmol × hr/L)	New guidelines recommend AUC-guided dosing rather than troughs (see text)

Data from References 55 and 56.

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 (ie, 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 total, ideal, or adjusted body weight administered every 24 hours, with the dose and/or interval adjusted based on renal function or observed mid-dose serum concentrations.[57] 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.

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 mid-interval 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.[57]

Vancomycin

12 Although IV vancomycin has been associated with oto- and nephrotoxicity in humans, most of these reports occur when vancomycin is combined with known nephrotoxic agents. Although serum peak and trough concentrations were previously recommended for monitoring vancomycin therapy, only trough concentrations have been monitored because of concentration-independent killing of bacteria by vancomycin. Specifically, vancomycin trough concentrations of 15 to 20 mg/L (10-14 µmol/L) have been recommended for more serious infections such as bloodstream infections, pneumonia, endocarditis, and meningitis caused by *S. aureus* because of a number of factors including (a) poor penetration into tissue such as the lung, (b) the association of the emergence of vancomycin-nonsusceptible strains in patients who had trough serum concentrations maintained below 10 mg/L (7 µmol/L), and (c) the trend of higher vancomycin MICs for most strains of staphylococci. However, the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists have recommended that vancomycin dosing be adjusted based on the AUC/MIC of vancomycin/S. aureus for serious iinfections caused by MRSA. The AUC/MIC is the parameter that best predicts efficacy as demonstrated by both animal and human data.[55] Achieving a free-fraction (unbound) AUC to MIC ratio (*f*AUC/MIC) of greater than or equal to 211 or a total AUC/MIC of greater than 400 improved outcomes of patients with *S. aureus* bacteremia and/or endocarditis. With trough concentrations of 15 to 20 mg/L (10-14 µmol/L) used as a surrogate marker for success, the assumption was that a therapeutic AUC/MIC was achieved.[56] However, newer data have demonstrated that the traditional trough-only approached underestimates up to 34% of vancomycin treated patients.[58,59] Additionally, nephrotoxicity rates are substantially lower when utilizing AUC versus trough dosing and monitoring.[49] The current version of the vancomycin dosing guidelines suggest an AUC/MIC target of 400 to 600 to be achieved within the first 24 to 48 hours of infection due to MRSA.[58] These monitoring recommendations for vancomycin extend to infections due to MRSA only. Infections due to other organisms do not have the data to support therapeutic drug monitoring of vancomycin.

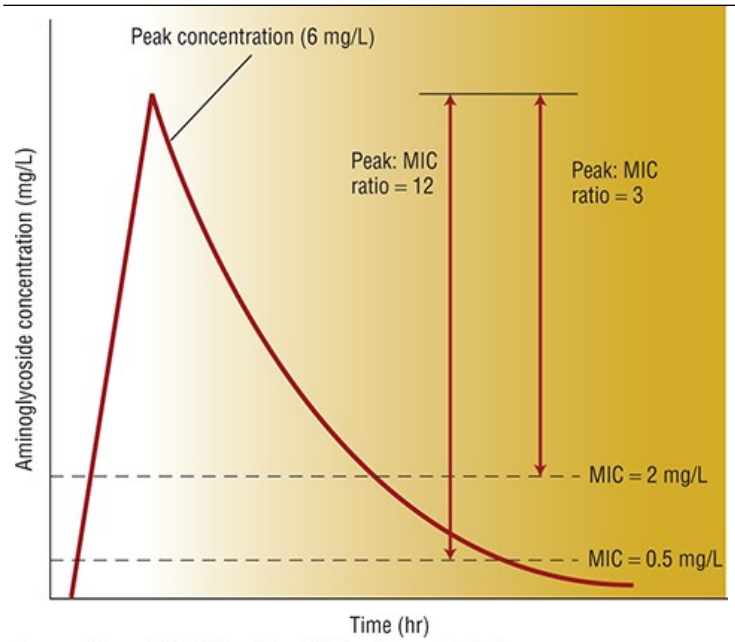
While the process of calculating AUC for each vancomycin dosing regimen may appear daunting, AUC can be calculated in multiple ways, and the guidelines recommend either of the following methodologies to determine AUC in clinical practice. In patients with two vancomycin serum concentrations, AUC can be calculated by dividing the dose received by the patient-specific clearance. Correct timing and documentation of blood draws and pharmacokinetic calculations are required for this strategy, which may require additional training and coordination of medical staff. For patients with only one concentration, an AUC can be estimated by maximum a posteriori (MAP) probability Bayesian software. While this methodology requires little calculation from a clinician, specialized software and clinician training is required to successfully employ this method of AUC monitoring. Precise timing of the vancomycin dose and concentration is needed for these estimations.

USING PHARMACODYNAMICS TO IMPROVE ANTIMICROBIAL THERAPY

13 14 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 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.[60] Similarly, the probability of clinical and/or microbiologic cure can be maximized if a fluoroquinolone is chosen that achieves an AUC-to-MIC ratio of 100 to 125 or greater for gram-negative bacteria (eg, *P. aeruginosa*) and 30 to 40 or greater for gram-positive bacteria (eg, *S. pneumoniae*) (Fig. e126-10).[60]

FIGURE e126-10

Illustration of the concept of peak concentration to the 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.



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

To date, most of the data on optimization of antimicrobial pharmacodynamics have been generated in *in vitro* models of infection, in animal models of infection, 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 has already resulted in therapeutic innovations such as (a) the expansion of serum concentration monitoring for select antimicrobials (eg, antiretroviral agents, antifungal agents), (b) 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 given infections, (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.

ABBREVIATIONS

AUC	area under the curve
CDC	Centers for Disease Control and Prevention
CFU	colony-forming unit
CLSI	Clinical and Laboratory Standards Institute
CMV	cytomegalovirus
CRP	C-reactive protein
CSF	cerebrospinal fluid
ELISA	enzyme-linked immunosorbent assay
ESBL	extended-spectrum β-lactamase

ESR	erythrocyte sedimentation rate
FIC	fractional inhibitory concentration
FISH	fluorescence in situ hybridization
FPIA	fluorescence-polarization immunoassay
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
IL	interleukin
KOH	potassium hydroxide
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
MBC	minimum bactericidal concentration
MCA	morphokinetic cellular analysis
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
NF	nuclear factor
NGS	next generation sequencing
PAE	postantibiotic effect
PCR	polymerase chain reaction
PCT	procalcitonin
PMN	polymorphonuclear leukocyte
RDT	rapid diagnostic tests
S-DD	susceptible dose-dependent
TNF	tumor necrosis factor
VRE	vancomycin-resistant enterococci
WBC	white blood cell

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SELF-ASSESSMENT QUESTIONS

1. Choose the correct statement(s) regarding the WBC count and differential.

   A. WBC count is usually elevated in response to infection.

   B. The normal range of the WBC count is 12,500 to 20,000 cells/mm^3 (12.5×10^9 - 20.0×10^9/L).

   C. WBC count is nonspecific and can be elevated in response to a number of noninfectious conditions.

   D. Neutrophils are the most common type of WBC in the blood.

   E. A, C, and D are correct statements.

2. The inflammatory process initiated by infection can set up a complex set of host responses which include:

   A. Activation of the complement cascade

   B. An increase in the erythrocyte sedimentation rate

   C. Elevations of C-reactive protein

D. Increased production of interleukins and tumor necrosis factor

E. All of the above

3. Which of the following circumstances would be considered the most consistent with colonization by normal microbiota?

A. *S. epidermidis* found on the skin

B. *S. aureus* in the bloodstream

C. *E. coli* in the urine

D. *Viridans* streptococci found in the nasopharynx

E. Both A and D

4. Choose the correct statement(s) regarding the identification of potential pathogens

A. The Gram stain may provide a presumptive diagnosis and identify whether the pathogen is gram-positive or gram-negative and a bacillus or coccus.

B. Cultures can provide definitive pathogen identification and differentiate organisms on the basis of biochemical characteristics more rapidly than molecular-based methods.

C. MALDI-TOF MS can rapidly speciate most microbes on the basis of genetic markers.

D. Even with automated systems, detection of bacteria or fungi within a few hours is not yet possible.

E. All of the above.

5. Although widely employed, the use of molecular methods is often limited by these tests' lack of sensitivity.

A. True

B. False

6. Which of the following is an appropriate description of an element of nucleic acid amplification?

A. Polymerase chain reaction (PCR) is based on the capability of a DNA polymerase to copy and elongate a targeted segment of DNA.

B. Each PCR cycle theoretically doubles the amount of DNA originally present in a sample.

C. PCR techniques are useful for detecting fastidious or slowing growing organisms.

D. Genetic markers for resistance for *M. tuberculosis* and methicillin-resistant *S. aureus* are two examples where PCR techniques have been employed.

E. All of the above.

7. Which of the following technologies is NOT currently used for rapid identification of potential pathogens?

A. Mass spectrometry.

B. Multiplex PCR

C. Enzyme-linked immunosorbent assays.

D. All of the above are used for rapid identification of potential pathogens.

8. Which of the following statements is most accurate regarding microtiter MIC testing?

   A. Microtiter MICs are difficult to perform via automated procedures.

   B. Microtiter MICs have little to no value in the contemporary management of infections.

   C. Microtiter MICs may overestimate or underestimate *in vivo* antimicrobial activity.

   D. Microtiter MICs are capable of reporting any number of potential minimum inhibitor concentrations and are not limited to doubling dilutions.

9. Which of the following statements describing susceptibility testing is most correct regarding susceptibility testing?

   A. MIC is defined as the lowest concentration of a given antimicrobial that will kill 99.9% of the patient's organism after 16 to 24 hours of incubation.

   B. MIC is defined as the lowest concentration of given antimicrobial that will inhibit visible growth of an organism after 16 to 24 hours of incubation.

   C. Kirby–Bauer test is a quantitative susceptibility test that can provide an MIC value.

   D. An Etest is a qualitative test used to measure susceptibility and resistance for an antibiotic against a given pathogen.

10. MM is a patient with pneumonia, a lung abscess, and stage 3 chronic kidney disease. Methicillin-resistant *Staphylococcus aureus* (MRSA; susceptible to vancomycin via microtiter MIC) grew from a bronchoalveolar lavage sample. Vancomycin therapy was started, but the patient has not responded to 7 days of therapy. Which of the following would be the MOST likely explanation for the failure?

    A. The presence of vancomycin-resistant *Staphylococcus aureus* (VRSA).

    B. The patient's reduced kidney function due to stage 3 chronic kidney disease is increasing vancomycin clearance.

    C. Inadequate penetration of the vancomycin to the site of the infection in the lung.

    D. Suboptimal peak to MIC ratio.

11. Which of the following statements is FALSE concerning antimicrobial susceptibility testing and its application to the management of infections?

    A. An infection due to an antimicrobial-resistant organism is unlikely to respond to treatment with even maximal doses of that antimicrobial.

    B. The Kirby–Bauer disc diffusion test results in quantifiable MIC data that allows dose optimization of antibiotic therapy.

    C. Automated susceptibility test systems can interface with pharmacy records to help assess appropriateness of antimicrobial therapy.

    D. Newly developed testing methods for mycobacteria have reduced susceptibility reporting time to less than 28 days

12. A patient has a lung infection due to *Pseudomonas aeruginosa*. He is currently receiving therapy with a fluoroquinolone that on average produces a peak serum concentration of 5 mcg/mL (mg/L) and an AUC of 100 mg x h/L. The MIC for this fluoroquinolone against his infecting pathogen is 2 mcg/mL (mg/L). Which of the following statements is TRUE.

    A. The data provided are incomplete, since fluoroquinolones demonstrate time-dependent pharmacodynamics and we need the trough value to determine the time above the MIC.

    B. The fluoroquinolones demonstrate concentration-dependent pharmacodynamics and the peak-to-MIC ratio is optimized for this pathogen at the current dose.

    C. The fluoroquinolones demonstrate exposure-dependent pharmacodynamics and the AUC-to-MIC ratio is optimized for this pathogen at the current dose.

D. The fluoroquinolone should be avoided in this instance due to suboptimal pharmacodynamics and a low probability of clinical cure.

13. Which of the following statements is FALSE concerning "once-daily" (extended interval) aminoglycoside dosage regimens?

A. The higher doses produce higher peak serum concentrations than conventional doses, which maximize antimicrobial activity.

B. Peak and/or mid-dose serum concentrations should be routinely monitored.

C. Nomograms can be used to determine dosage regimens.

D. Limitations in the clinical studies of "once daily" regimens have prevented widespread use.

14. Which of the following statements is FALSE concerning special *in vitro* tests of antimicrobial susceptibility?

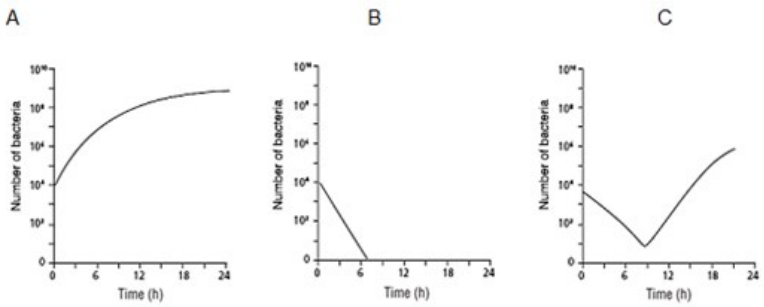
A. Demonstration of *in vitro* antagonism for two antimicrobials does not always correlate with poor clinical outcomes when those antimicrobials are used together in patients.

B. MBCs can be helpful to guide antimicrobial treatment of infections such as infective endocarditis or osteomyelitis.

C. Time-kill curve tests can be used to measure the effect of antimicrobial concentration on bacterial survival.

D. The administration interval can be prolonged for an antimicrobial with a postantibiotic effect.

15. Which figure(s) is/are the most appropriate representation of how bacteria are affected by the presence of an antibiotic to which they are fully susceptible?



A. Figure A only

B. Figure B only

C. Figure C only

D. Figures A and C

SELF-ASSESSMENT QUESTION-ANSWERS

1. **E**

2. **E**

3. **E**

4. **A**

5. **B**

6. **E**

7. **D**
8. **C**
9. **B**
10. **C**
11. **A**
12. **D**
13. **D**
14. **B**
15. **B**