Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

D Clinical Microbiology

16 The Clinician and the Microbiology Laboratory

*Patrick R. Murray**

The clinician and the microbiologist must actively work together to maximize the clinical value of diagnostic microbiology testing. Unfortunately, the current trend to consolidate laboratory services and to move them off-site has made timely communication between laboratory personnel and patient care providers more difficult.[1] In addition, these changes potentially increase the time between specimen collection and laboratory processing, compromising the specimen integrity and delaying the availability of critical test results. These changes, along with other cost-cutting measures, make it even more important that the infectious diseases physician and the microbiologist work together. The following expectations regarding the microbiologist's and the clinician's responsibilities should be recognized and addressed (Table 16-1).

The menu of tests offered by the laboratory should be sufficient to satisfy the patient care needs. Selection of these tests and the availability of infrequently offered tests should be determined after discussions with key members of the medical staff and should be revised as patient care needs change. If the performance of infrequent, expensive, or sophisticated testing is shifted to an external laboratory, the microbiologist should ensure that timely transport and appropriate test procedures for these send-out tests are maintained so that test accuracy is not compromised. The microbiologist should provide the medical staff with a menu of the offered tests, including cutoff times for receipt of specimens and turnaround times for test results. Guidelines for collection of appropriate specimens, transport of the specimens to the testing site, and laboratory policies (e.g., limits on the number of specimens that can be submitted for a specific test, list of pathogens routinely sought in stool cultures) should be available. The test menu and guidelines should be readily available on the hospital computerized information system and ideally integrated with electronic ordering of tests. The computer system should document receipt of specimens in the laboratory, provide a record of testing in progress, and report preliminary and final results. The microbiologist should coordinate with key members of the medical staff a policy of immediate telephone notification of critical laboratory results, such as positive blood or other normally sterile fluid and tissue cultures, isolation of highly infectious pathogens, and significant positive stain results from clinical specimens. In addition, the microbiologist and medical staff should develop guidelines for reporting antibiotic susceptibility results for bacteria, mycobacteria, and fungi. Periodic publication of antimicrobial susceptibility patterns for the most common bacteria can be used to guide empirical therapy.[2] The laboratory must ensure that its operation meets all current regulatory requirements (Clinical Laboratory Improvement Amendments), including initial verification and ongoing validation of procedures that are used by the laboratory.[3,4] Participation in external proficiency surveys, an effective program of quality control, and the use of established quality assurance benchmarks can ensure the accuracy of the laboratory test results. Minimizing testing costs is essential for an efficient laboratory operation but must be done without significant compromise of the quality of results. Finally, a system of short-term storage of all specimens and long-term storage of important isolates should be established to facilitate additional testing if required.

The responsibilities of the clinician, particularly of the infectious diseases physician, include acquiring a substantive understanding of the laboratory's test menu, specimen collection and transport guidelines, and testing policies. The clinician should know the appropriate specimens and diagnostic tests for detection of specific pathogens and be prepared to alert the laboratory personnel when a specific fastidious (e.g., *Corynebacterium diphtheriae, Bartonella* spp.) or highly pathogenic (e.g., *Francisella tularensis,* drug-resistant *Mycobacterium tuberculosis, Coccidioides immitis*) organism is considered. The clinician should also be prepared to prioritize test requests when only a limited quantity of specimen can be collected. In this situation, discussions with the laboratory director can facilitate modification of the routine laboratory practices to optimize the testing process (e.g., need for routine stains, selection of media, and testing conditions to optimize recovery of both bacteria and fungi on a limited number of media). Communication with the laboratory director is also important when the clinical testing needs are not satisfied by the available test menu or test turnaround time, when a laboratory policy causes patient care problems, or when test results are inconsistent with a patient's clinical presentation.

One inevitable difficulty that confronts both the microbiologist and clinician is the continuous revision of the taxonomic nomenclature, particularly for bacteria and fungi. Much of the taxonomic reorganization now under way is driven by our increased ability to classify organisms by using gene sequencing and, more recently, mass spectrometry. The advantages of using these tools to classify and identify organisms are greater precision than by using traditional phenotypic tests and a better understanding of the relationship between newly described organisms and disease. The disadvantages are the practical challenges of remembering a rapidly expanding list of new names and the difficulties this presents for performing searches of the medical literature. Fortunately, an up-to-date list of all validly published names of bacteria, as well as the historical names of the organisms, is accessible on the Internet (www.bacterio.cict.fr/). The International Committee of Taxonomy of Viruses publishes a similar database for viruses (www.ictvonline.org). The scientific names of fungi and parasites are defined by the International Code of Botanical Nomenclature (www.bgbm.org/iapt/nomenclature/code/default.htm) and the International Code of Zoological Nomenclature (www.iczn.org), respectively; however, up-to-date lists of validly named fungi and parasites do not currently exist.

Hospital epidemiology is an area of shared interest for the microbiology service and the hospital infection-control officers. Although a hospital surveillance program can monitor infection rates and noncompliance with established infection prevention policies, the most effective component to any infection control program is vigilance—on the part of the microbiologist and clinician. The microbiologist has the unique perspective of seeing all the organisms isolated from patients throughout the hospital and may be the first to recognize a small cluster of unique organisms. The clinician also has a unique perspective—the ability to assess the likelihood that an infection in his or her patient is nosocomial. Working in concert, the microbiologist and clinician play an important role in guiding a potential outbreak investigation.

KEYWORDS

antigen detection; bacteriology; clinical microbiology laboratory; culture; laboratory diagnosis; molecular diagnosis; mycobacteriology; mycology; parasitology; serology; virology

TABLE 16-1 Microbiologist and Clinician Partnership

Microbiologist's Responsibilities
Provide an appropriate, comprehensive test menu that is responsive to physician needs
Establish a relationship with an external laboratory for performance of infrequent, expensive, or sophisticated testing that cannot be performed on site
Provide cut-off times for processing test requests and turnaround times for reporting results
Provide guidelines for specimen collection and transport
Maintain an effective computerized system for acknowledging receipt of specimens, testing in-progress, and reporting of results; immediate notification to the physician of critical results should be a component of an overall communication system
Periodic publication of antimicrobial susceptibility patterns for the most commonly isolated bacteria in the institution
Maintain a program of quality control that ensures the accuracy of all offered tests
Establish a laboratory that conforms to regulatory standards
A system of short-term storage of all specimens and long-term storage of important isolates should be established to facilitate additional testing if required
Clinician's Responsibilities
Maintain knowledge of the laboratory test menu and specimen collection and transport guidelines
Alert the laboratory when a specific organism is sought (e.g., a fastidious or highly pathogenic organism)
Prioritize test requests when a limited quantity of specimen can be collected
Establish an open communication with the laboratory director when testing needs are not satisfied by the available test menu or special handling of a specimen is required

In the following sections, guidelines for specimen selection, collection, and transport are summarized, followed by a presentation of the general procedures that are used to process specimens and detect and identify medically important organisms.

SPECIMEN SELECTION, COLLECTION, TRANSPORT, AND INITIAL PROCESSING

General Considerations

Selection of the appropriate specimen and test for the detection of an organism responsible for the patient's disease is the ultimate objective. If a specific pathogen is suspected, then selection of the appropriate specimen for the specific diagnostic test is straightforward; however, the differential diagnosis is typically not so limited. A variety of pathogens are usually considered, which increases the types of specimens that must be collected and the number of tests ordered. Although this may not pose a problem if the quantity of specimen is sufficient (e.g., blood, urine, stool, expectorated sputum), this can present a significant problem if the quantity is limited or the specimen difficult to obtain (e.g., aspirated fluids, tissue biopsy). Thus, the physician has the responsibility to consider carefully potential pathogens and the diagnostic tests that should be ordered. The physician also has the responsibility to ensure specimens representative of the site of infection are collected in the appropriate container and transported to the laboratory in a timely fashion. Delays while specimens remain in the patient care area adversely affect diagnostic testing. The microbiologist has the responsibility to provide appropriate, readily available instructions and materials for collection and transport of the specimens.

Selection of the appropriate specimens depends on multiple factors. If the diagnostic test is isolation of the organism in culture, then the specimen must contain viable organisms; in addition, care should be taken to avoid organisms that may either suppress or overgrow the pathogen or confound the interpretation of the culture. Ideally, the specimens should be collected before administration of antimicrobials. An adequate volume of specimen should be collected to maximize recovery of the pathogen, and an adequate number of specimens should be collected if the organism is transiently present (i.e., blood cultures for detection of bacteremia or fungemia). Microscopy is a rapid but typically insensitive and nonspecific method for detection of pathogens. Thus, if the quantity of specimen is limited, the value of microscopy must be balanced with the need for other diagnostic tests. If microscopy is the primary diagnostic test (e.g., examination of peripheral blood smears for plasmodia, *Babesia, Borrelia*), then multiple specimens may need to be examined by using specialized stains or microscopy techniques. Selection of the appropriate specimen for detection of microbial antigens is determined by the suspected pathogen. For example, cerebrospinal fluid (CSF) would be appropriate for diagnosis of cryptococcal meningitis, but both CSF and urine should be collected for diagnosis of pneumococcal meningitis. Likewise, the optimum specimens for diagnosis of *Legionella* pneumonia are bronchial lavage for culture and urine for antigen testing. Serologic tests are useful if the collection of blood is properly timed to coincide with the peak level of antibodies or to demonstrate a significant rise in antibodies. Because these levels can vary for different pathogens, collections of samples should be coordinated carefully to optimize their usefulness and not as an afterthought when confronted with initial negative tests.

Transport of specimens to the microbiology laboratory must be done in a timely fashion, which is particularly important in the handling of urgent requests. The laboratory should determine in what manner and how often specimens should be delivered and should periodically monitor this process to make sure that delivery to the laboratory is occurring as expected. It is important to include, with the time required to transport a specimen to the laboratory, the delays between collection of the specimen and shipment to the laboratory and the time between receipt of the specimen in the laboratory and laboratory processing. Efforts to minimize transit time (e.g., use of pneumatic tube systems) are wasted if there are significant delays after collection or receipt in the laboratory. The physician has the responsibility to minimize the former and the microbiologist the latter. These transit times should be monitored systematically as part of a diagnostic quality assurance program. It is also important to time the collection and transport of specimens to ensure they arrive in the laboratory before an established cutoff time for tests that are performed once per day or less often.

Table 16-2 summarizes generally accepted guidelines for specimen collection and transport of common specimen types for bacterial and fungal stains and cultures. Because specialized transport systems are used for viruses and parasites, these will be discussed separately. Not all situations can be covered under general guidelines. For these instances, the physician should contact laboratory personnel to discuss the best procedures for ensuring optimal handling of the specimen. For more extensive guidelines or for recommendations that are beyond the scope of this chapter, other sources are available.[5]

Specific Specimen Guidelines and Initial Laboratory Processing

Blood Specimens

Blood cultured for bacteria and fungi is considered one of the most important specimen types processed by the microbiology laboratory. Because relatively few bacteria and fungi are present in the blood of septicemic patients, a large volume of blood is traditionally cultured in bottles containing nutrient broths. In the 1970s, the first automated system was developed that detected positive cultures by monitoring the broth for metabolic byproducts (i.e., radioactively labeled carbon dioxide) of microbial growth. In subsequent years, a number of commercial companies introduced a series of modifications of this broth-based system. The current fully automated systems monitor blood culture bottles for microbial growth every 10 to 20 minutes. Because this process can be done without entry into the bottles, contamination of the cultures resulting from laboratory processing is essentially eliminated. A variety of culture media are available for these systems, including aerobic and anaerobic broths, broths supplemented with resins to bind and remove antibiotics, broths for pediatric cultures, and fungal media. Use of resin-supplemented media significantly improves recovery of bacteria in patients who have been treated with antibiotics in the 48 hours before blood collection.[6] Decisions about which media should be routinely or selectively used should be made after the microbiologist and infectious disease specialists carefully consider the advantages and disadvantages of each medium.

TABLE 16-2 Guidelines for the Collection and Transport of Specimens Submitted for Bacterial and Fungal Stains and Cultures

SPECIMEN	COLLECTION GUIDELINES	OTHER COMMENTS
Abscess	Aspirate of pus or fluid in anaerobic transport vial is preferred; swabs usually have insufficient material for stains and culture. Clean surface of closed abscess with 70% alcohol; collect specimens at margins of abscess. Aspirates in anaerobic transport tubes are acceptable for aerobic and anaerobic bacterial, fungal, and mycobacterial cultures.	Specimen sent immediately in capped syringe after the air is excluded is also acceptable. Specify location of abscess for optimal processing; provide all other pertinent information (e.g., surgical infection, trauma, bite wound).
Blood for Culture		
Routine aerobic and anaerobic bacteria	Disinfect bottle tops with 70% isopropyl alcohol; disinfect phlebotomy site with alcohol, followed by tincture of iodine or chlorhexidine; allow the disinfectants to dry. Collect 10-20 mL/adults and 1-3 mL/child for each blood culture; divide blood into two blood culture bottles, preferably an aerobic and an anaerobic bottle; collect two to three cultures per 24-hr period.	Optimal recovery of bacteria from patients receiving antibiotics requires use of broths supplemented with resins designed to inactivate antibiotics. All blood cultures should be transported immediately to start incubation or processing.
Fastidious bacteria, mycobacteria, and fungi	Refer to Table 16-4.	Refer to Table 16-4.
Blood for Microscopic Examination		
Anaplasma, Ehrlichia	Prepare thick and thin smears from fingerstick or EDTA blood.	Transport rapidly for smear preparation; test insensitive for *Ehrlichia* species.
Borrelia	Prepare thick and thin smears from fingerstick or EDTA blood.	Transport rapidly for smear preparation; test insensitive for *Borrelia burgdorferi*.
Bone Marrow	Collect 1-2 mL aseptically; inoculate into blood culture bottle or 1.5-mL lysis-centrifugation tube.	Obtain for *Histoplasma,* mycobacteria, *Brucella*, *Salmonella enterica* serotype Typhi; aspiration of more than 2 mL will dilute marrow with peripheral blood.
Catheter		
Intravascular	Remove aseptically, cut at least a 2-inch segment from tip, and place segment in sterile container.	Transport rapidly to prevent drying out.
Foley	Not acceptable for culture	Growth represents distal urethal microbes.
Drainage	Culture of drainage catheter is not recommended.	Drainage catheters may be superficially contaminated.
Ear		
Inner	For intact eardrum, clean ear canal with soap solution or 70% alcohol, then aspirate by syringe. For ruptured eardrum, collect drainage with noncotton swab.	If the specimen is collected by aspiration, inoculate the media at the time of collection or immediately transport the syringe to the laboratory. Drainage collected from a perforated eardrum should not be cultured for anaerobes.
Outer	Remove crusted debris and then firmly scrape or swab the outer ear.	
Eye		
Conjunctiva	Roll premoistened swab over conjunctiva; stains should be prepared and media inoculated at the time of collection.	The number of stains prepared and media inoculated should be limited because of the small amount of material collected.
Cornea	A sterile spatula should be used to scrape the surface of the cornea; stains should be prepared and media inoculated at the time of collection.	The number of stains prepared and media inoculated should be limited because of the small amount of material collected. Anesthetics may inhibit growth of some bacteria.
Vitreous and anterior chamber fluids	Because a limited amount of fluid can be collected by aspiration, it should be transported immediately to the laboratory in the properly enclosed collection syringe.	The number of stains prepared and media inoculated should be limited because of the small amount of material collected.
Feces	Collect specimen directly in a sterile container and deliver immediately to laboratory; transport in Cary-Blair holding medium if delayed in transport.	Do not process for bacterial pathogens if patient has been hospitalized for more than 3 days unless approved by laboratory director; consider *Clostridium difficile* for hospitalized patients with diarrhea; multiple specimens per day are not indicated. Notify laboratory if specific pathogen is suspected (e.g., *Vibrio, Yersinia, Aeromonas*, enterohemorrhagic *Escherichia coli*).
Fluids (Body Fluids Other Than Blood, Urine)		
Pericardial, peritoneal, pleural, synovial	Send at least 2-5 mL for bacteria, >10 mL for fungi and/or mycobacteria; transport in an anaerobic collection system.	If sufficient material can be collected, inoculate aerobic and anaerobic blood culture bottles for bacterial and yeast cultures unless *Neisseria* is suspected (inhibited by anticoagulant in bottles).
Cerebrospinal	1-2 mL for bacteria 5-10 mL optimum for mycobacteria or fungi; more for chronic disease	Send immediately; notify laboratory if anaerobic culture or acid-fast stains are needed. Do not inoculate blood culture bottles.
Genital Tract (Commonly Submitted Specimens)		
Cervical, urethral	Remove mucus and secretions from the cervical os; using a fresh swab, sample the endocervical canal. Collect material expressed from the urethra on a sterile swab.	Specimens collected for culture or stains should be inoculated onto media at the time of collection or immediately transported to the laboratory; do not allow the swab to dry. If the specimen is processed by nucleic acid amplification technique, use the specific collection device designed for the system.
Vaginal	Obtain secretions from the vaginal wall with a sterile swab.	Gram stain and not culture is recommended for the diagnosis of bacterial vaginosis.
Ulcer	Collect exudate for microscopy (*Treponema pallidum*—darkfield; *Klebsiella [Calymmatobacterium] granulomatis*, *Haemophilus ducreyi*—Gram stain, *Chlamydia trachomatis*—DFA) and culture (*H. ducreyi, C. trachomatis*).	Discuss the appropriate transport system with the laboratory before specimen is collected.
Nails and Hair	Clip affected areas; transport to laboratory in envelope or dry, sterile container.	Culture for yeasts and dermatophytes.

Continued

TABLE 16-2 Guidelines for the Collection and Transport* of Specimens Submitted for Bacterial and Fungal Stains and Cultures—cont'd

SPECIMEN	COLLECTION GUIDELINES	OTHER COMMENTS
Respiratory, Upper		
Nose	Insert premoistened swab 1-2 cm into nares and rotate against nasal mucosa.	Generally used to detect carriage of *Staphylococcus aureus*; specify for laboratory.
Nasopharynx	Nasopharyngeal washings and swabs appropriate for *Bordetella pertussis, Corynebacterium diphtheriae, Neisseria gonorrhoeae, Neisseria meningitidis*.	Notify laboratory if a fastidious bacterial pathogen is considered.
Paranasal sinuses	Collect by aspiration; if bacteria are suspected, submit in anaerobic transport system.	Aerobic and anaerobic bacterial cultures should be performed if bacterial etiology is suspected; fungal stain and culture should be performed if fungal infection is suspected.
Throat or pharynx	Swab the posterior pharynx, avoiding saliva, for the recovery of *Streptococcus pyogenes*; appropriate for *B. pertussis, C. diphtheriae, N. gonorrhoeae, N. meningitidis*. Detection of yeast is generally restricted to microscopy (e.g., Gram stain, calcofluor white stain).	Notify the laboratory if a fastidious bacterial pathogen is considered.
Respiratory, Lower		
Bronchial alveolar lavage	A large volume of fluid may be collected; transport in sterile container.	Do not submit for anaerobic culture unless a special sheathed bronchoscopic brush is used; fluid should be concentrated for optimum yield from stains and cultures; some laboratories perform quantitative bacterial cultures to guide interpretation. Preferred specimen for detection of *Pneumocystis.*
Bronchial brushing or biopsy	Transport specimen in sterile container or 1 mL sterile saline; do not allow the specimen to dry.	A small volume of specimen is generally collected, and the number of tests should be limited to maximize sensitivity.
Sputum, expectorated	Have patient rinse or gargle with water to remove excess oral flora; instruct patient to cough deeply and expectorate secretions from lower airways; collect and transport in a sterile container. Collect 1 mL for bacterial culture; 5 mL or more for mycobacterial culture and molds.	Presence of epithelial cells is indicative of contamination with oral flora; a contaminated specimen is unacceptable for routine bacterial culture, but can be processed for mycobacteria or molds. Some laboratories will not process these specimens for molds other than dimorphics.
Sputum, induced	Induced with sterile saline using a nebulizer.	Contamination with oral bacteria is common; specimen is processed for bacteria despite the presence of epithelial cells. Specimen can be processed for *Pneumocystis.*
Tracheal aspirate	Acceptable for same tests as expectorated sputum; not screened for acceptability such as induced sputa.	Contamination with oral bacteria is common; specimen is processed for bacteria despite the presence of epithelial cells.
Skin Lesion	Scrape skin at active edge of lesion; avoid blood. Place in sterile petri dish; biopsy may be more definitive than swabs of lesion.	Transport swabs in transport media to prevent drying out; specify specific organism if one is suspected (e.g., dermatophyte, *Sporothrix, Mycobacterium*, etc.).
Tissues and Biopsies	Keep specimen moist and transport rapidly to laboratory. Do not use bacteriostatic saline or formalin. Biopsies are suitable specimens for routine, fungal, mycobacterial, and anaerobic cultures, depending on site of biopsy.	Always specify the type of tissue, and indicate the specific pathogen sought (e.g., *Nocardia, Bartonella, Histoplasma)* if other than routine bacteria.
Urine		
Midstream	Instruct women to hold labia apart, discard the first portion of voided urine, and collect a midstream portion in a sterile container. Instruct men to retract the foreskin, discard the first portion of voided urine, and collect a midstream portion in a sterile container. Collect first-voided urine for *C. trachomatis* and *N. gonorrhoeae* tests. Keep refrigerated and transport to laboratory promptly, or submit in urine tube with boric acid to prevent overgrowth of contaminating organisms.	Cleansing before voiding does not consistently improve the quality of the specimen; however, if the patient is unable to provide a proper specimen, cleansing and supervised collection may be necessary.
Catheterized	Discard the first portion of urine, which is likely to be contaminated with urethral flora or organisms that have colonized an indwelling catheter. Transport as for a midstream specimen.	Catheterization may result in an iatrogenic urinary tract infection.
Suprapubic aspirate	Transport aspirated urine promptly to the laboratory.	This is the only urine specimen acceptable for anaerobic culture. Urine is generally sterile, although transient colonization of the bladder may occur.
Wound (See "Abscess and Skin Lesion")	Decontaminate open lesion first. Aspirate if possible; if swabs must be used, be sure that quantity is sufficient for stains and cultures. Open, superficial wounds are not suitable for anaerobic culturing. Identify type/location of wound.	Transport in anaerobic transport media.

DFA, direct fluorescent antibody test; EDTA, ethylenediaminetetraacetic acid.
*It is assumed that appropriate transport media will be provided and used.

Supplementary systems, such as the lysis-centrifugation system (Isolator, Wampole Laboratories, Cranbury, NJ), can be used for recovery of fastidious organisms (Table 16-3). Although lysis-centrifugation offers advantages, such as quantitative cultures and isolated colonies for rapid identification, it remains a labor-intensive method that has rates of contamination higher than those of bottle systems and therefore should not be used for routine blood cultures.

For most common, nonfastidious pathogens, the most important factor that influences recovery of organisms is the volume of blood that is collected. Typically, the number of organisms in the blood of a septic patient is low (an average of less than 1 organism/mL) and the success of obtaining a positive blood culture is directly related to the volume of blood that is cultured.[7] The current recommendations for adult patients is 20 mL of blood should be collected and distributed into two

TABLE 16-3 Special Blood Culture Methods

ORGANISM	METHOD	COMMENTS
Abiotrophia and *Granulicatella*	Bottle systems	Subculture to chocolate agar or blood agar with pyridoxal
Bartonella	Lysis-centrifugation	More sensitive and faster than bottle systems. Use fresh blood agar; incubate 2-4 wk in humidified chamber
Brucella	Lysis-centrifugation	May be most sensitive system
	Bottle systems	Blind subculture may be required; incubate for 7-10 days
Campylobacter and *Helicobacter*	Lysis-centrifugation	Subculture to blood agar and Campy agar; incubate in microaerophilic atmosphere
	Bottle systems	Extend incubation for 7-10 days; may not be detected by monitoring system of automated blood culture instruments; stain broth with acridine orange
Dimorphic fungi and molds	Lysis-centrifugation	Subculture to media for recovery of fungi; extend incubation for 4 wk
Francisella	Lysis-centrifugation	Subculture to media with sulfhydryl compounds (e.g., chocolate agar, BCYE agar)
	Bottle systems	Blind subculture may be required; incubate for 7-10 days
Legionella	Lysis-centrifugation	Subculture onto BCYE agar
Leptospira	Fletcher's semisolid or EMJH medium	Incubate in air at 28° to 30°C for up to 3 mo; examine broth weekly by darkfield or phase-contrast microscopy
Mycobacterium	Lysis-centrifugation	Subculture to broth and solid media for isolation of mycobacteria
	Bottle systems	Use media specifically designed for isolation of mycobacteria
Nocardia	Lysis-centrifugation or bottle system	Incubate primary blood agar plates or of bottles for 7-10 days

BCYE, buffered charcoal yeast extract agar; EMJH, Elinghausen, McCullough, Johnson, and Harris medium for leptospires.

blood culture bottles. The routine use of a single blood culture bottle is never acceptable. For neonates and children, proportionately smaller volumes of blood should be collected. Ideally, two or three blood cultures should be collected in a 24-hour period. Additional "routine" blood cultures are generally not useful for septic patients with negative blood cultures, and alternative culture methods should be considered (see Table 16-3). It is recognized that there are practical limits on the volume of blood that can be collected from some patients, and this should be considered when interpreting the significance of negative blood cultures.

Blood cultures start with proper skin antisepsis before venipuncture: The skin surface is first cleansed with 70% alcohol, followed by 0.5% chlorhexidine gluconate (30 seconds), tincture of iodine (1 minute), or 10% povidone-iodine (2 minutes). Chlorhexidine is the most rapid, effective disinfectant.[8] The septum of the culture bottle or tube should also be wiped with 70% alcohol and allowed to air dry. Blood can be collected with a needle and syringe or directly into the culture bottles with a collection system. If a needle and syringe is used, the needle should not be changed between collection and inoculation of the blood culture system because this procedure poses an unnecessary risk of a needlestick and has not been demonstrated to reduce contaminated blood cultures significantly.[9] If a collection system is used, care must be taken to avoid overfilling the culture bottles. Separate venipunctures should be performed for each culture to minimize contamination with bacteria from the skin surface (e.g., *Staphylococcus epidermidis*, *Corynebacterium* spp., *Propionibacterium acnes*). True infections with these organisms are commonly associated with intravascular catheters. In this setting, a persistent bacteremia occurs, so all blood cultures should be positive. If only one of two or three cultures is positive, then this more likely represents contamination of the culture during collection than catheter-acquired sepsis.

Patients frequently have catheters for intravenous access, including long-term percutaneous catheters and subcutaneous implanted devices and short-term catheters used for a variety of limited vascular access needs. Although microbiologists have recommended that blood for culture should not be drawn through catheters, the reality is this is frequently done and in many cases represents the only practical access site for critically ill patients. If a culture is collected through a catheter, then the access port should be disinfected with 70% alcohol before the blood is removed. Intravascular catheters represent a source for bacteremia and fungemia with both organisms on the skin surface as well as opportunistic gram-negative rods (e.g., *Pseudomonas* spp., *Stenotrophomonas, Ochrabacterum, Methylobacterium*) and acid-fast organisms (e.g., rapidly growing mycobacteria, *Tsukamurella, Gordonia*). Typically, blood cultures collected through a contaminated line have a higher inoculum than blood collected from a peripheral vein, which can be used to determine if a line is the source. This can be documented either by using a quantitative blood culture system, such as the lysis-centrifugation system, or by demonstrating earlier detection of a positive culture collected from the catheter versus the peripheral site.[10] Thus, whenever possible, all blood cultures collected from a catheter should also include a blood culture from a peripheral venipuncture site.

Blood cultures should be incubated routinely for a minimum of 5 days. A number of studies have documented that most positive blood cultures are detected during the first 2 days of incubation, and extension of incubation beyond 5 days generally results in recovery of the more slow-growing skin contaminants.[11] An effort to reduce the incubation period further has been driven by the fact automated blood culture systems have a limited capacity for bottles. If culture bottles are incubated for a longer period of time, fewer cultures can be processed in each instrument, thus increasing the need for additional instruments. I believe that incubation for less than 5 days is not justified because a small number of significant pathogens will likely not be detected (e.g., *Aggregatibacter, Bartonella, Francisella, Campylobacter, Cryptococcus, Candida glabrata*, rapidly growing mycobacteria). Furthermore, the microbiologist should be willing to extend the incubation period for selected cultures at the request of the infectious disease specialist.

Blood culture systems have also been used to assess the sterility of platelets and cell therapy products (i.e., human cells and tissues processed in vitro and then administered for therapeutic purposes). The BacT/Alert 3D system (bioMérieux, Lyon, France) is the only system approved currently for sterility testing of platelets, but validations studies have been done with other culture systems; both the BACTEC 9240 (Becton Dickinson, Sparks, MD) and BacT/Alert 3D systems have been validated for cell therapy products.[12,13]

Intravenous Catheter Tips

Semiquantitative culture of catheter tips is most commonly performed by rolling the tip across a blood-agar plate. The presence of 15 or more colonies is indicative of a catheter infection,[14] although other investigators suggest a lower breakpoint, such as five colonies, may identify infection more accurately.[15] Other methods include culture of sonicates of segments or tips or intraluminal flushing of the catheter tip with broth to culture the inside of the catheter segment. Quantitative culture performed by flushing, sonicating, or vortexing the catheter segment and then preparing and plating a set of dilutions for colony counts is more labor intensive but may be a more accurate predictor of catheter-related sepsis.[16] Culture of the tip in nutrient broth should not be done because this will result in false-positive cultures because of contamination of the catheter during removal or handling before culture. Guidelines have been established for the prevention of catheter-related infection; these include definitions for different types of catheter-related infections.[17] Use of these definitions should help in the standardization and improvement of analyses of catheter-related infections.

Cerebrospinal, Peritoneal, Pleural, Synovial, and Pericardial Fluids

Laboratory processing of normally sterile body fluids is considered critically important. The specimens should be collected in a manner to avoid contamination and transported immediately to the laboratory. Upon receipt in the laboratory, processing these specimens should

receive priority, and the results of microscopy and antigen testing should be directly called to the clinician. An adequate quantity of fluid should be collected for the requested tests. This is generally not a problem with pleural and peritoneal fluids, but may pose a problem with CSF, synovial, and pericardial fluids. Gram staining and routine culture may require only 1 to 2 mL of fluid; however, if fungal or mycobacterial infection is suspected, larger volumes of fluid, preferably more than 5 mL for each test, increase the likelihood of successful culture. If a large quantity of pleural or peritoneal fluid is collected, then aerobic and anaerobic blood culture bottles should be inoculated, and additional fluid for microscopy, antigen testing, and nucleic acid amplification (NAA) tests should be submitted to the laboratory in a sterile container. The sensitivity of microscopy can be improved by concentrating the specimen by centrifugation when it is received in the laboratory. Although microscopy is generally positive for patients with bacterial meningitis (infection with *Listeria* is the exception), prior exposure to antibiotics can rapidly eliminate organisms in CSF or alter their Gram stain properties. Use of direct antigen tests for bacteria in CSF has fallen into disfavor in recent years because the incidence of *Haemophilus influenzae* meningitis in young children has significantly decreased with the introduction of the *H. influenzae* type B conjugated vaccine and the lack of a reliable *Neisseria meningitidis* serotype B antigen test. Laboratories need to determine the real benefit, if any, of maintaining these tests in their hospital setting because they rarely affect patient management for acute bacterial meningitis. If the direct Gram stain is negative, initiation of antibiotic therapy based on the most appropriate empirical coverage, rather than in response to antigen testing, is a safer course of action in life-threatening situations. In contrast with the bacterial tests, antigen tests for *Cryptococcus neoformans* are sensitive and specific. Cultures for the most common causes of meningitis (e.g., *Streptococcus agalactiae* [group B], *Streptococcus pneumoniae, N. meningitidis, Listeria monocytogenes, C. neoformans*) are generally positive within 1 to 2 days; however, the sensitive of culture can be significantly decreased by prior exposure to antibiotics. Detection of other organisms in culture may require extended incubation (e.g., *Nocardia, Mycobacterium*) or specialized media (e.g., *Leptospira*). Serologic testing is the test of choice for *Treponema pallidum* (neurosyphilis) and *Borrelia burgdorferi* (neuroborreliosis). Although the use of multitarget NAA tests for the detection of all bacterial pathogens in CSF would provide a rapid, specific diagnosis of meningitis, these tests are currently available only as research tools.

Processing synovial fluid and pericardial fluid is similar to CSF—generally only a small volume of fluid is available, and infection is caused by a single organism. In contrast, large volumes of pleural fluid and peritoneal fluid may be collected, and multiple organisms, including anaerobes, may be present in the specimen. Under no circumstance should a swab of these fluids be submitted to the laboratory. If a large volume of fluid is collected, it should be concentrated by centrifugation before microscopy and culture is performed. In addition, if only a limited quantity of specimen can be obtained, then the requested tests should be carefully selected and prioritized to maximize yield. Although any growth from these fluids must be considered significant and reported immediately, clinical judgment is required in assessing real significance because contamination during collection and processing of the specimen occasionally occurs. If a specific organism is suspected, then the laboratory should be notified so testing can be optimized.

Respiratory Tract Specimens

Laboratories subdivide respiratory tract specimens into two major categories: upper respiratory and lower respiratory. Common upper respiratory tract specimens include throat swabs, nasopharyngeal swabs or washings, and mouth or oral cavity swabs or scrapings. Aspirates of paranasal sinuses or the middle ear are submitted only occasionally for specific problematic cases because empirical therapy without culture is generally effective. Nasopharyngeal swabs or washings are an unreliable substitute for paranasal aspirates and should not be submitted for the diagnosis of sinusitis. Specimens submitted for the diagnosis of lower respiratory tract specimens include expectorated and induced sputa, tracheal aspirates, and bronchial lavages, brushings, and biopsies. Fine-needle lung aspirates and open lung biopsies may also be collected.

Throat swabs submitted from patients with bacterial pharyngitis are only processed for *Streptococcus pyogenes* (group A *Streptococcus*) unless a specific request is submitted to look for other agents.[18] Although it is recognized that *Streptococcus dysgalactiae* (members of group C and G β-hemolytic streptococci) may also cause pharyngitis, the lack of proven rheumatic fever sequelae, as well as only rare reports of associated poststreptococcal glomerulonephritis, has deterred laboratories from screening for and reporting these isolates. Likewise, *Arcanobacterium haemolyticum* can cause pharyngitis and a scarlet fever–like rash, but most laboratories do not screen for this uncommon organism. NAA assays and direct antigen tests are used for the rapid detection of *S. pyogenes* in throat swabs. The nucleic acid assays are highly accurate compared with culture methods, and results are available in approximately 1 hour. Antigen tests (enzyme immunoassays [EIAs]) are widely used because they are specific, relatively inexpensive, and rapid, with results available within 10 to 20 minutes; however, these tests vary in their sensitivity, especially for detecting very low numbers of organisms. The recent introduction of lateral flow chromatographic immunoassays, interpreted with small digital readers, has significantly improved the sensitivity of these antigen tests. Although it would be expected that large numbers of organisms should be recovered in patients with disease, the quality of the specimen varies according to the effectiveness of the sampling process; therefore, very low numbers of organisms may still be significant. It is currently recommended that a negative antigen test should be confirmed with a traditional culture or nucleic acid assay, although this may be unnecessary with the new generation of immunoassays. Antibiotic susceptibility testing is not routinely done on *S. pyogenes* because all isolates remain universally susceptible to penicillin.

If other agents of pharyngitis are sought (e.g., *S. dysgalactiae*, *A. haemolyticum*, *Neisseria gonorrhoeae, Corynebacterium diphtheriae*, *F. tularensis*, *Mycoplasma pneumoniae*) the laboratory must be notified because tests for agents other than *S. pyogenes* are not routinely done. For some organisms (i.e., *S. dysgalactiae*, *A. haemolyticum*) no specialized testing procedure is required; for other organisms, specialized culture media or alternative test methods are used. For example, selective media should be used for the recovery of *C. diphtheriae,* and toxin testing is required to demonstrate diphtheria toxin. Likewise, although *Bordetella pertussis* can be cultured on specialized media, nucleic acid amplification is the test of choice. Dacron, rayon, or calcium alginate swabs can be used to collect specimens for *Bordetella* culture, but calcium alginate swabs should be avoided if the specimen is submitted for nucleic acid amplification.[19]

Some laboratories screen high-risk patients for methicillin-resistant *Staphylococcus aureus* (MRSA) using nares swabs. A variety of selective, differential media have been developed for this purpose, although all are relatively insensitive unless the swab is incubated in an enrichment broth before the agar media are inoculated. Commercially produced nucleic acid amplification tests are also available for the rapid detection of this organism.[20,21]

Expectorated and induced sputa and tracheal aspirates are the most common specimens submitted for diagnosis of lower respiratory tract infections. Although these specimens are readily obtained, the significance of recovering potential pathogens that are isolated is sometimes difficult to assess because pathogens colonizing the upper airways can contaminate the specimen. It is difficult to avoid contamination of induced sputa and tracheal aspirates; however, this can be minimized in expectorated sputa by instructing the patient to cough deeply and expectorate secretions from his or her lower chest directly into a clean container. A single coughed specimen is sufficient for bacterial cultures, and repeated expectorated specimens will only increase the risk of contamination. Care should be taken to submit the proper specimen because laboratories are required to screen and reject expectorated specimens submitted for bacterial culture that are contaminated with oral secretions (identified by the presence of squamous epithelial cells). Although not ideal, contaminated lower tract specimens can be processed for *Legionella*, *Nocardia*, *Mycobacterium*, and molds because selective media can be used to suppress the growth of contaminants.

Lower respiratory tract specimens obtained by bronchoscopic procedures (lavage, brushing) or lung biopsy are important specimens that

require prompt transport to the laboratory for processing. Diagnoses obtained by the use of bronchoalveolar lavage and transbronchial biopsy have significantly decreased the need for open lung biopsies. Some laboratories use quantitative cultures of bronchoalveolar lavage specimens to assess the significance of an isolated pathogen.[22] The value of this compared with traditional semiquantitative cultures (i.e., the presence of few, moderate, or abundant colonies of a pathogen) is controversial, so the use of quantitative respiratory cultures should be determined after discussions with the infectious disease physician and pulmonary specialists. Isolation of respiratory pathogens in biopsy specimens or fine-needle aspirates of the lungs is almost always considered significant. Likewise, regardless of the collection method or number of organisms present, some respiratory pathogens are never found to colonize the upper or lower airways and detection should always be considered significant (e.g., *Legionella, M. pneumoniae, Chlamydia, Nocardia, Mycobacterium tuberculosis, Histoplasma, Blastomyces, Coccidioides, Cryptococcus*).

Infections with some respiratory pathogens (e.g., *S. pneumoniae, Legionella*) can be diagnosed by detecting specific antigens in urine; however, these tests are insensitive and should be supplemented with culture. Microscopy (e.g., Gram stain, acid-fast stains, calcofluor white stain, specific fluorescent antibody test) can provide a rapid diagnosis if positive, but are insensitive, and alternative test methods should also be used. The use of specific NAA tests is rapidly becoming the diagnostic test of choice for some pathogens and an important supplementary test for other pathogens (refer to Table 16-4). These tests are particularly important when a specific organism is considered in the differential diagnosis (e.g., *M. tuberculosis, Pneumocystis jirovecii, M. pneumoniae, Legionella pneumophila*).

Urinary Tract Specimens

Urine specimens, including voided midstream and catheterized specimens, are the most commonly processed specimens in the microbiology laboratory. Reliable test results are determined by the quality of the specimen and how it is transported to the laboratory. Although urine is typically sterile or transiently colonized with small numbers of organisms, the urethra is colonized with large numbers of organisms (e.g., *Lactobacillus*, streptococci, staphylococci). Introduction of these organisms into the specimen during collection or contamination of the specimen with vaginal or fecal organisms can compromise the specimen. This is particularly a problem if the urine is not transported to the laboratory promptly. Specific, detailed instructions should be given to each patient at the time of urine collection to minimize contamination with bacteria. This would include discarding the first portion of the voided or catheterized specimen, which would be contaminated with the urethral flora. The one exception to this rule is collection of urine for the diagnosis of urethritis caused by *N. gonorrhoeae* or *Chlamydia trachomatis*. In this situation, the first-voided urine should be sent to the laboratory. Laboratories should ensure that specimen delivery occurs within 2 hours of collection or, if this is not possible, the urine is transferred to specific tubes with agents that inhibit bacterial overgrowth (e.g., boric acid). Refrigeration of the specimen can also be done, although this is impractical if the specimen is going to be shipped to an outside laboratory for processing. Suprapubic aspirates, done primarily in infants or other patients in whom assessment of clean-voided urine is difficult or an anaerobic infection is suspected, should be clearly labeled as such so that all growth is identified and reported. This recommendation also applies to specimens obtained at cystoscopy or by other invasive procedures.

Rapid screening techniques for urinary tract infection include direct Gram stains and a variety of commercially available products, such as dipstick methods, bioluminescence, and filtration devices. A Gram stain is prepared by placing a drop of well-mixed, uncentrifuged urine onto a slide, air drying, and then staining. The slide is read with the oil immersion lens; one or more organisms per oil immersion field are equivalent to 10^5 or more colony-forming units per milliliter (CFU/mL). The presence of mixed bacterial types or a moderate amount of squamous epithelial cells usually indicates contamination with normal genital flora. The number of organisms in a urine specimen will decrease by 1000-fold or more from the first-voided morning specimen to ones collected in the evening.[23] Because randomly collected specimens from an infected patient will frequently have 10^4 CFU/mL or fewer, Gram staining is a relatively insensitive screening method for these specimens. Likewise, use of other rapid screening methods is generally insensitive with randomly collected urines.

Fortunately, the most common urinary tract pathogens grow rapidly. These organisms include *Escherichia, Klebsiella, Enterobacter, Proteus, Pseudomonas, Enterococcus,* and *Staphylococcus*. Contaminants that are generally disregarded include lactobacilli, diphtheroids, nonenterococcal α-hemolytic streptococci, and coagulase-negative staphylococci other than *Staphylococcus saprophyticus.* In some patient populations, high colony counts of *S. epidermidis* may also be considered significant. Yeast, particularly *Candida* species, may be isolated from routine midstream urine cultures. Quantitation of the yeasts is not useful in assessing the significance of the isolate, so determination of true urinary tract infection may require more invasively obtained specimens. Urine collected for mycobacterial culture should be greater than 50 mL of a first-voided specimen, and a similar volume or greater for recovery of *Cryptococcus, Blastomyces, Histoplasma*, or *Coccidioides*.

Gastrointestinal Tract Specimens

Acute diarrhea can have a wide variety of etiologies, including bacteria, viruses, and parasites. Stool specimens are preferred to rectal swabs and should be transported to the laboratory quickly. Recommendations for minimizing unnecessary stool cultures include rejection of routine bacterial stool cultures in patients who have been hospitalized for 3 days or longer.[24] Most laboratories also have guidelines that limit specimen submissions to one per day and no more than three specimens (on successive days) for initial screening of acute gastroenteritis.

The spectrum of bacterial diarrheal pathogens is relatively well defined, and all laboratories have a "routine" setup to look for the common agents, which should include at a minimum *Campylobacter, Salmonella,* and *Shigella*. The laboratory should be notified if other pathogens are suspected because selective culture methods or the use of antigen or NAA tests may be required. A comprehensive workup of fecal specimens requires the use of a variety of selective, differential culture media as well as microscopy, antigen tests, and NAA tests. Enrichment broths to detect small numbers of organisms may or may not be used. Thus, rectal swabs provide an insufficient quantity of specimen and should not be submitted. Feces should be immediately sent to the laboratory or mixed in Cary-Blair transport medium after the specimen is collected.

Some physicians have found the presence of leukocytes useful for differentiating between invasive inflammatory diarrheal disease (e.g., *Campylobacter, Shigella,* enteroinvasive *E. coli*) and secretory diarrhea (e.g., toxin-producing bacteria, viruses, parasites). Although Gram stains can be used to detect leukocytes, a more reliable test is detection of lactoferrin as a marker for leukocytes. The cells, but not lactoferrin, are degraded during delays between collection of the specimen and examination in the laboratory.

Shiga toxin–producing enterohemorrhagic *E. coli* (EHEC), the most common of which is *E. coli* O157:H7, is an important cause of colitis, frequently with gross blood in the stool specimen. Detection of this organism can be by culture on selective media (i.e., sorbitol-MacConkey agar) or detection of the Shiga toxin. Commercially available enzyme-linked immunosorbent assays (ELISAs) can be used directly on stool specimens or on cultured isolates. Laboratory tests are not widely available for other *E. coli* strains responsible for gastroenteritis (including enterotoxigenic *E. coli* [ETEC], enteroinvasive *E. coli* [EIEC], enteropathogenic *E. coli* [EPEC], enteroaggregative *E. coli* [EAEC]).

Clostridium difficile is the most common cause of hospital-acquired bacterial gastroenteritis. In addition, strains of this organism are also found in the community, either in individuals who were infected previously during a hospitalization or who were exposed to the organism in the community. Thus, *C. difficile* disease is more widespread than previously recognized.[25] Documentation of *C. difficile* disease has been problematic. The diagnostic gold standard is to isolate the organism by culture of feces and demonstrate production of enterotoxin and

TABLE 16-4 Methods for the Detection of Selected Bacteria in Clinical Specimens

BACTERIA	DETECTION METHOD[†] Microscopy	Culture	Antigen Detection	Nucleic Acid Detection	Antibody Detection	COMMENTS
Acinetobacter	A	A	C	C	C	Microscopy and culture tests of choice; characteristic microscopic morphology useful for preliminary identification
Actinomyces	A	A	C	C	C	Microscopy and culture tests of choice; extended incubation in anaerobic atmosphere required for isolation
Aggregatibacter	A	A	C	C	C	Extended incubation may be required for isolation in culture
Anaplasma phagocytophilum	B	C	C	A	A	NAA test of choice during active infection; serology positive in >90% of patients but delayed response; Giemsa stain of buffy coat smears positive in 60% of patients
Bacillus anthracis	B	A	C	B	B	Microscopy and culture used in most laboratories for initial detection; NAA tests commercially available (sensitive and specific); serologic assays for antibodies vs. protective antigen useful in culture-negative patients
Bartonella	B	A	C	B	A	Microscopy only useful for early stage of CSD and in patients with bacillary angiomatosis; culture useful primarily for immunocompromised patients with bacteremia; direct immunofluorescent antibody tests used for patients with acute trench fever; NAA tests primarily used in reference laboratories; serology (IFA, EIA) test of choice for CSD, endocarditis, and bacteremia
Bordetella pertussis	B	A	C	A	A	Microscopy (DFA) insensitive and nonspecific (if polyclonal antibodies used); culture insensitive and requires use of specialized media and prolonged incubation; NAA test of choice, sensitive and specific if appropriate controls used; serology (ELISA) useful in unvaccinated children if increases in IgG or IgA to pertussis toxin or filamentous hemagglutinin demonstrated
Borrelia	B	B	C	A	A	Culture generally not performed except in laboratories with experience; microscopy useful only for relapsing fever—test of choice; NAA test used in reference laboratories for diagnosis of Lyme disease; serology test of choice for Lyme disease—patients screened with EIA or IFA and confirmed with immunoblotting
Brucella	B	A	C	C	A	Culture is a sensitive test if prolonged incubation is used (e.g., 2 wk); microscopy generally not useful; antigen tests and NAA tests not available except in reference laboratories; serology helpful adjunct to culture; a significant rise in antibodies or titer ≥160 is highly suggestive but cross-reactivity occurs (e.g., *Francisella, Vibrio, Yersinia*)
Burkholderia cepacia complex	B	A	C	C	C	Culture on selective media test of choice; microscopy sensitive but nonspecific
Burkholderia pseudomallei	B	A	B	B	B	Culture test of choice; microscopy sensitive but nonspecific; antigen tests available with sensitivity 60%-85%; NAA tests and serology restricted to reference laboratories in endemic areas
Campylobacter spp.	B	A	A	B	C	Culture is sensitive if fresh media are used; thin organisms difficult to detect by microscopy; commercial antigen tests sensitive (80%-95%) and specific; serology primarily restricted to epidemiologic investigations
Chlamydia pneumoniae	B	C	B	B	A	Serology (MIF) is test of choice (positive IgM titer ≥16; IgG titer ≥512); NAA test sensitive and specific but not commercially available; microscopy (Giemsa, DFA) insensitive
Chlamydia psittaci	B	C	B	B	A	Serology (MIF) is test of choice; NAA test sensitive and specific but not commercially available; microscopy (Giemsa, DFA) insensitive
Chlamydia trachomatis	B	B	B	A	C	Commercial NAA test of choice; culture sensitive but infrequently used; microscopy and antigen tests have low sensitivity (70%-80%); serology (MIF test) sensitive and specific but increase in titer may not be detected in chronic or recurrent disease
Clostridium botulinum	B	A	B	C	C	Diagnosis by clinical presentation and culture from food or stool/wound, or detection of toxin in serum, stool, or food; toxin assays insensitive (<60% positive) except in children (>90%)
Clostridium difficile	B	A	B	A	C	NAA amplification of the toxin genes is the test of choice; culture is sensitive but additional tests required to determine toxin production; recent studies show antigen tests for toxins are insensitive
Clostridium tetani	B	B	C	C	C	Diagnosis primarily by clinical presentation: culture and microscopy commonly negative (>50%)

TABLE 16-4 Methods for the Detection of Selected Bacteria in Clinical Specimens*—cont'd

BACTERIA	DETECTION METHOD†					COMMENTS
	Microscopy	Culture	Antigen Detection	Nucleic Acid Detection	Antibody Detection	
Corynebacterium diphtheriae	B	A	C	B	C	Microscopy and culture tests of choice; NAA test for detection of diphtheria toxin in isolates and directly in clinical specimens is available in reference laboratories
Coxiella burnetii	C	C	C	B	A	Serology (IFA) test of choice; acute Q fever: fourfold increased titer to phase II antigen, or IgM titer ≥50 and IgG titer ≥200; chronic Q fever: IgG titer ≥800 to phase I antigen; NAA tests sensitive but not commercially available
Ehrlichia chaffeensis	B	C	C	A	A	NAA test of choice for active infection; serology (IFA) most common test; positive serology: 4-fold increase in titer or IgG ≥64
Ehrlichia ewingii	B	C	C	A	B	NAA test of choice for active infection; specific serology not available but cross reacts with *E. chaffeensis* serology
Eikenella	A	A	C	C	C	Microscopy and culture tests of choice; extended incubation may be required
Erysipelothrix rhusiopathiae	B	A	C	B	C	Culture test of choice; microscopy generally insensitive; NAA tests available in reference laboratories
Escherichia coli	A	A	B	B	C	Microscopy and culture most common tests; antigen tests for Shiga toxin sensitive and specific; NAA tests for enteric pathogens available in reference laboratories
Francisella tularensis	B	A	C	C	A	Culture on selective media with prolonged incubation and serology tests of choice; microscopy insensitive, and antigen and NAA tests not widely available; antibodies detected within 1 wk of symptoms but persist for months to years, so seroconversion or an elevated titer (agglutination ≥128) required
Haemophilus ducreyi	B	A	C	C	C	Microscopy insensitive (<50%) and nonspecific; use selective, differential media for culture
Haemophilus influenzae	A	A	B	B	C	Microscopy and culture tests of choice; antigen tests for serotype b capsular antigen in CSF less useful in vaccinated child; NAA tests available only in reference laboratories
Helicobacter pylori	C	B	A	B	A	Culture requires specialized incubation conditions (not commonly performed); microscopy restricted to histopathology; antigen detection test of choice; ELISA tests for fecal antigens sensitive and specific (>90%); urea breath tests also widely used; serum IgG tests more sensitive than whole-blood IgG tests that are used in office practices; IgA tests not recommended (too insensitive)
Kingella	A	A	C	C	C	Microscopy and culture tests of choice; extended incubation may be required
Klebsiella granulomatis	A	C	C	C	C	Microscopy (Giemsa stain, not Gram stain) test of choice; does not grow on conventional media
Legionella	C	A	A	A	B	Microscopy not recommended (including DFA); urine antigen tests sensitive (>80% for *Legionella pneumophila* serogroup 1 but insensitive or negative for other *Legionella;* culture and NAA tests tests of choice (sensitive and specific); serology less sensitive, antibodies may develop slowly and IgM may persist for 1 yr or more
Leptospira	B	B	C	B	A	Culture requires specialized techniques with organisms isolated in blood during first week of illness and urine thereafter; microscopy (darkfield) insensitive and requires experienced observer; serology most frequently performed test; microagglutination titer ≥200 or seroconversion diagnostic; serology is insensitive in early stages of disease and seroconversion may be delayed
Listeria monocytogenes	A	A	C	C	C	Microscopy and culture tests of choice; molecular probes available for identification of isolates
Mycobacterium tuberculosis	A	A	B	A	A	Microscopy and culture most commonly performed; skin test (PPD) and interferon-γ assays (e.g., QuantiFERON-TB) are most common delayed hypersensitivity tests; antigen assays available primarily in high-risk countries; NAA tests for direct detection in clinical specimens are sensitive and specific; molecular probe assays used to identify cultured isolates
Mycobacterium, other spp.	A	A	C	B	C	Microscopy and culture most commonly performed; molecular probes used to identify cultured isolates
Mycoplasma pneumoniae	C	B	B	A	A	NAA test of choice but not commercially available; serology (EIA) most commonly used; seroconversion may be delayed; cold agglutinin test not recommended

Continued

TABLE 16-4 Methods for the Detection of Selected Bacteria in Clinical Specimens*—cont'd

BACTERIA	DETECTION METHOD†					COMMENTS
	Microscopy	Culture	Antigen Detection	Nucleic Acid Detection	Antibody Detection	
Neisseria gonorrhoeae	A	A	C	A	C	NAA test of choice; high sensitivity and good specificity; microscopy and culture are less sensitive compared with NAA
Neisseria meningitidis	A	A	B	C	C	Microscopy and culture tests of choice; antigen detection tests for capsular polysaccharides in CSF, serum, and urine less sensitive than microscopy and cross react with *E. coli* K1
Nocardia spp.	A	A	C	C	C	Microscopy and culture tests of choice; extended incubation generally required for isolation
Pasteurella spp.	A	A	C	C	C	Microscopy and culture tests of choice; extended incubation may be required
Pseudomonas	A	A	C	C	C	Microscopy and culture tests of choice
Rhodococcus equi	A	A	C	C	C	Microscopy and culture tests of choice; extended incubation generally required for isolation
Rickettsia akari, R. rickettsii (spotted fever group)	B	C	C	B	A	Serology (IFA) using spotted fever group antigens sensitive but seroconversion delayed; NAA test sensitive but not commercially available
Rickettsia prowazekii, R. typhi (typhus group)	B	C	C	B	A	Serology (IFA) using typhus group antigens sensitive but seroconversion delayed; NAA test sensitive but not commercially available
Salmonella	B	A	C	B	C	Microscopy and culture tests of choice; serology (Widal test) insensitive and nonspecific—not recommended
Shigella	B	A	B	B	C	Microscopy and culture tests of choice; antigen tests for Shiga toxin useful for *S. dysenteriae* serotype 1.
Staphylococcus aureus	A	A	C	A	C	Microscopy and culture most commonly used; commercial NAA tests for identification of *S. aureus* and detection of MRSA in clinical specimens
Stenotrophomonas maltophilia	A	A	C	C	C	Microscopy and culture tests of choice
Streptococcus agalactiae (group B)	A	A	C	A	C	Microscopy and culture most commonly used tests: antigen tests used to detect group-specific antigen in specimens are not recommended (sensitivity, 60%); commercial NAA tests detect organism in specimens
Streptococcus pneumoniae	A	A	B	B	C	Microscopy and culture most commonly used tests; antigen tests used to detect C polysaccharide in urine (sensitivity, 50%-80%); commercial NAA tests only for identification of isolates in culture
Streptococcus pyogenes (group A)	A	A	A	A	B	Microscopy and culture most commonly used tests: antigen tests used to detect group-specific antigen in specimens (sensitivity, 80%); commercial NAA tests detect organism in specimens; serology used for diagnosis of poststreptococcal disease
Treponema pallidum	B	C	C	B	A	Unable to grow in culture; darkfield microscopy and DFA sensitive in primary syphilis; NAA tests recently introduced for primary and secondary disease (sensitive and specific); serology is most commonly performed test and test of choice; nontreponemal and treponemal tests are sensitive and generally specific (refer to text)
Tropheryma whipplei	B	C	C	A	C	NAA test of choice, available in reference laboratories;
Vibrio spp.	A	A	B	B	C	Microscopy and culture most common tests; tests for O1 antigen are available in endemic areas; NAA tests available in reference laboratories
Yersinia enterocolitica	A	A	C	B	C	Microscopy and culture tests of choice; extended incubation may be useful
Yersinia pestis	A	A	A	A	C	Microscopy, culture, antigen tests, and NAA tests all sensitive; direct antigen tests provide rapid, sensitive (90%-100%) diagnosis

*Organisms not listed are generally detected primarily by culture and microscopy.

†A, Test is generally useful for the indicated diagnosis; B, test is useful under certain circumstances or for the diagnosis of specific forms of infection; C, test is seldom useful for general diagnostic purposes.

CSD, cat-scratch disease; CSF, cerebrospinal fluid; DFA, direct fluorescent antibody test; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IFA, indirect fluorescent antibody test; IgA, IgG, and IgM, immmunoglobulin A, G, and M, respectively; MIF, microimmunofluorescence; MRSA, methicillin-resistant *Staphylococcus aureus;* NAA, nucleic acid amplification (includes polymerase chain reaction); PPD, purified protein derivative.

cytotoxin; however, this assay ("toxigenic culture") requires incubation for up to 5 days and is not clinically practical. Various immunoassays are commercially available for detection of the *C. difficile* enterotoxin and cytotoxin, but these assays are insensitive and nonspecific and should not be performed. Controversy surrounds use of immunoassays for glutamate dehydrogenase (GDH), a cytoplasmic enzyme present in *C. difficile* as well as some other bacteria. Because GDH is not a unique marker for *C. difficile* and does not determine if toxin is produced, a positive GDH assay must be confirmed with a toxin-specific NAA test. The controversy with this approach relates to the GDH sensitivity, which has been reported to vary from 75% to 100%. Because a screening test must have very high sensitivity and the inherent delays

associated with multistep testing, the current diagnostic test of choice is use of a commercial toxin-specific NAA test with high sensitivity and specificity.[26-28]

Some hospitals have established a program of screening high-risk patients for fecal colonization with vancomycin-resistant enterococci (VRE). Selective media, such as bile esculin azide agar (Enterococcosel agar) supplemented with 6 μg/mL vancomycin, are effective. Alternative, commercial NAA tests are available for detecting the genes responsible for this resistance (*vanA, vanB*). The *vanA* test appears to be sensitive and specific; however, reactivity with nonenterococcal enteric bacteria carrying the *vanB* gene has compromised the utility of the *vanB* test.

The discovery of *Helicobacter pylori* and its association with gastritis has resulted in the need for diagnostic verification of the presence or absence of this organism. Although microscopy and culture were important in the original studies demonstrating this organism in gastric biopsies, diagnosis now is made by antigen testing or serology. Commercial immunoassays are available for detecting *H. pylori* antigens in fecal specimens and have a high sensitivity and specificity (>90%). Detection of serum immunoglobulin G (IgG) antibodies to *H. pylori* is also widely used to provide evidence of past or recent infection.

Genital Specimens

The genital tract normally contains many organisms, including coagulase-negative staphylococci, lactobacilli, corynebacteria, streptococci, anaerobes, and yeast. Genital tract infection may be caused by members of this endogenous population (e.g., *S. agalactiae*) or exogenous sexually-transmitted pathogens (e.g., *N. gonorrhoeae, C. trachomatis, Haemophilus ducreyi, T. pallidum, Klebsiella [Calymmatobacterium] granulomatis*). Commonly submitted specimens include vaginal or penile discharge, urine, genital ulcers, and urethral, cervical, and anorectal swabs. Certain pathogens are known to be associated with specific types of genital infection; thus, the physician should select the appropriate diagnostic specimens and tests based upon the likely pathogen.

Vaginitis with accompanying discharge can be caused by *Trichomonas vaginalis, Candida,* or bacteria. *T. vaginalis* is discussed later in this chapter. Vaginal candidiasis is most frequently diagnosed by direct smears that show many budding yeast and pseudohyphae. Although culture of *Candida* can be accomplished rapidly and easily, growth of *Candida* is not necessarily diagnostic of candidal infection because many asymptomatic women harbor *Candida* spp. Bacterial vaginosis results from a disruption of the normal bacterial population in the vagina with a decrease in lactobacilli and a proliferation of a mixture of bacteria, including *Mobiluncus*, *Mycoplasma hominis, Gardnerella, Prevotella,* and *Atopobium*. The diagnosis of bacterial vaginosis is not based on bacterial culture because growth and identification of these presumed pathogens is difficult, slow, and unnecessary. Gram staining of vaginal secretions suggests bacterial vaginosis when the normally predominant gram-positive rods (i.e., lactobacilli) are absent and replaced with a prevalence of delicate gram-negative and gram-variable curved or fusiform bacteria (i.e., *Mobiluncus*) and gram-variable coccobacilli on epithelial cells (*Gardnerella vaginalis* on "clue" cells). The watery character of the discharge, a pH greater than 4.5, and the characteristic fishy amine odor after addition of potassium hydroxide (KOH) are also used to support this diagnosis.[29]

N. gonorrhoeae is a major pathogen in genital infections such as cervicitis, salpingitis, urethritis, and epididymitis. This organism is labile; therefore, if culture is performed, then the specimen should be sent promptly to the laboratory in appropriate transport media and should never be refrigerated. Specimens should also not be transported to the laboratory on swabs. NAA tests have replaced culture in most laboratories because the tests are more sensitive and not influenced by transport delays. Culture and most NAA tests can also be used with anorectal or oropharyngeal specimens. Attempts to culture *C. trachomatis* have also been replaced by NAA tests, and tests for both *N. gonorrhoeae* and *C. trachomatis* can be performed on the same specimen. A rapid presumptive diagnosis of gonorrhea in symptomatic men can be made by examining a Gram stain of urethral discharge. The sensitivity and specificity of urethral smears in this setting are as high as 95%. The sensitivity decreases when urethral specimens from asymptomatic men are examined, although the specificity remains good. Gram stains of cervical discharge are thought to be less reliable because of the presence of other organisms, which can decrease the test specificity.

Detection of *Haemophilus ducreyi* requires the use of specialized media and extended incubation. The laboratory must be notified if this organism is suspected. *Klebsiella granulomatis* is an organism that does not grow on conventional media. Diagnosis is made by observing the organism in tissue with the Giemsa stain. This is a test more commonly performed in the anatomic pathology laboratory rather than a microbiology laboratory. Diagnosis of *T. pallidum* infection can be made by examining a freshly collected specimen by darkfield microscopy (must be examined immediately after collection while the spirochetes are still motile), a direct fluorescent antibody test (reagents are not readily available), or most commonly by serology.

Skin, Skeletal, and Soft Tissue Specimens

Specimens sent to the laboratory include tissue biopsies, aspirates or swabs of abscesses, wound swabs, material obtained by surgical débridement, and drainage samples. The usefulness of processing these specimens by microscopy and culture is limited by the site of the lesion. Lesions that communicate directly with the skin or mucosal surfaces are commonly contaminated with a mixed population of organisms. For meaningful test results, laboratories prefer surgically obtained tissue samples, aspirates of closed abscesses, and an aliquot of pus or fluid rather than swab samples. When anaerobic bacteria are expected, the specimen should be inoculated into an anaerobic transport container and delivered promptly to the laboratory. Swabs of superficial skin ulcers, from the skin surface of a sinus tract, or from open abscesses commonly yield mixed bacterial flora and often do not reflect the organisms of true infectious significance and should be avoided.

Wound infections and abscesses are caused by many different organisms, including aerobic, facultatively anaerobic, and strict anaerobic bacteria, mycobacteria, and fungi. It is important for the clinician and the laboratory technician to recognize that certain organisms are often associated with particular types of wounds or abscesses. An infected animal bite may yield *Pasteurella multocida* or *Capnocytophaga*; a post-traumatic hand infection may yield *S. aureus, Mycobacterium marinum,* or *Sporothrix schenckii,* depending on the source of trauma; a postoperative wound infection might yield *Pseudomonas* or *Acinetobacter*, among others. Fungal, mycobacterial, and anaerobic cultures must be specifically requested if these organisms are suspected. When fastidious organisms such as *Francisella, Brucella,* or *Bartonella* are sought, the laboratory should be notified so that cultures can be set up appropriately and held for prolonged incubation as needed. Providing the laboratory with the location or type of wound, abscess, or tissue is often useful because it may hasten the recognition of specific pathogens known to be associated with a particular type of infection (e.g., cat bite, brain abscess) or anatomic site.

Whenever an ample amount of specimen is available, microscopy should be performed to obtain some preliminary indication of the infecting organism(s). If antibiotic therapy has been initiated, direct smear may be the only available guide to the etiology because growth may be inhibited. Impression smears are made by gentle pressing of a freshly cut surface of the tissue onto a slide, which can then be stained for bacteria, mycobacteria, and fungi. Direct examination of specimens by microscopy can provide preliminary information about the quality of the specimen (e.g., many epithelial cells in a wound specimen indicates skin contamination), guides empirical therapy by the physician, and directs culture strategies for the microbiologist. For example, if organisms resembling nocardia or mycobacteria are observed in a Gram-stained specimen submitted for routine culture, additional processing to recover these organisms can be performed. The organisms observed by microscopy should be correlated with culture results. Discrepancies may help identify an organism that would not otherwise be detected. For example, faintly staining gram-negative rods that do not grow on routine aerobic plates should suggest the possibility of an anaerobic organism, such as *Bacteroides.*

In some hospital settings, quantitative wound, burn eschar, or tissue biopsy specimen cultures have been used to help predict the likelihood of wound sepsis, burn infection, or the success of skin grafting. Quantitative culture requires weighing and careful preparation of the specimen for serial dilutions to determine whether the colony count is greater than 10^5 CFU/g of tissue. Such colony counts are correlated with a greater likelihood of infection associated with wound closure. Direct Gram smears of known quantities of specimen can also be used to give an immediate assessment of organism load. Because quantitative cultures are time consuming and labor intensive, not all laboratories have procedures in place for performing these assays. The availability of quantitative wound cultures should be checked by consultation with the laboratory before tests are ordered.

DETECTION METHODS

Five general approaches are used to detect organisms and the host response to specific infections: microscopy, culture, detection of bacterial antigens or nucleic acids, and detection of antibodies directed against the organism (see Table 16-4). An overview of detection methods for bacteria is summarized in this section.

Microscopy

Table 16-5 is a summary of the stains used to detect bacteria, fungi, and parasites. Bacteria are detected most commonly with differential (e.g., Gram, acid-fast, Giemsa) and fluorescent stains (acridine orange, auramine-rhodamine stains). The Gram stain is a simple, yet reliable stain that can provide important preliminary information on whether a bacterial infection is caused by a gram-negative or a gram-positive organism and whether the organism is a rod or a coccus. An experienced microscopist can further classify organisms based on subtle differences in morphology or special arrangements of the cells. For example, staphylococci are gram-positive cocci arranged in clusters; streptococci are gram-positive cocci arranged in chains; *S. pneumoniae* and *Enterococcus* are gram-positive cocci arranged in pairs; Enterobacteriaceae are gram-negative rods that stain more intensely at the ends rather than the center [bipolar staining]; *Pseudomonas*, *Stenotrophomonas*, and *Burkholderia* are small gram-negative rods frequently arranged in pairs; *Acinetobacter* are fat, coccobacilli that retain crystal violet when stained and resemble gram-positive cocci. *Nocardia*, mycobacteria, yeasts, and molds may also stain with the Gram stain.

The acid-fast stain is used to detect bacteria with medium- and long-chain mycolic acids in their cell wall. Only a limited number of genera responsible for human disease retain acid-fast stains: *Mycobacterium, Nocardia, Rhodococcus, Tsukamurella,* and *Gordonia*. The genera other than *Mycobacterium* generally stain weakly with acid-fast stains and are referred to as partially or weakly acid-fast. The Giemsa and Wright-Giemsa stains are differential stains used primarily to detect bacteria (*Borrelia*) or bacterial inclusions (*Anaplasma, Ehrlichia*), as well as fungi and parasites, in blood specimens. Although the stains are insensitive, a positive stain is rapid confirmation of the presence of the organism. Fluorescent stains are generally the most sensitive microscopic stains because bacteria stained with fluorescent dyes are easy to detect in an otherwise black background. Auramine and rhodamine dyes are used in the variation of the acid-fast stain to detect mycobacteria. This stain is more sensitive than traditional acid-fast stains and is equally specific. The acridine-orange stain is used to detect bacteria in positive blood cultures when the Gram stain is negative and when certain very thin organisms are difficult to detect with the Gram stain (e.g., *Campylobacter, Helicobacter*). Specific fluorescent antibody stains with antiserum conjugated to a fluorescent label are used both for direct detection of bacteria in patient specimens as well as identification of isolated organisms. At present, direct fluorescent antibody (DFA) stains are more commonly used for the detection and identification of viruses rather than bacteria. DFA tests are still used in some laboratories for selected bacteria (e.g., *S. pyogenes*, *T. pallidum*, *Legionella,* and *B. pertussis*); however, the DFA test for *S. pyogenes* (group A *Streptococcus*) has primarily been replaced by antigen tests; the DFA test for *T. pallidum* is sensitive and specific for primary syphilis, but the reagents are not widely available; the DFA test for *Legionella* has low sensitivity; and the DFA tests for *B. pertussis* has low sensitivity and specificity.[30]

TABLE 16-5 Microscopic Stains for Bacteria, Fungi, and Parasites

STAIN	APPLICATION
Wet Mount and Contrasting Stains	
Wet mount	Used to directly examine aspirates, feces, vaginal discharge, urine sediment for fungi and parasites; bacteria can be observed using darkfield microscopy
Potassium hydroxide (KOH)	KOH digests host cell components (e.g., skin cells) and enables detection of fungi
India ink	Contrasting dye to detect encapsulated bacteria (e.g., *Bacillus anthracis*) and fungi (e.g., *Cryptococcus*)
Lugol's iodine	Contrasting dye for detection of protozoa in feces
Methylene blue	Contrasting dye for detecting bacteria and fungi
Differential Stains	
Acid-fast stains (Ziehl-Neelsen, Kinyoun, modified Kinyoun)	Used to detect acid-fast and partially acid-fast bacteria (e.g., *Mycobacterium, Nocardia, Rhodococcus, Tsukamurella, Gordonia, Legionella micdadei*), microsporidia, and parasites (*Cryptosporidium, Cyclospora, Cystoisospora, Sarcocystis*)
Giemsa and Wright-Giemsa stains	Differential stain for detection of bacteria (e.g., *Borrelia, Ehrlichia, Anaplasma, Chlamydia*), fungi (e.g., *Toxoplasma, Pneumocystis*), and parasites (e.g., microfilariae, *Babesia, Plasmodium*) in blood
Methenamine silver	Performed in histology labs rather than in microbiology. Used for detection of fungal elements in tissues, although other organisms such as bacteria can be detected
Gram stain	Differential stain for bacteria and yeasts; mycobacteria and nocardia will partially stain
Iron-hematoxylin stain (Delafield's)	Differential stain for detection of microfilariae in blood
Iron-hematoxylin stain (Tompkins-Miller method)	Differential stain for detection of intestinal protozoa cysts and trophozoites
Spore stain	Stain for detection of spores in *Clostridium* and *Bacillus*
Trichrome and modified trichrome stains	Differential stain for detection of intestinal protozoa cysts and trophozoites and microsporidia
Fluorescent Stains	
Acridine-orange stain	Used to detect bacteria, particularly thin ones (e.g., *Helicobacter, Campylobacter*), and fungi
Auramine-rhodamine stain	Used to detect acid-fast and partially acid-fast bacteria
Calcofluor white stain	Nonspecific fluorescent stain for fungi (including *Pneumocystis, microsporidia*) and parasites (e.g., protozoa cysts)
Direct fluorescent antibody	Specific antibody-mediated, fluorescent stain used primarily to detect viruses in clinical specimens

Culture

The foundation for much of what we know about bacteria is based on the ability to grow the organisms in vitro. A variety of media, including broth media and media solidified with agar, have been developed for this purpose: general enriched, selective, differential, and specialized media (Table 16-6). Use of broth media allows the detection of small numbers of organisms or anaerobic organisms from a specimen where anaerobes are infrequently recovered (e.g., CSF). General enriched media (e.g., blood agar, chocolate agar) are designed to support the growth of most organisms. Selective media (e.g., MacConkey agar, phenylethyl alcohol agar) are designed to support the growth of certain organisms (e.g., gram-negative or gram-positive bacteria) and suppress the growth of other organisms. These media are particularly useful if a mixed population of organisms is present in a specimen. Differential media typically are selective media that contain substrates facilitating recognition of specific organisms (e.g., lactose-fermenting gram-negative rods growing on MacConkey agar, *Salmonella* or *Shigella* from a stool specimen inoculated onto xylose-lysine-deoxycholate [XLD] agar). Some organisms have very specific growth requirements (e.g., *Legionella* requires iron and cysteine; *B. pertussis* requires media with inhibitors of fatty acids, sulfides, and peroxides), so specialized media

TABLE 16-6 Selected Media for Recovery of Bacteria, Fungi, and Parasites

ORGANISM	MEDIA	COMMENTS
Bacteria		
Aeromonas spp.	Cefsulodin-Irgasan-novobiocin (CIN) agar	Selective, differential agar; will grow on routine media
Bordetella pertussis, B. parapertussis	Regan-Lowe medium; Bordet-Gengou medium	Selective medium; organisms will not grow on routine media
Borrelia burgdorferi	Barbour-Stoenner-Kelly medium	Incubate at 30° to 33°C in microaerophilic atmosphere for 6 wk or more
Burkholderia cepacia	*Burkholderia cepacia* selective agar; *Pseudomonas cepacia* (PC) agar; oxidative-fermentative polymyxin B-bacitracin-lactose (OPBL) medium	Selective, differential agar; will grow on routine media
Campylobacter spp.	Campy-CVA medium, charcoal cefoperazone desoxycholate agar (CCDA)	Selective media; recovery optimal using multiple media; incubation at 37° to 42°C in microaerophilic atmosphere
Clostridium difficile	Cycloserine-cefoxitin-fructose agar (CCFA)	Selective, differential agar; will grow on routine media
Corynebacterium diphtheriae	Cystine-tellurite blood agar; Tinsdale agar	Selective, differential media; will grow on routine media
Escherichia coli O157	Sorbitol-MacConkey agar	Selective, differential agar; will grow on routine media
Francisella tularensis	Chocolate agar; buffered charcoal yeast extract (BCYE) agar	Requires sulfhydryl compounds (cysteine, cystine, thiosulfate, IsoVitaleX)
Helicobacter spp.	Multiple enriched media	Incubate in microaerophilic atmosphere
Legionella spp.	BCYE agar	Requires L-cysteine; growth enhanced by iron; supplemented with antibiotics to suppress oral flora
Leptospira spp.	Ellinghausen-McCullough-Johnson-Harris (EMJH), Fletcher's medium with rabbit serum	Selective medium; incubation at 28° to 30°C for up to 3 mo; will not grow on routine media
Mycobacterium spp.	Dubos broth; Lowenstein-Jensen medium; Middlebrook agar and 7H9 broth	Enriched media; will grow slowly on routine media
Neisseria gonorrhoeae	Modified Thayer-Martin agar, New York City agar	Selective media; will grow on chocolate agar but not blood agar
Nocardia spp.	BCYE agar	Enriched medium; will grow slowly on routine media
Salmonella spp.	Hektoen enteric (HE) agar; *Salmonella-Shigella* (SS) agar; xylose-lysine-desoxycholate (XLD) agar; chromogenic agar	Selective, differential agars; will grow on routine media
Shigella spp.	Hektoen enteric (HE) agar; *Salmonella-Shigella* (SS) agar; xylose-lysine-desoxycholate (XLD) agar	Selective, differential agars; will grow on routine media
Staphylococcus aureus	Mannitol salt agar (MSA); chromogenic agar	Selective, differential agar; will grow on routine media
S. aureus, methicillin-resistant (MRSA)	MSA with 4 µg/mL methicillin (oxacillin); chromogenic agar	Selective, differential agar; will grow on routine media
Streptococcus agalactiae (group B)	Lim broth; StrepB carrot broth	Selective, differential broths; will grow on routine media
Vancomycin-resistant *Enterococcus* (VRE)	Bile esculin azide agar (Enterococcosel) supplemented with 6 µg/mL vancomycin	Selective, differential agar; will grow on routine media
Vibrio cholerae, V. parahaemolyticus	Thiosulfate citrate bile salt sucrose (TCBS) agar	Selective, differential agar; will grow on most media
Yersinia enterocolitica	CIN agar	Selective, differential agar; will grow on routine media
Fungi		
Cryptococcus neoformans	Birdseed (niger seed) agar	Selective, differential agar for presumptive identification
Malassezia furfur	Agar medium supplemented with lipids	Requires lipids for growth (e.g., whole milk, malt extract, ox bile, Tween 40, oleic acid, olive oil)
Yeasts	Chromogenic agar	Selective, differential agar for presumptive identification
Dermatophytes	Dermatophyte test medium (DTM)	Selective, differential agar; stimulates sporulation
Parasites		
Acanthamoeba	Non-nutrient agar overlaid with *E. coli* or *Enterobacter aerogenes*	Ameba digests bacteria as the protozoa moves over the agar surface
Leishmania	Novy-MacNeal-Nicolle (NNN) medium	
Trichomonas	Diamond's complete medium; InPouch TV	InPouch TV is commercially available
Trypanosoma	Novy-MacNeal-Nicolle (NNN) medium	

have been developed for recovery of these organisms. Particularly important specialized media are used for performing antimicrobial susceptibility tests. The success of culture methods is dependent on the incubation conditions. The obvious example is recovery of most anaerobes requires that the inoculated agar plates be incubated in an anaerobic atmosphere. Likewise, some organisms, such as *Campylobacter* and *Helicobacter,* grow only in a microaerophilic atmosphere. Some organisms grow slowly and require extended incubation (e.g., *Bartonella, B. pertussis, Brucella, Francisella, Legionella*). It is impractical and unnecessary for the microbiologist to inoculate all specimens onto the large variety of available media and incubate the cultures in the numerous incubation conditions. Indeed, the microbiologist must frequently make decisions about how to best culture a specimen of limited quantity. Thus, it is critical for the clinician to clearly describe the source of the specimen and the suspected pathogens. If a specimen such as a biopsy or normally sterile fluid is collected, and collection of additional material is not practical, then it is important for the clinician to talk with the microbiologist to avoid mishandling the specimen. All laboratories should have detailed procedures for processing specimens from a variety of sources. These protocols should be developed using standard reference manuals[5] and after discussions with clinicians.

It should be recognized that knowledge of the microbes that colonize the human body is rapidly evolving with the introduction of DNA sequencing techniques that can detect previously uncultured organisms. As this work expands, it is likely that previously unrecognized organisms will be associated with infections.

Antigen Detection

Table 16-7 summarizes the available antigen tests for detection of bacteria, fungi, viruses, or parasites in clinical specimens. The advantages of these tests are they can be rapidly performed and are relatively inexpensive. The disadvantages are poor sensitivity or specificity for certain organisms and limited availability of some assays (e.g., assays for *Yersinia pestis*, Norovirus, *Plasmodium falciparum*). Antigen tests

TABLE 16-7 Antigen Tests for Detection of Bacteria, Fungi, Viruses, and Parasites in Clinical Specimens

Bacteria
Campylobacter jejuni and *C. coli*
Chlamydia trachomatis
Clostridium difficile
Escherichia coli (Shiga toxin)
Haemophilus influenzae type B
Helicobacter pylori
Legionella pneumophila serogroup 1
Neisseria meningitidis
Shigella dysenteriae (Shiga toxin)
Streptococcus pneumoniae
Streptococcus pyogenes (group A)
Yersinia pestis
Fungi
Aspergillus spp.
Blastomyces dermatitidis
Candida albicans
Coccidioides spp.
Cryptococcus neoformans
Histoplasma capsulatum
Panfungal*
Viruses
Adenovirus
Cytomegalovirus
Dengue virus
Hepatitis B virus
Herpes simplex virus (HSV-1, HSV-2)
Human immunodeficiency virus (HIV-1, HIV-2)
Human metapneumovirus
Influenza virus A, B
Norovirus
Respiratory syncytial virus
Rotavirus
Parasites
Cryptosporidium parvum (hominis)
Entamoeba histolytica/E. dispar
Giardia lamblia (duodenalis)
Plasmodium spp.
Trichomonas vaginalis

*Panfungal, beta-D-glucan assay that detects cell wall antigen in all fungi except Zygomycetes (not present) and *Cryptococcus* (low level).

for bacteria responsible for meningitis (i.e., *S. agalactiae, S. pneumoniae, N. meningitidis, H. influenzae* type B) are not recommended as a screening assay because they are no more sensitive than a Gram stain; however, these assays can be useful for the presumptive identification of an organism observed on Gram staining. A diagnosis of pneumonia caused by *S. pneumoniae* or *L. pneumophila* can be confirmed by detection of specific antigens in urine; however, negative assays must be confirmed by culture. Likewise, although group A *Streptococcus* antigen tests are widely used, negative assays must also be confirmed by either culture or a nucleic acid amplification assay. Antigen tests for the detection of *Campylobacter* or *Helicobacter* antigens in stool specimens or Shiga toxin produced by *E. coli* are rapid, sensitive assays for diagnosing infections caused by these organisms. Antigen tests are also widely used for detection of *C. difficile* toxins in stool specimens; however, the tests are insensitive and nonspecific and should not be used.[31] Immunoassays for *C. trachomatis* antigens have now been replaced in most laboratories by NAA tests. Thus, the value of antigen tests must be carefully assessed based on the performance characteristics of the individual assays and the availability of alternative detection methods.

Nucleic Acid–Based Tests

The commercial development of nucleic acid–based tests has increased dramatically since the last edition of this reference book. Previously, tests were restricted primarily to the detection of *N. gonorrhoeae*, *C. trachomatis*, and *M. tuberculosis* in clinical specimens,; screening blood products for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV); and the use of nonamplified probes for identification of selected bacteria and fungi in clinical specimens. In the last few years, the number of companies that have entered the field of molecular diagnostics has increased, as have the variety of diagnostic tests that are approved by the U.S. Food and Drug Administration (FDA) (Table 16-8). Particularly noteworthy is the development of multiplex tests for detection of the most common pathogens of the respiratory and intestinal tracts. Despite the complexity of tests, the testing platforms are also undergoing a transformation, moving from the need to have multiple rooms and instruments for specimen processing, nucleic acid amplification, and amplified product analysis to the use of disposable, sealed kits that allow testing on simplified instruments by minimally trained technologists. Leading examples of this approach include BD MAX (Becton-Dickinson, Sparks, MD), BioFire FilmArray (Biofire Diagnostics, Salt Lake City, UT), GeneXpert (Cepheid, Sunnyvale, CA), and Verigene (Nanosphere, Northbrook, IL). It is anticipated that this technology will continue to be refined, the menu of approved tests expanded, and the cost and technical ease permitting nucleic acid amplification tests to be performed by most clinical microbiology laboratories.

Serology

Serology has been used historically to confirm infections with bacteria, fungi, and viruses that are difficult to detect by other methods. The difficulty with serology is that some immunocompromised patients will not mount an adequate antibody response to infection, a significant increase in antibody titer may not be detected until weeks or months after the initial presentation, persistence of antibodies may make it difficult to differentiate between a recent and a past infection, and cross-reactions may compromise the specificity of the antibody response. In general, serology should be used to confirm other diagnostic tests whenever possible.

IDENTIFICATION METHODS FOR BACTERIA

We believe that most readers of this chapter would either have minimal interest in identification methods for bacteria, would consult specific chapters in this book for an overview of the subject, or would refer to a microbiology reference book for detailed information. For that reason, the information covered in this section is restricted to general comments concerning identification methods. For more detailed information, please refer to the individual chapters in this book or to a microbiology reference book.[5]

Identification by Biochemical Methods

The microscopic morphology and characteristics, such as colony size, color, and shape, and the presence or absence of hemolytic activity, are used by the microbiologist in selection of the appropriate identification procedure. An experienced microbiologist often is able to provide a reliable presumptive identification of common bacteria on the basis of these early characteristics and some preliminary identification tests. For example, an opaque white or light yellow colony that appears as gram-positive cocci found predominantly in clusters will be tested with a rapid coagulase test to identify the isolate as *S. aureus* or a coagulase-negative *Staphylococcus* species. Table 16-9 describes key tests that are commonly used, along with colony and Gram stain morphology, to identify selected species of bacteria.[32]

The historical classification of bacteria was based on their microscopic properties (e.g., gram-positive, gram-negative, acid-fast; cocci, rods, spiral-shaped), oxygen tolerance (i.e., aerobic, anaerobic), and patterns of biochemical reactivity (Table 16-10). The biochemical tests included fermentation or oxidation of carbohydrates (e.g., fermentation of glucose or lactose), use of carbon sources, presence of specific enzymes, or production of volatile fatty acids. A minimum number of these tests performed in test tubes could identify most of the

TABLE 16-8 Nucleic Acid–Based Tests for Detection of Bacteria, Fungi, Viruses, and Parasites in Clinical Specimens

TECHNOLOGY	MANUFACTURER	PRODUCT	TARGETS
Nonamplified Tests			
Peptide nucleic acid probes	AdvanDx (Woburn, MA)	PNA FISH	*Staphylococcus aureus/CNS; Enterococcus faecalis/other Enterococcus; Escherichia coli/Pseudomonas aeruginosa; E. coli/P. aeruginosa/Klebsiella pneumoniae; Candida albicans/C. glabrata; C. albicans/C. parapsilosis/C. krusei/C. glabrata/C. tropicalis*
DNA target probes	BD Diagnostics (Sparks, MD)	Affirm VPIII	*Candida* spp./*Gardnerella vaginalis, Trichomonas vaginalis*
rRNA target probes	Hologic Gen-Probe (San Diego, CA)	AccuProbe	Direct from specimen: group A *Streptococcus* Culture ID: group B *Streptococcus, S. pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis* complex, *M. kansasii, M. avium, M. intracellulare, M. avium* complex, *M. gordonae, Mycoplasma/Acholeplasma/Ureaplasma, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum*
		PACE	*Neisseria gonorrhoeae, Chlamydia trachomatis*
DNA target probes	Nanosphere (Northbrook, IL)	Verigene	Blood culture ID: *Staphylococcus* spp., *S. aureus, S. lugdunensis, S. epidermidis, Streptococcus* spp., groups A and B *Streptococcus, S. anginosus* group, *S. pneumoniae, Micrococcus* spp., *E. faecalis, E. faecium, Listeria* spp., and resistance markers *mecA,* van*A, vanB*
Amplified Tests			
Polymerase chain reaction (PCR)	Abbott Molecular Diagnostics (Abbott Park, IL)	RealTime PCR	*N. gonorrhoeae, C. trachomatis,* HIV-1, HBV, HCV
	BD Diagnostics	BD GeneOhm	MRSA, StaphSR, group B *Streptococcus, Clostridium difficile*
		BD MAX	MRSA, StaphSR, group B *Streptococcus, C. difficile*
	BioFire (formerly Idaho Technology; Salt Lake City, Utah)	FilmArray	*Bacillus anthracis, Francisella tularensis* Multiplex respiratory panel: adenovirus, coronavirus (HKU1, NL63, 229E, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza virus (A, A/H1, A/H3, A/H1-2009, B), parainfluenza virus (1-4), respiratory syncytial virus, *Bordetella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae*
	Cepheid (Sunnyvale, CA)	GeneXpert	*S. aureus,* MRSA, SSTI, group B *Streptococcus, Enterococcus vanA* gene, *N. gonorrhoeae, C. trachomatis,* influenza virus (A, A/H1-2009, B), enterovirus
	Focus Diagnostics (Cypress, CA)	Simplexa	*C. difficile*, influenza virus (A, A/H1N1 2009), influenza virus (A, B) + respiratory syncytial virus
	Hologic Gen-Probe	Prodesse	*C. difficile*, human metapneumovirus, adenovirus, influenza virus (A/H1, A/H3, A/H1N1 2009), parainfluenza virus (1-3), influenza A/B + respiratory syncytial virus
	Luminex (Austin, TX)	xTAG	Respiratory virus panel: influenza virus (A, A/H1, A/H3, B), respiratory syncytial virus, human metapneumovirus, rhinovirus, adenovirus) Gastrointestinal pathogen panel: *C. difficile, Campylobacter, Salmonella, Shigella, E. coli,* rotavirus A, Norovirus
	Nanosphere (Northbrook IL)	Verigene	*C. difficile* Respiratory virus panel: influenza virus (A, H1, H3, H1N1 2009, B), respiratory syncytial virus (A, B)
	Roche Molecular Diagnostics (Pleasanton, CA)	Amplicor	*N. gonorrhoeae, C. trachomatis,* human papillomavirus, HIV-1, HCV
		AmpliPrep/COBAS	CMV, HIV-1, HBV, HCV
		AmpliScreen	HIV-1, HBV, HCV
		COBAS TaqMan	HIV-1, HBV, HCV
		COBAS TaqScreen	West Nile virus Multiplex: HIV-1 (M, O), HIV-2, HBV, HCV
		LightCycler	MRSA
Strand displacement amplification (SDA)	BD Diagnostics	BD ProbeTec	*N. gonorrhoeae, C. trachomatis*
		BD Viper	*N. gonorrhoeae, C. trachomatis, Trichomonas,* HPV
Transcription-mediated amplification (TMA)	Hologic Gen-Probe	Amplified MTB	*M. tuberculosis* complex
		Aptima	*N. gonorrhoeae, C. trachomatis,* HIV-1, HCV, HPV, *Trichomonas vaginalis*
	Siemens Healthcare Diagnostics (Tarrytown, NY)	VERSANT	HCV
Branched DNA amplification (bDNA)	Siemens Healthcare Diagnostics	VERSANT	HIV-1, HCV
Hybrid capture	Qiagen	Digene	*N. gonorrhoeae, C. trachomatis,* HPV
Loop-mediated, isothermal DNA amplification (LAMP)	Meridian Biosciences (Cincinnati, OH)	Illumigene	*C. difficile,* group A *Streptococcus,* group B *Streptococcus*
Nucleic acid sequence–based amplification (NASBA)	bioMérieux (Lyon, France)	NucliSens	HIV-1

*U.S. Food and Drug Administration–approved for in vitro diagnostic use.

CMV, cytomegalovirus; CNS, coagulase-negative *Staphylococcus*; DNA, deoxyribonucleic acid; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, HIV-2, human immunodeficiency virus 1, 2; HPV, human papillomavirus; MRSA, methicillin-resistant *Staphylococcus aureus;* rRNA, ribosomal ribonucleic acid; SSTI, skin and soft tissue infection.

TABLE 16-9 Common Biochemical Reactions Used to Identify Bacteria

BIOCHEMICAL TEST	PRIMARY USE OF TEST
Bacitracin (A) disk	For presumptive identification of *Streptococcus pyogenes*, which is sensitive to low concentrations of bacitracin
Bile solubility	For rapid differentiation of *Streptococcus pneumoniae* (which are bile soluble) from other streptococci
Catalase	An important characteristic that defines major groups of gram-positive cocci (e.g., staphylococci [catalase positive], streptococci and enterococci [catalase negative])
Coagulase	A positive coagulase test is the key criterion for identification of *Staphylococcus aureus*
Hippurate hydrolysis	A positive test is used for presumptive identification of group B streptococci
Indole	A rapid test used for presumptive identification of *Escherichia coli* from urine specimens
Optochin (P) disk	Susceptibility to optochin is presumptive identification of *Streptococcus pneumoniae*
Oxidase	A key reaction to help differentiate gram-negative rods (e.g., Enterobacteriaceae, *Acinetobacter*, and *Stenotrophomonas* are oxidase negative; *Pseudomonas* and *Burkholderia* are oxidase positive).
PYR hydrolysis (L-pyrrolidonyl-β-naphthylamide)	A rapid test for presumptive identification of *Streptococcus pyogenes* and *Enterococcus*
Urease	A rapid test for *Proteus mirabilis* and *Helicobacter pylori*

TABLE 16-10 Classification of Common Bacteria

GRAM-POSITIVE			GRAM-NEGATIVE			
Cocci	**Rods**	**Acid-Fast**	**Cocci**	**Rods**	**SPIRAL/CURVED RODS**	**OTHER**
Aerobic	**Aerobic**	*Gordonia*	**Aerobic**	**Aerobic**	*Arcobacter*	*Anaplasma*
Abiotrophia	*Arcanobacterium*	*Mycobacterium*	*Neisseria*	*Acinetobacter*	*Borrelia*	*Chlamydia*
Aerococcus	*Bacillus*	*Nocardia*		*Actinobacillus*	*Campylobacter*	*Coxiella*
Enterococcus	*Brevibacterium*	*Rhodococcus*		*Aggregatibacter*	*Helicobacter*	*Ehrlichia*
Granulicatella	*Corynebacterium*	*Tsukamurella*		*Bartonella*	*Leptospira*	*Mycoplasma*
Leuconostoc	*Erysipelothrix*			*Bordetella*	*Treponema*	*Orientia*
Pediococcus	*Listeria*			*Brucella*		*Rickettsia*
Staphylococcus	*Rothia*			*Burkholderia*		*Tropheryma*
Streptococcus	*Streptomyces*			*Capnocytophaga*		*Ureaplasma*
				Citrobacter		
				Comamonas		
				Cronobacter		
				Delftia		
				Eikenella		
				Enterobacter		
				Escherichia		
				Francisella		
				Haemophilus		
				Hafnia		
				Kingella		
				Klebsiella		
				Legionella		
				Moraxella		
				Morganella		
				Pantoea		
				Pasteurella		
				Proteus		
				Providencia		
				Pseudomonas		
				Ralstonia		
				Raoultella		
				Salmonella		
				Serratia		
				Shigella		
				Stenotrophomonas		
				Vibrio		
				Yersinia		
Anaerobic	**Anaerobic**		**Anaerobic**	**Anaerobic**		
Anaerococcus	*Actinomyces*		*Acidaminococcus*	*Bacteroides*		
Finegoldia	*Bifidobacterium*		*Megasphaera*	*Fusobacterium*		
Peptococcus	*Clostridium*		*Veillonella*	*Leptotrichia*		
Peptostreptococcus	*Eubacterium*			*Porphyromonas*		
Peptoniphilus	*Lactobacillus*			*Prevotella*		
	Mobiluncus					
	Propionibacterium					

commonly recognized bacteria. For example, until the early 1970s, most laboratories identified all known members of the family Enterobacteriaceae by using reactions in six test tubes. As the number of clinically important organisms expanded, the number of additional tests that needed to be performed made this approach impractical and prohibitively expensive. The first commercial kits for organism identification were introduced in the 1970s. These consisted of a series of reactions that were incorporated in disposable strips or microwell plates. The pattern of reactions could be converted into a "metabolic profile" that was compared with an established database for the organism identification. Refinements of this approach included selection of reactions that could be interpreted after a few hours of incubation and the introduction of instrumentation for the interpretation of the individual reactions and metabolic profile. Further improvements include the development of fully automated systems for the inoculation, incubation, and interpretation of these tests. Extensive databases exist that allow the theoretical identification of most clinically important bacteria. These systems have also been integrated with antimicrobial susceptibility tests (to be discussed later). A detailed discussion of the manual and automated commercial identification systems is beyond the scope of this chapter; however, most laboratories use a combination of these systems.

TABLE 16-11 Serologic Identification of Selected Bacteria

BACTERIA	COMMENTS
Streptococcus, β-hemolytic	Lancefield grouping based on cell wall carbohydrates; A, B, C, F, and G most common
Streptococcus pneumoniae	Subtyping based on more than 90 capsule polysaccharides; polyvalent antisera used to identify *S. pneumoniae*; subtyping used to select most common polysaccharides for vaccine development
Neisseria meningitidis	Thirteen capsule polysaccharides; A, B, C, Y, and W135 most common clinical isolates
Haemophilus influenzae	Capsule polysaccharides used to classify species into 6 subtypes (A to F); subtype B most important
Escherichia coli	Subtyped by many cell wall (O) polysaccharides and flagella (H) proteins; O157:H7 most important
Salmonella enterica	Subtyped using more than 2500 cell wall (O) polysaccharides
Shigella spp.	Cell wall (O) polysaccharides used to divide 4 species into 45 subtypes
Legionella pneumophila	Polyvalent antisera used to subdivide species into 16 serogroups; serogroup 1 most important

Identification Using Specific Antisera

In addition to biochemical methods of identification, laboratories use antisera to confirm the identification of some organisms and for subtyping bacteria for epidemiologic purposes (e.g., classification of *Salmonella* strains). Agglutination with the use of whole-organism suspensions or latex agglutination by means of antibody bound to latex beads is technically simple to perform and takes a few minutes to complete. Examples of organisms for which serologic identification is useful are listed in Table 16-11.

Identification Using Genomic Techniques

The first molecular identification techniques were probe-based tests that relied on detection of nucleic acid sequences that were specific for a particular genus or species. These probe assays (see Table 16-8) became the method of choice for rapid, accurate identification of a variety of bacteria, mycobacteria, and fungi isolated in culture.

DNA sequence–based identification is another molecular technique that can be used to improve both the speed and accuracy of identification of bacteria that are difficult to identify by phenotypic tests. Gene sequencing techniques that target ribosomal ribonucleic acid (rRNA) genes and various housekeeping genes permit precise identification of organisms, but the current sequencing costs and technical demands limit the widespread use. Furthermore, proteomic identification methods (see later) are rapidly replacing sequencing methods and will likely restrict sequencing for characterization of selected organisms and use for epidemiologic investigations.

Whereas most subtyping of most organisms was previously performed using specific antisera, this is now more efficiently accomplished with molecular techniques. Availability of accurate typing helps to control outbreaks more rapidly and can help in the identification of sources, carriers, and patterns of spread. The main genotypic methods that are currently in use include pulsed-field gel electrophoresis (PFGE), restriction fragment length polymorphism (RFLP) of chromosomal DNA, and polymerase chain reaction (PCR)-based methods, such as random amplification of polymorphic DNA (RAPD) and multilocus sequence typing (MLST).[33] The different methods vary in reproducibility, ease of performance, turnaround time, robustness, and discriminatory power, with PFGE considered by many the gold standard. It is important to recognize that all these methods are relatively crude techniques to assess the similarities or differences in genetic composition among strains of bacteria. A more precise method for accomplishing this is whole-genome sequencing. With the dramatic improvements in sequencing techniques, whole-genome sequencing has enabled a level of discrimination among strain previously unattainable in epidemiologic investigations. As these sequencing techniques are further automated, it is anticipated that genome sequencing will replace the earlier genotypic methods.

Identification Using Proteomic Methods

The fundamental procedures for identification of organisms are rapidly being replaced by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, a method currently in widespread use in Europe for the identification of bacteria, mycobacteria, yeasts, and molds.[34-36] Use of MALDI-TOF is limited in the United States only until FDA approval is obtained. The method is technically simple and inexpensive. Bacteria or yeast are selected from a culture plate or positive broth culture (e.g., blood culture), transferred to a target plate, pretreated with formic acid, overlayed with a matrix, and then analyzed. Upon exposure to a laser beam, the matrix ionizes proteins present in the sample, which are then separated by size as they move through a vacuum tube, creating a unique protein spectrum. Processing mycobacteria and molds is slightly more complex, but identification of all organisms can be achieved in less than an hour, with an accuracy that exceeds all previous methods. MALDI-TOF mass spectrometry alters workflow in the microbiology laboratory, eliminating the need for Gram stains, subcultures, and delays associated with biochemical testing, and it dramatically improves time to definitive identification results.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

One of the most important functions of the microbiology laboratory is to determine the susceptibility of a presumed pathogen to antimicrobial agents. Although some organisms are predictably susceptible to effective antibiotics, treatment options are frequently limited for other organisms because of antimicrobial resistance or drug toxicity. In vitro susceptibility testing is one of the most complex tasks performed in the clinical laboratory: the tests must be reproducibly and accurately performed, clinically relevant, and timely. Methods for performing the tests are defined by standards published and annually reviewed by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]), a consensus group representing clinical laboratories, industry, and government agencies (www.CLSI.org). The standards define with exacting detail how each susceptibility test method should be performed and the results interpreted and reported. The documents also provide guidelines for the selection of specific classes of drugs to be tested against specific organisms, although the final decision of which antibiotics should be reported for a specific organism should be made by the microbiologist and clinician.

A number of different methods have been used to assess the activity of antibiotics against bacteria. Historically, the methods have been divided into dilution and diffusion methods, quantitative and qualitative methods, manual and automated methods, and overnight and rapid methods. Methods have also been developed to measure specific resistance mechanisms, such as production of β-lactamases or the presence of specific genes encoding resistance.

Dilution Methods

Dilution methods were the first susceptibility test procedures that were developed. In these procedures, serial dilutions of antibiotics are prepared in either a liquid or agar medium. Typically, a twofold dilution scheme is used (e.g., 100, 50, 25, 12.5, 6.25, 3.1, 1.5, 0.75, etc., μg/mL). Each dilution is then inoculated with a standardized concentration of the test organism and, after a specific incubation period, the lowest (minimum) concentration of antibiotic that inhibits the organism is determined. These methods are referred to as minimal inhibitory concentration (MIC) tests or quantitative susceptibility tests because the results are expressed numerically.[37]

The quantitative values can be converted into qualitative interpretations—susceptible, intermediate, resistant—based on interpretive criteria defined by CLSI for each organism-antibiotic combination.[38] An organism is defined as susceptible to an antibiotic when the MIC value is at or below the concentration achieved with the usual recommended dose of the antibiotic, intermediate when the MIC value is at the concentration of the antibiotic achieved with higher doses or in body sites where the active antibiotic is concentrated (e.g., urinary tract), and resistant when the MIC value is greater than the

concentration achieved in therapeutic doses or in situations where special resistance mechanisms are present (e.g., bacterium carries genes encoding resistance to the antibiotic).

Agar dilution tests performed in Petri dishes and broth dilution tests performed in test tubes are used primarily in research laboratories because the tests are technically cumbersome and not adapted to high-volume testing. However, broth dilution tests performed in microdilution trays are very common. These are referred to as broth microdilution MIC tests. Automated versions of these microdilution tests have been developed by a number of companies and are widely used in most clinical laboratories. Microdilution panels with a large number of antibiotics are available commercially and can be combined with biochemical tests for the simultaneous performance of identification and antimicrobial susceptibility tests. The antibiotics and biochemical substrates are dried in many test panels so they can be stored for an extended period of time.

Agar Diffusion Methods

The agar diffusion method was developed as a practical alternative to the agar and broth tube dilution procedures. The most popular agar diffusion method is the Kirby-Bauer disk diffusion method.[39] In this method, the test agar plate is swabbed with a standardized concentration of the test organism, and then paper disks containing a defined antibiotic concentration are placed on the lawn of bacteria. After overnight incubation, the diameter of the zone of inhibited growth around the disk is measured. This zone is influenced by a number of variables, including the susceptibility test medium (Mueller-Hinton agar is the standard for bacterial tests), the concentration of the test organism, the rate of growth of the test organism, the concentration of antibiotic in the disk, the diffusion of the antibiotic in the agar, and the susceptibility of the organism to the antibiotic. The first five variables are standardized by CLSI; therefore, if the test is properly performed, the size of the zone of inhibited growth is directly related to the susceptibility of the organism—the larger the zone, the more susceptible the organism is to the antibiotic. As would be expected, the results of the dilution tests and diffusion tests are related. There is an inverse linear relationship between the size of the zone and the MIC value—the larger the zone of inhibited growth (more susceptible the organism to the antibiotic), the smaller the MIC value. Thus, it is possible to extrapolate from the measured size of the inhibitory zone to the corresponding MIC value. In addition, the interpretive criteria that are applied to MIC tests apply to the diffusion tests. Thus, for most organism-antibiotic tests, the diffusion tests and dilution tests are equally accurate in predicting antimicrobial susceptibility.

The dilution tests are commonly referred to as quantitative tests, whereas the diffusion tests are referred to as qualitative tests. That is, the dilution tests are usually reported as a number or MIC value, whereas the diffusion test results are reported as susceptible, intermediate, or resistant. This is an oversimplification. Both tests are quantitative; in fact, the diffusion tests assess a greater range of quantitative values (zone sizes from 6 mm [diameter of the paper disk] to about 40 mm), compared with dilution tests, which typically measure two to five concentrations of a drug in most microdilution panels. So, the definition of a quantitative or qualitative test is determined by the reporting method rather than the testing method.

One variation of the Kirby-Bauer disk diffusion test is the E test (bioMérieux, Lyon, France), which is a gradient diffusion test. In this test, the antibiotic is in a commercially prepared strip, with a concentration gradient extending from the top to the bottom of the strip. When this is placed on a lawn of bacterial growth, an elliptic pattern of inhibited growth develops (greater inhibition at the end with a higher concentration of antibiotic). The strips are calibrated so that the area where the bacterial growth meets the strip corresponds to the organism's MIC value for the antibiotic. The advantage of the E test is that MIC results can be obtained easily for one or two antibiotics. However, when a large number of organism-antibiotic combinations need to be tested, the broth microdilution method is preferred.

Special Methods

Specialized methods have been developed to detect some specific resistance mechanisms. An example of this is the detection of β-lactamase production by both gram-positive and gram-negative organisms. There are more than 250 different β-lactamases produced by bacteria, so it is logical that a variety of methods have been developed. Two examples will illustrate important applications of these tests. Organisms such as *Staphylococcus, Neisseria, Haemophilus*, and *Bacteroides* produce a common β-lactamase that will degrade most penicillins and selected cephalosporins. The β-lactamase activity can be detected by measuring the byproducts of these degraded antibiotics. The most common method measures degradation of the chromogenic cephalosporin nitrocefin, which produces a red byproduct. Another important group of β-lactamases are the extended-spectrum β-lactamases (ESBLs). ESBL-producing organisms are resistant to all penicillins, cephalosporins, and monobactams, even when the in vitro tests indicate susceptibility. Diffusion and dilution tests have been developed to detect ESBL production. Reduced activity to cefpodoxime, ceftazidime, ceftriaxone, cefotaxime, or aztreonam is used to screen for ESBL production, and then inhibition of this β-lactamase activity by β-lactamase inhibitors (i.e., sulbactam, clavulanic acid) is used to confirm the screening test.[38] Resistance to carbapenem antibiotics in gram-negative rods is mediated by a number of novel β-lactamases. This resistance is initially detected by elevated MICs to one or more carbapenems and then confirmed by detection of the specific resistance gene by molecular tests. Carbapenem-resistant gram-negative rods should be considered resistant to all β-lactam antibiotics.

All species of staphylococci that are oxacillin (or methicillin) resistant should be considered resistant to all β-lactam antibiotics, including carbapenems, regardless of the actual in vitro result. For either MIC or disk testing of staphylococcal susceptibility to oxacillin and vancomycin, a full 24 hours of incubation at 35°C is required to maximize the likelihood of detection of resistance except in automated broth dilution systems that use specialized interpretive algorithms that allow a definitive result in 6 to 8 hours. Oxacillin resistance is encoded in the *mecA* or *mecC* genes. Molecular-based assays for one or both genes are widely used in clinical laboratories and offer a rapid method for determining resistance.

Infections with enterococci are commonly treated with a combination of an aminoglycoside and a cell wall–active antibiotic. Aminoglycosides have poor activity against enterococci when used alone because of poor uptake of the drug. Acquired resistance to aminoglycosides (that is, combination treatment will be ineffective) corresponds to high MIC values for the aminoglycosides. Thus, high-level resistance is defined by MIC values greater than or equal to 2000 μg/mL for streptomycin and greater than or equal to 500 μg/mL for gentamycin. Vancomycin resistance is also common in enterococci, particularly in *E. faecium* and less commonly in *E. faecalis*. This can be measured by MIC tests, growth on media with vancomycin, or by molecular tests that measure for the presence of genes associated with vancomycin resistance, *vanA* or *vanB* genes. Commercial assays for molecular testing for *vanA* are widely available (see Table 16-8).

MYCOBACTERIA

Mycobacteria are aerobic, nonmotile, rod-shaped bacteria that are acid fast; that is, once stained with certain dyes, they resist decolorization with acid-alcohol because of their cell wall structure.

Safety Issues

When concentrated direct smears are made, bleach (5% sodium hypochlorite) can be used to inactivate mycobacteria that may be present in patient specimens. Non–aerosol-producing procedures, such as manipulating specimens for preparation of smears, can be performed using biosafety level (BSL)-2 practices and a biologic safety cabinet. The Centers for Disease Control and Prevention (CDC) recommends that all other procedures that potentially generate aerosols should be performed in a BSL-3 facility. Such a facility includes restricted access; directional airflow maintaining the laboratory under negative pressure; and the use of special gowns, gloves, and masks.[40]

Specimen Collection and Transport

Specimens for smear and culture for mycobacteria should be collected and transported in closed, leakproof, sterile containers. Containers contaminated with specimen on the outside are not acceptable because

of the hazard posed to personnel. Gastric aspirates require pH neutralization soon after collection to ensure the viability of any mycobacteria that may be present; arrangements should be made with the laboratory in advance to ensure optimal specimen handling. Biopsies are preferable to swab specimens of tissue lesions for the isolation of mycobacteria. No special procedures are usually necessary for the collection and transport of sterile fluids, urine, and stool. However, organism concentrations in sterile fluids may be low, so a minimum of several milliliters (in the case of CSF, more than 5 mL if possible) should be obtained whenever mycobacterial infection is a serious consideration.[41] Blood may be collected for mycobacterial culture, either in a blood collection tube that contains anticoagulant or in a lysis-centrifugation tube, or it may be inoculated directly into a special bottle for mycobacterial blood culture, depending on laboratory protocol. Twenty-four–hour collections of sputum and urine are unacceptable because of the likelihood of bacterial overgrowth. For sputum and urine, it is recommended that at least three first-morning specimens be obtained and that a minimum of 40 mL of midstream urine be processed for each culture.[41] Protocols for handling different specimen types vary significantly among laboratories, and it is important for physicians to be aware of the procedures used by each of the laboratories they use.

Direct Organism Detection

Mycobacteria may be detected directly in patient specimens through visualization of stained organisms or by detection of specific mycobacterial nucleic acids or other cellular constituents (see Tables 16-5 and 16-8).

The Gram stain is insensitive for the detection of mycobacteria. If visible at all, mycobacteria may appear as finely beaded, gram-positive rods with only the beads visible (gram-positive) and the rest of the organism appearing gram-negative, or they may appear as negative images (unstained rodlike outlines) in the specimen. Specific mycobacterial stains are based on the ability of mycobacteria to retain certain dyes after washing with an acid-alcohol decolorizer (hence, "acid fast"), unlike most other bacteria. The primary stain in the Ziehl-Neelsen and Kinyoun stains is carbolfuchsin, staining mycobacteria red. The Ziehl-Neelsen stain requires a heating step and has been replaced in many laboratories by the Kinyoun stain, which is a "cold" acid-fast stain. The auramine O and auramine-rhodamine stains use fluorescent compounds; although fluorescence microscopy is required for visualization of mycobacteria, these stains do not involve the use of an antibody but are based on the acid-fast properties of mycobacteria. Fluorescent stains are more sensitive for the detection of mycobacteria, particularly in direct specimens, because the organisms stain brightly and can be clearly distinguished from background material. In addition, because slides stained with a fluorescent dye can be examined reliably with an objective lens of lower magnification (25×) than with the oil immersion lens (100×) required for carbolfuchsin-stained smears, reading of slides can be done faster. Rapidly growing mycobacteria may be less avidly acid fast than are slow growers and may be more readily visualized with a modified acid-fast stain that uses a weaker decolorizing step than is used with a regular carbolfuchsin stain. Some species of mycobacteria differ from others in the length, width, or arrangement of individual cells. *Mycobacterium kansasii,* for example, tends to be long and broad, sometimes with a banded rather than a beaded appearance, whereas *Mycobacterium avium* complex may appear coccobacillary, especially on a smear from a liquid medium. Particularly when grown in a liquid medium, cells of *M. tuberculosis* often group together in "cords" that are composed of long strands of organisms with their long axes parallel. However, the microscopic appearance of individual cells and groups of cells should at best be considered only suggestive of a certain species or group of species.[42]

The CDC currently recommends that a nucleic acid amplification test (NAAT) should be performed on the first sputum from all patients suspected with a *M. tuberculosis* infection for which the test result would alter case management or tuberculosis (TB) control activities. Despite this recommendation, there is only one FDA-approved test currently available (see Table 16-8) for respiratory specimens, and the test sensitivity is high only for smear-positive specimens.[43] Other NAATs are available for research use only or for clinical testing outside the United States. Laboratory-developed tests (e.g., DNA sequencing, real-time PCR, loop-mediated isothermal amplification) must be validated before they can be used for diagnostic testing. One gene target, the *secA1* gene, is useful for the detection and identification of a large number of mycobacterial species in clinical specimens.[44]

Although direct detection of both mycobacterial antigens and tuberculostearic acid may have some usefulness for the diagnosis of tuberculosis, particularly tuberculous meningitis, such testing is not generally available. Nucleic acid amplification may be more sensitive than tuberculostearic acid detection for the diagnosis of both pulmonary and meningeal tuberculosis.[45]

Specimen Processing and Plating

Specimens such as sputum, urine, and stool, which can be expected to contain considerable normal microbial flora, must be digested and decontaminated to prevent overgrowth of any mycobacteria present. These processing steps are inevitably somewhat toxic to mycobacteria, and a balance must be struck to minimize the loss of mycobacteria while simultaneously maximizing the elimination of as many other microorganisms as possible.[41] Concentration of the specimen occurs as part of the digestion and decontamination procedures, and smears of a specimen are prepared from such concentrates. The most commonly used digestion and decontamination procedure entails the use of a mixture of *N*-acetyl-L-cysteine and sodium hydroxide (NaOH), although other procedures are occasionally used, such as the oxalic acid procedure for specimens heavily contaminated with *Pseudomonas aeruginosa.*[41] Sterile fluids, such as CSF, may be concentrated for smear preparation and plating without prior digestion and decontamination. Biopsies specimens obtained from normally sterile sites must be ground before plating but also do not require prior digestion and decontamination. Blood processed by a lysis-centrifugation method can be planted onto solid media, from which blood organism concentration can be determined. Currently, several types of mycobacterial blood culture bottles that can be used with automated instruments are available for direct inoculation, but quantitation cannot be obtained with liquid culture media.[41]

It is recommended that a liquid, in addition to a solid medium, be used for plating specimens whenever practical; the liquid media may enhance both the sensitivity of culture and the rapidity of organism detection.[41,46] Use of a liquid medium often results in the isolation of *M. tuberculosis* within 2 weeks and isolation of other slowly growing mycobacteria in even less time. Consequently, when one of these liquid media has been used, one cannot assume that an organism detected within a week of incubation is necessarily a rapid grower in the traditional sense. Antimicrobial agents may be added to liquid and solid media to help prevent overgrowth of contaminants; some of these agents may be inhibitory to some mycobacteria (e.g., nalidixic acid may inhibit some strains of rapidly growing mycobacteria and *M. kansasii*); hence, if media with and without antimicrobials are used, the chance of isolating any mycobacteria present may be enhanced. If no growth is obtained from a smear-positive specimen from an untreated patient, the possibility that optimal growth conditions for the organism have not been used or that the organism is uncultivable should be considered. Many liquid-based systems use an instrument for automated detection of organism growth, but some nonautomated systems are also available. Detection is based on such features as carbon dioxide (CO_2) production (BacT/ALERT 3D, bioMérieux, Lyon, France), oxygen consumption (BACTEC 9000MB System and Mycobacteria Growth Indicator Tube [MGIT], Becton-Dickinson, Sparks, MD), and monitoring the rate of oxygen consumption by measuring pressure changes in the culture vial (VersaTREK, Magellan Biosciences, Cleveland, OH).[41]

Mycobacterial cultures are generally incubated at 36° C ± 1° C in an atmosphere of approximately 8% CO_2, the optimum conditions for isolation of *M. tuberculosis.* However, several species of pathogenic mycobacteria have different growth requirements or preferences that may have to be satisfied to ensure their isolation (Table 16-12). Given the preference of several skin and subcutaneous pathogens to grow at 30° C (including *Mycobacterium haemophilum, M. marinum, M. ulcerans,* and rapid growers), at least a portion of all skin biopsy specimens (and perhaps other specimen types obtained from cooler areas of the body such as the extremities) submitted for mycobacterial culture

TABLE 16-12 Pathogenic Mycobacteria with Unusual Growth Requirements or Characteristics

Mycobacterium bovis
Growth said to be enhanced by 0.4% pyruvate, but there is no apparent need for supplementation with the use of newer media
Mycobacterium haemophilum
Requires ferric iron (provided as ferric ammonium citrate, hemin, or blood)
Requires approximately 30°C for initial growth
Mycobacterium genavense
Grows best in BD BACTEC media
May grow on Middlebrook 7H11 medium supplemented with mycobactin J or on acidified Middlebrook medium supplemented with blood and charcoal
Mycobacterium marinum
May require approximately 30°C for initial growth
Mycobacterium paratuberculosis
May require many months to grow, even on special media
Cultivation is not feasible in the diagnostic laboratory
May be killed by routinely used digestion and decontamination procedures
Mycobacterium ulcerans
May require approximately 30°C for initial growth
Requires prolonged (up to 3 mo) incubation for detection
Mycobacterium xenopi
Grows best at 42°C, but will grow, at least slowly, at 36°C
Rapidly Growing Mycobacteria
Often grow best at approximately 30°C, but usually also grow well at 36°C

should be incubated at approximately 30°C. It is also important that the clinician notify the laboratory if a pathogen with specific growth requirements, such as *M. haemophilum,* is suspected so that optimal culturing procedures can be used. *Mycobacterium avium* subsp. *paratuberculosis,* reported from some patients with Crohn's disease and the etiologic agent of Johne's disease in ruminants, cannot reliably be isolated in the routine diagnostic laboratory.

Significance of Isolation of Mycobacteria from Clinical Specimens

Isolation of certain species of mycobacteria, such as *M. avium* complex or a rapidly growing mycobacterium, may represent colonization or specimen contamination rather than active disease, but the potential significance of repeated isolation of the same species from the same source requires careful evaluation.[47] Most isolates of *M. gordonae,* a water organism, are regarded as contaminants; only rarely is it a proven pathogen. Laboratory cross-contamination, although fortunately rare, has been well documented and requires sustained vigilance for its detection and prevention.[48]

The molecular methods used in epidemiologic investigations can also be used in assessing the likelihood of cross-contamination. *M. tuberculosis* isolated from a one of several smear-negative specimens, especially in the absence of consistent histopathology or a highly consistent clinical picture, requires consideration of the possibility of specimen cross-contamination.[49]

Mycobacterial Identification

Traditional Categories and Methods

To aid their further identification to the species level, nontuberculous mycobacteria were divided into several groups by Runyoun. Rapid growers (Runyoun group IV) take less than 7 days for growth after subculture to a solid medium from a dilute suspension; however, they generally also take less than 7 days to grow on solid or in liquid medium on initial isolation. Slowly growing mycobacteria are further divided into photochromogens, scotochromogens, and nonphotochromogens (Runyoun groups I, II, and III, respectively), depending on their ability to produce pigment and the relationship of pigment production to light stimulation. Photochromogens produce pigment after light stimulation but not when grown continuously in the dark with no light exposure. Scotochromogens produce pigment when grown in the dark as well as after light exposure. Nonphotochromogens do not produce pigment even after light stimulation. However, not all isolates of some species will fall into the same category based on growth rate or pigment production. For example, most isolates of *M. avium* complex are nonphotochromogenic, but some are scotochromogenic; many isolates of *M. marinum* (a photochromogen) grow rapidly, and isolates of *M. szulgai* are usually photochromogenic at 25°C but scotochromogenic at 36°C. *M. tuberculosis* complex, although by definition not a member of any of the Runyoun groups, is in fact a nonphotochromogen. This is important because if a pigmented, acid-fast colony is isolated in culture, this effectively excludes *M. tuberculosis*.

Some mycobacterial species that are closely related and/or difficult to distinguish in the diagnostic laboratory are grouped into complexes. The two most frequently encountered complexes are *M. tuberculosis* complex (consisting *of M. tuberculosis, M. africanum, M. bovis, M. canettii*, and several other species primarily of animal origin) and *M. avium* complex (MAC, consisting of *M. avium, M. intracellulare*, and probably at least one other species). Several species of rapid growers are also placed into different complexes, but these groupings are not so widely recognized.

Because more than 100 species of mycobacteria are recognized, use of biochemical tests cannot reliably identify most isolates. A practical method for preliminary identification of *M. tuberculosis* is the demonstration of niacin production and reduction of nitrate. However, for the definitive identification of this and other species, genomic or proteomic methods should be used.

Molecular Identification Methods

Commercially available probes (AccuProbe [Hologic Gen-Probe; San Diego, CA]) are available for the identification of *M. avium* complex, *M. avium*, *M. gordonae, M. intracellulare, M. kansasii,* and *M. tuberculosis* complex (see Table 16-8). These chemiluminescent compound–labeled probes are DNA probes for rRNA, which exists in multiple copies in each cell; they can be used only with organisms grown in culture, not directly on patient specimens. The procedure can be performed within a matter of hours from the time of organism isolation. Laboratories not doing further testing of isolates of the *M. avium* complex and the *M. tuberculosis* complex should make it clear that their identifications have not gone beyond the level of the complex (e.g., they should report "*M. tuberculosis* complex," not "*M. tuberculosis,*" unless additional appropriate testing such as niacin and nitrate reactions has been done). Other nucleic acid–based methods for identification of mycobacterial species include amplification of a portion of the genome common to all mycobacteria (such as 16S rRNA, *HSP*, or *secA1* genes), followed by the use of species-specific probes or sequencing of the amplified material. Identification of mycobacteria with MALDI-TOF mass spectrometry has recently been reported to be rapid and highly accurate. Conventional MALDI-TOF methods (see earlier) were modified for mycobacteria by inactivation of isolates by heat treatment, followed by use of sonication with glass beads in the presence of formic acid and acetonitrile. This method is very promising because definitive identification of isolates could be accomplished in less than 1 hour.

Susceptibility Testing

In the United States, susceptibility testing of *M. tuberculosis* complex is done either by the proportion method, using Middlebrook 7H10 agar or in a liquid medium.[50] It is currently recommended that all initial isolates of *M. tuberculosis* complex be tested, as well as isolates from patients who are still culture positive after 3 months of therapy or who are clinically failing to respond. The use of liquid medium is recommended for testing the four primary antituberculous drugs (ethambutol, isoniazid, pyrazinamide, and rifampin) because results are usually available within a week, as opposed to the 3 weeks required with solid medium. In the proportion method, an isolate is considered resistant to a specific concentration of antimicrobial if the number of colonies growing on the corresponding drug-containing quadrant of the plate is greater than 1% of the number of colonies on a drug-free quadrant. Testing that uses liquid media has been adjusted to provide results that are in accordance with those obtained with the standardized procedure using a solid medium. Testing of the "critical

concentration" of each drug is considered most important. These "critical" concentrations may differ somewhat between solid and liquid media for a given agent (e.g., the critical concentration for isoniazid in 7H10 agar is 0.2 µg/mL; in BACTEC 12B and ESP II [Trek Diagnostics, Westlake, OH] liquid media, it is 0.1 µg/mL). CLSI has published an approved standard for susceptibility testing of *M. tuberculosis*, which also includes recommendations for the testing of most other pathogenic species of mycobacteria and aerobic actinomycetes.[50] The document includes discussions of the circumstances under which susceptibility testing of the various species may be most useful. Rapidly growing mycobacteria do not generally respond to agents used for the treatment of tuberculosis. The CLSI document describes a modification of the broth microdilution procedure used for bacterial susceptibility testing for use with rapid growers, including a variety of species-specific procedural and interpretive guidelines. Molecular tests are also available for the direct detection of resistance genes, but these should be considered presumptive and should be confirmed by phenotypic tests.

Epidemiology

Molecular typing can now be performed by a variety of methods to determine the relatedness of different isolates of *M. tuberculosis*. A widely used method is based on the insertion sequence IS*6110,* which is found in 0 to approximately 25 copies scattered in different locations in the chromosome in different strains. The DNA fragments produced by a specific endonuclease (which cleaves at only one site in each insertion sequence) are separated by gel electrophoresis, and the DNA fragment patterns obtained from different isolates are compared to determine relatedness.[51] The procedure has been used for such purposes as investigating episodes of possible laboratory cross-contamination, tracing sources of infection in outbreak situations, determining whether a second episode of disease is due to a previously isolated strain or to a newly infecting strain, and determining whether an infection is caused by more than one strain of organism. Other techniques can be used when the IS*6110*-based technique is not practicable, such as for isolates with too few copies of the insertion sequence. Among the molecular techniques used for evaluation of the strain relatedness of nontuberculous mycobacteria are PFGE and RAPD. As with epidemiologic investigation of bacterial outbreaks, whole-genome sequencing may rapidly render all other techniques obsolete.

Latent Tuberculosis: Laboratory Diagnosis

The tuberculin skin test is the traditional method for assessing exposure to *M. tuberculosis*. Interferon-γ (IFN-γ) is critical in regulating cell-mediated immunity, and in 2001, the first IFN-γ release assay (IGRA) for the detection of *M. tuberculosis* infection (QuantiFERON-TB test; Cellestis, Carnegie, Victoria, Australia) was approved by the FDA. A second-generation assay, QuantiFERON-TB Gold test, was approved in 2005, and the T-SPOT TB test (Oxford Immunotec, Marlborough, MA) was approved in 2008. The tests involve the detection of IFN-γ released in fresh heparinized whole blood from lymphocytes of sensitized individuals after incubation with synthetic peptides similar to the early secretory antigens of *M. tuberculosis*. Detailed guidelines on the use and interpretation of this test have been published recently by the CDC.[52] The CDC recommends that the IGRA tests can be used for patients previously immunized with bacillus Calmette-Guérin (BCG) (in contrast with the tuberculin skin test, there is no cross-reactivity) and for patients where skin test follow-up is unreliable.

AEROBIC ACTINOMYCETES

Aerobic actinomycetes are gram-positive rods (in most species also beaded and conspicuously branching) that grow best under aerobic conditions. They are similar in certain respects to both corynebacteria and mycobacteria, which are often also included with the aerobic actinomycetes.[53] The taxonomy of this group is under active revision, and considerable nomenclatural change is occurring. The morphologically similar anaerobic actinomycetes (including the genus *Actinomyces*), which have a preference or requirement for anaerobic growth conditions, are included with gram-positive anaerobic bacteria. In addition to *Corynebacterium* and *Mycobacterium*, genera of aerobic actinomycetes that contain species pathogenic for humans include *Actinomadura, Dermatophilus, Gordonia, Nocardia, Nocardiopsis, Rhodococcus, Streptomyces, Tropheryma,* and *Tsukamurella.*[53] *Tropheryma whipplei,* the causative agent of Whipple's disease, is not cultivatable in the routine laboratory. Organisms in the genera *Gordonia, Nocardia, Rhodococcus,* and *Tsukamurella* are almost always modified acid-fast positive; of these, only isolates of *Nocardia* regularly produce an aerial mycelium. The modified acid-fast stain for aerobic actinomycetes is similar to the Kinyoun stain for mycobacteria but differs particularly in that a weaker decolorizing solution, a shorter time period, or both are used for decolorization. The stain requires some expertise for performance and interpretation and should always be done with appropriate positive and negative controls. Although they are true bacteria and not molds, these organisms have traditionally been handled in the mycology section of the laboratory, perhaps because they resemble molds both in their relatively slow growth rate and in their tendency to form long, branched structures reminiscent of hyphae. Because these organisms are widespread in the environment, colonization and specimen contamination can occur. Single colonies of *Streptomyces,* especially from nonsterile sites and from nonmycetomatous lesions, are very likely to represent contamination. However, most aerobic actinomycetes are very uncommon as laboratory contaminants, and the potential clinical significance of their isolation should be carefully assessed.

Specimen Collection and Transport

Collection and transport procedures suitable for bacterial and fungal cultures are also adequate for the aerobic actinomycetes, but refrigeration of specimens should be avoided because some *Nocardia* strains lose viability at low temperatures.[53] If infection with an organism in this group is suspected, alerting the laboratory is advisable because special staining and plating procedures are useful for enhancing the likelihood that the organism will be detected. Because these organisms may cause deep-seated infections, such as brain abscess, biopsy may be necessary for isolation.

Direct Organism Detection

In some infections caused by these organisms, such as mycetoma, the organisms may grow in clinical lesions as dense masses visible macroscopically as "grains" in purulent material. Detection of these "grains" may greatly facilitate isolation of the etiologic agent; hence, the laboratory should be notified in advance when such an infection is suspected. The only generally available means of direct detection of these organisms in patient specimens is staining (see Table 16-5). On Gram stain, *Nocardia* tends to be especially thin and long and may appear to be composed mainly of tiny noncontiguous gram-positive beads. Organisms in other genera, such as *Streptomyces* (and the anaerobic actinomycetes), tend to appear wider and more avidly gram-positive, but a definite genus assignment of any organism other than *Dermatophilus* cannot be made with certainty on the basis of organism morphology alone. *Nocardia* may also be invisible on Gram stain or may appear only as negative images, and a modified acid-fast smear should always be requested if *Nocardia* infection is suspected. *Gordonia, Rhodococcus, and Tsukamurella* spp. may be unbranched, much shorter than *Nocardia,* and overall more coryneform in appearance. *Dermatophilus congolensis* produces branching filaments with divisions that occur both parallel and perpendicular to the long axis of the filament. In Gram-stained material, the organism may appear too darkly stained for adequate visualization of structural detail, and some other stain, such as a Giemsa stain, may be necessary.

Specimen Processing and Plating

No special specimen processing is required for isolation of the aerobic actinomycetes, which grow on many different types of media. However, they may not survive the specimen processing used to eliminate bacterial flora from specimens for mycobacterial culture. Because they may be present in low numbers and are relatively slow growing, isolation of aerobic actinomycetes may be enhanced with the use of media that contain antimicrobial agents to suppress the normal flora. A medium that has been found to be particularly useful for the isolation of

Nocardia is buffered charcoal-yeast extract (BCYE) agar, originally developed for the recovery of *Legionella* (see Table 16-6); the same medium with antimicrobial agents inhibitory to most other bacteria can be used for specimens such as sputum, which may also contain normal flora.[53] Cultures for *Nocardia* should be held for 2 weeks, although most isolates grow within 3 to 5 days.

Identification of Aerobic Actinomycetes

Colonies of *Nocardia* typically have a powdery, whitish surface because of the production of aerial mycelia and a tan-to-orange reverse surface. When stained from colonies, the organism often breaks up into coccobacillary fragments, with long, branching forms much less conspicuous than in direct patient material. Organisms stained from culture may be less acid fast than those in stains prepared from the original specimen. Growth on traditional mycobacterial media (e.g., Middlebrook agar, Lowenstein-Jensen medium) enhances acid-fastness. Isolates that form aerial hyphae and are acid fast can be presumptively identified as *Nocardia*.

The taxonomy of the genus *Nocardia* has become increasingly complex as new pathogenic species are recognized and formerly recognized species are subdivided into additional species. Conventional identification procedures for *Nocardia* spp., as performed in most laboratories, involve only a small number of phenotypic tests, and these are insufficient to distinguish accurately among the different species in the genus. Differences in the results of testing for susceptibility to certain antimicrobial agents may help in discriminating among some of these species, but such testing has limited value.

Colonies of *R. equi,* which is the principal pathogen in the genus *Rhodococcus,* most commonly are somewhat mucoid and acquire a pink color after 4 days of incubation. The isolate initially is rodlike but will evolve to a coccoid form with extended incubation.

Molecular methods, such as amplification by PCR of a portion of the genome, followed by DNA sequencing of the 16S rRNA gene (and/or other genes such as *secA*), is presently the most reliable method for identification of aerobic actinomycetes to the species level.[53] Preliminary work using MALDI-TOF, with processing of the isolate in a manner similar to mycobacteria, is very promising and will likely become the identification method of choice.

Susceptibility Testing

It is hoped that the use of the CLSI-recommended procedure for susceptibility testing of aerobic actinomycetes will produce greater comparability of results from different laboratories, but experience with the procedure is needed to ensure intralaboratory and interlaboratory reproducibility.[50] It may, however, prove to be the case that isolates in the genera *Rhodococcus* and probably *Gordonia* are best tested for susceptibility by using the same procedures as for rapidly growing, aerobic bacteria. In the past, different investigators used different susceptibility testing procedures; caution must be used when attempting to compare published results obtained by differing methodologies.

Epidemiology

Because infection with aerobic actinomycetes generally occurs on a sporadic basis, epidemiologic investigations are only rarely needed. A variety of molecular techniques have been found useful for epidemiologic investigations.[53]

FUNGI

Unlike bacteria, fungi are eukaryotic. It is estimated that there may be several million species of fungi, of which less than 500 have been reported to be human or animal pathogens with any frequency. However, additional pathogenic species are reported in the literature regularly, as the result of both already-known organisms being recognized as pathogens and the description of new species. The members of the genus *Pneumocystis,* formerly considered to be parasites, are now considered fungi, and recent taxonomic studies have demonstrated that members of the phylum Microsporidia belong to the kingdom Fungi.[54] The genus *Prototheca* is thought to be an achlorophyllous alga; organisms in this genus can be pathogenic and are usually included in discussions of fungi because they grow well on most fungal culture media.

Terminology

The diagnostic mycology laboratory uses terminology that, although it has little taxonomic significance, is useful in terms of determining both clinical relevance and identification. Yeasts are unicellular organisms that grow as relatively smooth, creamy colonies, whereas molds produce fuzzy colonies because of the production of aerial hyphae. Some organisms may initially produce yeastlike colonies that become more moldlike with continued incubation. Microscopically, yeasts appear as round or oval cells that reproduce by budding. Many yeasts, such as most species of *Candida,* also produce hyphal structures. Hyphae are morphologically subdivided into pseudohyphae and true hyphae. Pseudohyphae resemble chains of sausages in that the hyphae tend to be constricted at the septa, and each cell originates as a bud. The cell at the tip tends to be small and rounded, and a septum is often found at each branch point. In contrast, true hyphae tend to have straighter, more parallel walls without constrictions at the septa and usually without a septum at the branch point. True hyphae may be either septate or aseptate. True hyphae that are septate suggest organisms such as *Aspergillus, Fusarium,* and *Scedosporium.* True aseptate hyphae may be broad and have a ribbon-like appearance; their presence suggests one of the Mucorales (aseptate molds) such as *Rhizomucor.* Brown pigment in hyphae suggests that the organism is a dematiaceous (black) mold; hyaline molds do not produce pigmented hyphae. These simplified distinctions are not absolute. Thus, some *Candida* species can form true hyphae, and the "aseptate" molds may have an occasional septum. Dermatophytes and dimorphic molds are usually considered separate categories of mold distinct from the other hyaline septate molds. The likelihood that an organism may be a dermatophyte is usually suggested by the specimen source (e.g., skin, hair, nails). Dimorphic molds have a nonmold form when they are growing in the patient but grow as a mold in the laboratory at 30° C. Table 16-13 summarizes some of the more significant and more commonly isolated genera and species according to the categories frequently used in the diagnostic laboratory. Of the clinically significant dimorphic molds, *Blastomyces dermatitidis, Histoplasma capsulatum, Paracoccidioides brasiliensis, Penicillium marneffei,* and *Sporothrix schenckii* grow as yeasts or yeastlike structures in the patient at 37° C and hence are thermally dimorphic. *Coccidioides* species most commonly grow in the patient as thick-walled spherules and require a special medium that is not widely available to be grown in this form in vitro; otherwise, these species grow as a mold both at 30° C and at 35° C and hence are not thermally dimorphic. Two species of *Coccidioides* are now recognized, *C. immitis* and *C. posadasii,* and can be distinguished by molecular methods, although it is not clear that there are any clinically significant differences between them.[55]

Many molds have a morphologic form that results from sexual reproduction; these differ from the form that results from asexual reproduction. The form that results from sexual reproduction is known as the *teleomorph,* or perfect state; the form that results from asexual reproduction is known as the *anamorph,* or imperfect state. In the past, each form has been given a different scientific name; for example, the sexual form of *H. capsulatum* is named *Ajellomyces capsulatum,* and the sexual form of *Scedosporium apiospermum* is named *Pseudallescheria boydii.* Now, all fungi will be given a single name, using historical precedent and common use.

Safety Issues

Virtually all molds release their reproductive structures (conidia or spores) into the air, and for many fungal pathogens, the respiratory tract is the initial portal of entry. Thus, it is not surprising that certain organisms, particularly *Coccidioides* spp. and *H. capsulatum,* can be significant laboratory hazards. Containment facilities (e.g., a BSL-2 laboratory for specimen handling, initial isolation, and identification; BSL-3 is for further propagation of such agents) are needed to process specimens and to work with cultures of these organisms.[56] Because infectious spores are not present in patient specimens such as sputa or biopsy specimens, these specimens are not considered hazardous by the aerosol route, but they would be infectious by accidental inoculation. Because several of the dimorphic molds can be significant laboratory hazards when grown in mold phase, the clinician should alert laboratory personnel whenever a patient has a significant probability

TABLE 16-13 Laboratory Categorization of Selected Clinically Significant and/or Commonly Isolated Fungi

	MOLDS				
YEASTS	**Dematiaceous**	**Hyaline Septate**			**Hyaline Aseptate**
		Dermatophyte	**Dimorphic**	**Other**	
Candida	*Alternaria*	**Dermatophyte**	**Dimorphic**	**Other**	*Absidia*
Cryptococcus	*Bipolaris*	*Epidermophyton*	*Blastomyces dermatitidis*	*Aspergillus*	*Apophysomyces*
Blastoschizomyces	*Cladophialophora*	*Microsporum*	*Coccidioides* spp.	*Fusarium*	*Basidiobolus*
Malassezia	*Curvularia*	*Trichophyton*	*Histoplasma capsulatum*	*Paecilomyces*	*Cunninghamella*
Saccharomyces	*Exophiala*		*Paracoccidioides brasiliensis*	*Penicillium*	*Conidiobolus*
Rhodotorula	*Exserohilum*		*Penicillium marneffei*	*Scedosporium apiospermum**	*Mucor*
Trichosporon	*Wangiella*		*Sporothrix schenckii**	*Scedosporium prolificans**	*Rhizomucor*
					Rhizopus
					Saksenaea

*Often considered a dematiaceous mold.

Modified from Koneman EW, Roberts GD. Practical Laboratory Mycology. *3rd ed. Baltimore: Williams & Wilkins; 1985.*

of a dimorphic mold infection. Such advance notification allows the laboratory to take additional precautions with the cultures of such patients' specimens.

Specimen Collection, Transport, and Processing

The procedures used for the collection of specimens for bacterial culture will suffice for fungal culture (see Table 16-2); special media may be used to enhance fungal isolation and suppress associated bacteria and yeasts, depending upon the specimen source (see Table 16-6). It is important to be sure that an adequate volume of specimen has been collected, as for example, in the cases of CSF (up to 5 mL or even 30 mL for chronic meningitis) and urine (up to 200 mL). Twenty-four–hour collections of urine or sputa are not suitable for fungal culture because of bacterial overgrowth; first morning specimens are considered optimal. Aspirated material can be transported to the laboratory in an anaerobic transport vial; as long as the specimen is received and processed promptly by the laboratory, little loss of fungal viability appears to occur.[57] Blood culture performed by lysis-centrifugation is still considered to be the most sensitive procedure for the isolation of *H. capsulatum* from blood and is perhaps the best technique to use for the isolation of all molds from blood (see Table 16-4).[57] Commercially available broth and biphasic systems have been shown to be quite sensitive for the isolation of yeasts from blood.[31] A source of fatty acids, such as olive oil, must be added to media to recover *Malassezia* spp.; clinicians should therefore notify the laboratory when the presence of this organism is suspected. Fungal cultures are generally incubated at 30°C, and until recently, they were generally held for 4 weeks, or even longer, when the presence of a slowly growing mold such as *H. capsulatum* was suspected. However, it has been shown that shorter periods of incubation suffice for the isolation of most fungal pathogens, depending on the type of specimen and the organisms being sought; these shorter incubation periods are likely to be used by a growing number of laboratories.[58] Thus, if a slowly growing mold, such as *H. capsulatum,* is suspected, it is useful for the clinician to notify the laboratory. Certain specimen types are more useful than others for the detection of particular pathogens; these issues are dealt with in the chapters that pertain to specific organisms.

Direct Organism Detection

Fungi are generally detected in clinical specimens either by direct visualization of the organisms or by detection of substances produced by or contained in the organism; the use of molecular methods for direct detection of fungi other than *Pneumocystis jirovecii* is still primarily a research tool. Real-time PCR for diagnosis of *Pneumocystis* pneumonia is a sensitive assay that may be useful for patients in whom respiratory specimens are negative for *Pneumocystis* by traditional stains, but in whom there is a high clinical suspicion of infection. The assay, which can detect *Pneumocystis* in oral washes or sputum, may also be of benefit in patients who are too ill to undergo invasive procedures such as bronchoalveolar lavage, although PCR cannot distinguish colonization from infection.[59] Fungi may be visualized histopathologically or cytologically by a variety of special stains, such as the methenamine silver, periodic acid–Schiff, and Papanicolaou stains. In the microbiology laboratory, fungi are usually visualized directly by a Gram stain, a KOH wet mount, or a calcofluor white stain (see Table 16-6). With the Gram stain, yeast cells and pseudohyphae of *Candida* species usually stain uniformly throughout as gram-positive, whereas the cells of *C. neoformans* may be unevenly speckled with crystal violet and may be surrounded by an orange halo that is presumably capsular material. The true hyphae of organisms such as *Aspergillus* spp. or *Rhizopus* spp. are gram-negative, visible as unstained negative images, or Gram-stain invisible. In clinical specimens, the aseptate molds tend to appear as broad, branching, ribbon-like structures of rather uneven width that tend to fold on themselves, whereas hyaline septate molds tend to be smaller and more regular in width, and do not exhibit the folding often found in aseptate molds. Although KOH wet mounts allow visualization of most fungi, organisms may actually be quite difficult to discern because they do not stand out prominently from the background; in addition, tissue components, such as blood vessels, can be mistaken for fungal elements, so considerable care and expertise are required for the reading of such preparations. In the calcofluor white stain, a fluorescent compound binds to certain cell wall polysaccharides found in all fungi; organisms stained by this dye can be readily visualized with the use of a fluorescence microscope.

Among the procedures that can be used for visualization of *P. jirovecii* are calcofluor white, toluidine blue O, methenamine silver, Giemsa, and monoclonal antibody stains.[25] Calcofluor white, toluidine blue O, and methenamine silver stains stain only the cyst wall; a drawback of these stains is that they also stain yeast cells, which can be morphologically similar to *Pneumocystis* cysts. Giemsa stain allows visualization of intracystic structures and trophozoites, but the cyst wall appears only as a negative image. Monoclonal antibody stains allow the visualization of both cysts and trophozoites of *P. jirovecii,* and provide specificity not available with the other stains; however, these stains are relatively insensitive and will be falsely negative if relatively few organisms are present in the specimen.

Antigen detection tests for *B. dermatitidis*, *C. neoformans*, *Coccidioides* spp., and *H. capsulatum* are available and have clearly demonstrated their usefulness for the diagnosis of cryptococcosis and histoplasmosis. Sensitivity and specificity of these tests for the diagnosis of blastomycosis and coccidioidomycosis are still being assessed. These tests can be performed on a variety of body fluids. Detection of cryptococcal antigen has been demonstrated to be a more sensitive technique for the diagnosis of cryptococcal meningitis than is examination of CSF for cryptococci by the India ink procedure.

The detection of galactomannan in blood has been found to be helpful for the diagnosis of invasive aspergillosis.[42] However, care is needed in interpreting the significance of these test results in that sensitivity and specificity have varied in different studies. The test for β-D-glucan may be positive in a variety of invasive fungal infections, such as those caused by *Candida* spp.; not all fungi contain β-D-glucan, and a variety of substances can cause a false-positive result.

The development of molecular methods for the diagnosis of fungal infection, particularly with *Aspergillus,* is an area of active research. Such methods are still largely investigational for use with direct specimens.

Significance of Isolation of Fungi from Clinical Specimens

It is often difficult to assess the significance of the isolation of fungi other than dimorphic molds from clinical specimens, particularly if they are isolated in small quantity from nonsterile sites, such as the respiratory tract. It is usually impossible to attach clinical significance to the isolation of *Candida* from pulmonary material other than a lung biopsy because *Candida* can be part of the normal upper respiratory tract flora. Even *C. neoformans* may occasionally be a colonizer and not a pathogen in the respiratory tract. Isolation of opportunistic pathogens, such as *Aspergillus* spp. from the respiratory tract of an immunocompromised patient, presents a particularly difficult interpretive problem for which no unambiguous guidelines exist. In addition to the clinical and radiographic aspects of each situation, it may be useful to consider (1) whether the organism was seen directly in the specimen, (2) the quantity of organism that grew in culture, (3) if the organism is isolated in multiple cultures, and (4) the particular genus or species recovered (e.g., *Aspergillus niger* is less likely to be a pathogen than is *Aspergillus fumigatus* or *Aspergillus flavus*). *Penicillium* species other than *P. marneffei* are common laboratory contaminants; their isolation is almost never indicative of disease. However, because many different fungi are capable of causing at least occasional cases of infection, each situation needs to be assessed individually. The laboratory should never assume a particular isolate is a contaminant and not report recovering the organism. Rather, the laboratory concerns should be discussed with the ordering physician and the isolate reported with the qualification that it is a possible laboratory contaminant.

Identification of Fungi

Candida albicans, the most commonly isolated yeast species in clinical laboratories, is identified by performing a simple test, such as a germ tube test. It has recently been found that another *Candida* species, *C. dubliniensis,* is also germ tube positive; however, because it is not clear that there is any clinically significant difference between these two species, most laboratories do not attempt to distinguish between them. Both manual kits and automated systems are available for the identification of clinically significant yeasts. In addition, MALDI-TOF mass spectrometry is highly accurate for identification of yeasts at the species level, making the need for phenotypic tests unnecessary.[60] Identification of most molds is based largely on the morphologic features of the organism, but for certain organisms, other features, such as the ability to grow at higher temperatures or the need for specific nutritional supplements, are also used. For some fungi, assignment to a particular genus may be straightforward, whereas identification to the species level may require a mycologist with expertise in the particular group in question; examples include organisms that belong to the genera *Curvularia* and *Fusarium.* Chemiluminescent probes are available for the identification of isolates of *B. dermatitidis, Coccidioides* spp., and *H. capsulatum* (see Table 16-8). As with similar probes for mycobacterial identification, they are intended for use with isolates, not with direct patient specimens; the probe is a DNA probe for organism ribosomal RNA. These probes can be used with either the yeast or mold phase, thus allowing early identification of isolates and obviating the need for conversion of the mold to the yeast phase for *B. dermatitidis* and *H. capsulatum.* Use of these probes also eliminates the need for extensive manipulation of cultures of these hazardous organisms. As with other organisms, the number of recognized species is increasing rapidly. When confronted with an unfamiliar fungal name, the clinician should consult with the laboratory and/or search for relevant information on an Internet site such as PubMed (www.pubmedcentral.nih.gov/). A further complication is that some teleomorphs, such as *Neosartorya fischeri*, may have an anamorph (in this case, *Aspergillus fischerianus*) that is morphologically virtually indistinguishable from *A. fumigatus*.[61] There may prove to be differences in pathogenicity and antifungal susceptibility among such morphologically similar organisms.[44] Molecular methods, such as sequencing ribosomal genes or the internal transcribed spacer (ITS) region, are particularly useful for distinguishing accurately between such similar species; however, such procedures are thus far available in few clinical laboratories. As with bacteria, mycobacteria, and yeasts, the use of MALDI-TOF mass spectrometry promises to alter traditional approaches to mold identification. Lau and coworkers[62] demonstrated accuracy comparable to gene sequencing, with results available in less than 1 hour.

Susceptibility Testing

The CLSI has published standardized procedures for susceptibility testing of yeast and filamentous fungi.[63,64] Numerous caveats pertain to the assessment of the clinical relevance of such results; these vary with the drug, organism, and site of infection. For example, currently available interpretive guidelines are available only for *Candida* spp., but CLSI document M27-S3 notes that *Candida krusei* is assumed to be intrinsically resistant to fluconazole, and MICs of isolates of that species should not be interpreted with the available fluconazole scale.[63] For itraconazole, the interpretive data are based solely on results with mucosal infections, and sufficient data are not available to provide breakpoints for invasive infections. In the case of filamentous fungi, the way the MIC should be read is different for various organism–antifungal agent pairs. For example, for fluconazole and ketoconazole, complete absence of growth at the MIC is not required. Rather, "the turbidity allowed corresponds to approximately 50% or more (nondermatophyte isolates) to 80% or more (dermatophyte isolates) reduction in growth compared with the growth in the control well (drug-free medium)."[64] For the echinocandins, the concept of (minimal effective concentration" is used; this is "the lowest concentration of antimicrobial agent that leads to the growth of small, rounded, compact hyphal forms, compared with the hyphal growth seen in the growth control well."[64] With regard to the relevance of the end points obtained in the testing of filamentous fungi, the CLSI document states, "The clinical relevance of testing this group of fungal pathogens remains uncertain, and breakpoints with proven relevance have yet to be identified by CLSI or any regulatory agency."[64]

Epidemiology

A variety of molecular techniques have been applied to different fungal species for determination of strain relatedness. Such studies are available only in larger laboratories or in reference centers.

Serology

The usefulness of serologic determinations for the diagnosis of infection has been investigated for many different fungi. Often, several different methodologies, such as complement fixation and immunodiffusion, have been developed for the same organism. Kits for detection of antibody to certain organisms are commercially available. Testing for the presence of antibody to assist in the diagnosis of invasive disease has been used for the various dimorphic molds; such testing may be particularly useful for the diagnosis of coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. Antibody testing for the diagnosis of invasive infection caused by other fungal pathogens generally has not been found to be useful. However, testing for antibody in noninvasive disease has been found helpful for the diagnosis of allergic bronchopulmonary aspergillosis and aspergilloma. For details regarding optimal diagnostic methodology for different agents and for problems relating to result interpretation, see relevant chapters in this text that pertain to specific etiologic agents.

VIRUSES

The International Committee on Taxonomy of Viruses (ICTV; www.ictvdb.org) recognizes more than 2300 species of viruses. As with bacteria and fungi, viruses are classified by their morphologic and genomic properties. Specifically, viruses are classified by the type and organization of their genome (DNA or RNA, single- or double-stranded, positive or negative strand, linear or circular), replication (in cytoplasm or nucleus), and structure of the virion (size, helical or icosahedral, enveloped or not). Of the 87 families of viruses, 26 contain human pathogens (Table 16-14).

Virology Specimen Collection and Transport

The appropriate specimen for viral diagnosis is determined by the pathogen, site of infection, timing related to disease onset, and specific diagnostic test. For example, serologic diagnosis requires collection of blood specimens early in the course of disease and 2 or more weeks

TABLE 16-14 Classification of Viruses

DNA VIRUSES		RNA VIRUSES		
Single-Stranded	**Double-Stranded**	**Single-Stranded**	**Double-Stranded**	**OTHER**
Anelloviridae Parvoviridae	Adenoviridae Hepadnaviridae* Herpesviridae Papillomaviridae* Polyomaviridae* Poxviridae	**Negative-Strand RNA** Arenaviridae* Bornaviridae Bunyaviridae Deltaviridae Filoviridae Orthomyxoviridae Paramyxoviridae Rhabdoviridae **Positive-Strand RNA** Astroviridae Caliciviridae Coronaviridae Flaviviridae Hepeviridae Picornaviridae Retroviridae Togaviridae	Picobirnaviridae Reoviridae	Prions

*Circular nucleic acid; all other viruses with linear nucleic acids.
DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

TABLE 16-15 Specimen Collection for Viruses Associated with Human Infections

BODY SITES	POTENTIAL VIRUSES	SPECIMEN COLLECTION
Amniotic fluid	CMV, HIV, HSV, parvovirus B19, rubella virus	Collect by amniocentesis and transport in sterile container without VTM
Blood	Arbovirus, adenovirus, arenavirus, BK virus, coronavirus, CMV, enterovirus, EBV, filovirus, hepatitis virus (A, B, C, D, E), HSV, HHV-6, HHV-7, HHV-8, LCM, measles virus, parvovirus B19, rabies, retroviruses, rubella virus, VZV	Collect blood for culture in sterile vacuum tube or EDTA tube; avoid using tube with heparin; blood for serology should be collected at onset of symptoms and 2-4 wk later; consult laboratory for use of whole blood or plasma for NAA tests
Bone marrow	CMV, EBV, HHV-6, parvovirus B19, rubella, VZV	Aspirate transferred to EDTA tube and transported to laboratory
CSF, brain tissue	Adenovirus, arboviruses, arenavirus, CMV, coxsackie A virus, enterovirus, EBV, HHV-6, HSV, JC virus, LCM, measles virus, mumps virus, parechovirus, prions, rabies virus, retroviruses, VZV	Collect CSF and tissue in sterile leakproof container; do not dilute in VTM
Eye	Adenovirus, enterovirus, coxsackie A virus, CMV, HSV, VZV	Conjunctival swab collect on Dacron or rayon swab moistened with saline and place in VTM; aqueous and vitreous fluid placed in sterile container without VTM
Feces	Adenovirus, astrovirus, coronavirus, CMV, enterovirus, Norovirus, rotavirus, sapovirus	Fecal specimens (preferred) in leakproof container; fecal swabs in VTM
Genital	Adenovirus, CMV, HSV, papillomavirus, retrovirus	Collect on swabs and transport in VTM
Respiratory	Adenovirus, arenavirus, coronavirus, filovirus, hantavirus, CMV, HSV, influenza virus, parainfluenza virus, metapneumovirus, measles virus, parechovirus, rhinovirus, RSV, rubella virus, VZV	Nasopharyngeal aspirates (NPA) are specimen of choice from children; NPA or nasopharyngeal swabs collected from adults; bronchoalveolar lavage for diagnosis of lower respiratory tract infections
Skin	Enterovirus, HHV-8, HSV, measles virus, parvovirus B19, poxvirus, rubella virus, VZV	Swab or aspirate fluid in vesicle and scrape cells at base of lesion; place fluid in VTM
Tissue	Adenovirus, CMV, HSV, many other viruses	Place in VTM
Urine	Adenovirus, BK virus, CMV, filovirus, HSV, enterovirus, measles virus, mumps virus, rubella virus	Collect midstream specimen in sterile container; VTM not necessary

CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; EDTA, ethylenediaminetetraacetic acid; HHV-6, -7, -8, human herpesvirus 6, 7, 8, respectively; HSV, herpes simplex virus; LCM, lymphocytic choriomeningitis virus; NAA, nucleic acid amplification; RSV, repiratory syncytial virus; VTM, viral transport medium; VZV, varicella-zoster virus.

later to demonstrate seroconversion. Microscopy requires collection of specimens with infected cells, whereas antigen tests and nucleic acid amplification tests can be performed on specimens with cell-free viruses. Culture can be performed with specimens collected from the site of active disease or, if impractical, the site of initial replication (e.g., upper respiratory tract) or secondary colonization (e.g., stool, urine). Specimens should not be sent to the virology laboratory with the generalized request for "viral studies." The most likely viruses responsible for the patient's symptoms should be determined by the patient's physician or infectious disease specialist and, in collaboration with the clinical microbiologist, the appropriate specimens collected for laboratory confirmation. Table 16-15 provides a guide for selection of specimens for diagnosis of viruses associated with human infections.

The timing for collection of specimens for viral diagnosis is determined by the properties of the virus and the host. For many viral infections (e.g., influenza virus, measles virus, mumps virus, rhinovirus, West Nile virus), viral shedding begins shortly before symptoms appear and then rapidly decreases. For other chronic infections (e.g., cytomegalovirus [CMV], hepatitis B and C viruses, human immunodeficiency virus [HIV]), viral shedding can be prolonged even when the patient appears asymptomatic. Some viral shedding may be short lived in immunocompetent patients and persistent in immunocompromised patients (e.g., CMV, Norovirus, respiratory syncytial virus [RSV]). In general, collection of specimens for most diagnostic tests, with the exception of serology tests, should be at the onset of symptoms. The exception to this rule is collection of blood for serologic diagnosis. An acute-phase serum should be collected during the first week of illness and a second, convalescent serum collected 2 to 4 weeks later.

Swab and tissue specimens for viral culture should be placed in a viral transport medium that is buffered, contains protein (serum, albumin, or gelatin), and antibiotics. Antibiotics are usually incorporated in viral transport media to suppress the growth of contaminating bacteria and fungi, so separate specimens from the same site must be collected if bacterial or fungal cultures are requested. Certain types of

swab materials are not recommended; specifically, cotton swabs or wooden shafts may be toxic for culture, and calcium alginate-aluminum shaft swabs may interfere with culture of certain viruses, immunofluorescence tests, and nucleic acid amplification tests. Liquid specimens such as CSF, bronchoalveolar lavage fluid, or urine should not be diluted in viral transport media. Blood should be collected in a sterile vacuum tube or tube with the anticoagulant ethylenediaminetetraacetic acid (EDTA) or citrate. The anticoagulant heparin should be avoided if nucleic acid amplification tests are performed because heparin is a nonspecific polymerase inhibitor.[65] All specimens for virologic testing should be kept cold after collection and during transport to the laboratory. Delays in specimen transport or processing should be avoided because loss of viral viability and possibly antigen or nucleic acid degradation may occur. Specimens should not be frozen unless transport or processing is delayed by more than 5 days. Freezing at −70°C is preferable to freezing at −20°C.[66]

Detection and Identification Methods for Viruses

As with bacteria, five general approaches are used for the detection of viruses: microscopy, culture, detection of viral antigens or nucleic acids, and detection of antibodies against the organism (Table 16-16). In contrast with bacteria, microscopy and culture are generally less useful than alternative detection methods. In addition, the tests used for the detection of viruses are usually considered definitive, and supplementary identification tests are not performed.

Microscopy

Microscopy should be considered a supplementary test for viral diagnosis. Because of the small size of viruses, individual viruses cannot be seen with the light microscope. Two approaches are currently used for the microscopic detection of viruses: electron microscopy to observe individual viral particles and light microscopy to observe intracellular viral clumps or "inclusions." Although electron microscopy provides a relatively rapid detection of viral particles, few diagnostic laboratories currently use this technique. The test is useful for certain viruses (e.g., rotavirus, poxviruses, polyomavirus); however, equally rapid and more sensitive antigen and NAA tests are available for these agents. Histologic examination of tissues for viral inclusions is a rapid diagnostic test for some viruses, such as measles virus (Warthin-Finkeldey giant cells), rabies virus (Negri bodies), herpes simplex virus (HSV), varicella-zoster virus (VZV), and CMV. Wright-Giemsa smears of skin vesicles, showing multinucleated giant cells, have been used to diagnose HSV or VZV (Tzanck preparation). Specificity of microscopic examination of tissues and fluids from lesions is improved with staining techniques that use fluorescein-labeled antibodies directed against the virus (DFA tests). Commercial reagents for DFA tests are available for the herpesviruses (CMV, HSV, VZV) and respiratory viruses (influenza viruses A and B, parainfluenza virus, RSV, human metapneumovirus). The DFA test is particularly effective for VZV, where culture is insensitive; however, microscopy for most other viruses is less sensitive than alternative detection methods.[67] Fluorescein-labeled antibodies are also used to identify infected cells in tissue culture.

Culture

Viruses are strict intracellular pathogens, requiring host cells for their replication. In some infections, this can be a symbiotic relationship, where viral replication does not compromise host cell survival, and in other cases, viral replication leads to cell death. In vitro tissue culture systems were developed to mimic the natural environment for viral replication. These cell culture techniques allow detection of a wide range of viruses, including infections with a mixture of viruses. Tissue culture cells can be primary (divided only a few times), diploid (capable of 20-50 passages), or heteroploid (able to be maintained indefinitely). Because no one cell line will support the replication of all viruses, diagnostic virology laboratories use multiple cell lines. For example, influenza and parainfluenza viruses replicate in primary cell lines such as RhMK (primary rhesus monkey kidney) cells or heteroploid cell lines such as LLC-Mk2 (rhesus monkey kidney), whereas adenoviruses and RSV replicate in other cell lines such as A549 (human lung carcinoma) or HEp-2 (human epidermoid larynx carcinoma). Cocultured cell mixtures (e.g., R-Mix, H&V Mix, Super E-Mix, ELVIS [enzyme-linked virus-inducible system]) that support the growth of a broader range of viruses are also available. For some viruses, replication in traditional cell cultures may not be obvious for many days (cell death caused by viral replication is termed *cytopathic effect*). In addition, some viruses such as influenza and parainfluenza viruses, may produce little or no cytopathic effect. Detection of growth of these respiratory viruses is by staining cell cultures with fluorescein-labeled antibodies directed against the viral antigens or reactivity with erythrocytes that bind to cells expressing hemagglutinating viral antigens on their cell surface.

Many microbiology laboratories that culture viruses have replaced traditional cell culture techniques with shell vial spin amplification (SVA) cultures. This system uses 1-dram vials that contain cell culture monolayers on 12-mm round coverslips immersed in a tissue culture medium. As with traditional viral cultures, multiple shell vials that contain different cell lines are required. Specimens are added to the monolayer, centrifuged at low speed, and incubated. Instead of an examination for cytopathic effect, fluorescein-labeled monoclonal antibodies are used to detect viral antigens of replicating viruses. Shell vial cultures are incubated for up to 5 days, but most viruses can be detected in as little as 24 hours because visual cytopathic effect is not necessary for detection. A pool of monoclonal antibodies is first used to detect the presence of a virus; then individual monoclonal antibodies are used to identify the specific virus. This has the inherent limitation of only detecting viruses that are targeted by the antibodies.

Antigen Detection

A large number of commercial ELISA, EIA, and agglutination tests are currently available for viral antigen detection and are widely used in clinical laboratories (see Table 16-7). The tests are technically easy, inexpensive, and generally can be performed at the point of care or when the specimen is received in the laboratory, permitting a rapid test turnaround time. For example, detection of viral antigens provides for more rapid identification of RSV and influenza A and B infections than alternative test methods, with results available within 1 hour or less after receipt in the laboratory. The tests are highly specific (>95%), and a positive test result is particularly useful during the peak months of transmission; however, some of these respiratory virus assays are insensitive (6%-80%), and negative tests must be confirmed with alternative tests. Direct antigen tests are the test of choice for enteric viruses, such as rotavirus, adenovirus serotypes 40 and 41, and noroviruses if NAA tests are not available. In addition, antigen tests for CMV have proved useful for monitoring infections in immunocompromised patients, although this assay has been replaced by NAA tests in many laboratories. A note of caution regarding the use of rapid assays is that most assays target a single viral pathogen and, if used alone, will not detect other viruses or mixed viral infections.

Nucleic Acid–Based Tests

Nucleic acid–based amplification tests have dramatically changed viral diagnosis, with commercial assays available for the most common viruses (see Table 16-8) and home-brew assays developed for many other viruses. For many of the viruses (e.g., HIV-1 and HIV-2; hepatitis A, B, and C viruses [HAV, HBV, HCV]; CMV; human papillomavirus), these tests represent the diagnostic test of choice. NAA (specifically PCR) is the method of choice for the detection of HSV in CSF.[68] In the past, definitive diagnosis of CNS infection caused by HSV relied on the use of brain biopsy. Because detection in CSF by PCR is as sensitive as histologic stains of brain biopsy specimens, CSF PCR is now the recommended test for establishing this diagnosis. Traditional laboratory methods for the detection of HSV, CMV, and VZV in CSF, such as cell culture, antigen detection, and CSF or serum antibody detection, are known to be insensitive and are no longer recommended.[69]

Multiplex assays for the simultaneous detection of a number of respiratory viruses are available as commercial kits: FilmArray (BioFire Diagnostics), Prodesse (Hologic Gen-Probe), xTAG (Luminex, Austin, TX), and Verigene (Nanosphere). Additional multiplex assays for enteric and CNS pathogens are under development. Many of these assays are relatively simple to perform and can be processed by

TABLE 16-16 Methods for Detection and Identification of Viruses

VIRUS	DETECTION METHOD[†] Electron Microscopy	Culture	Antigen Detection	Nucleic Acid Detection	Antibody Detection	COMMENTS
Adenoviruses	C	A	A	A	C	IHC for tissue specimens; IFA and culture widely used for respiratory specimens. Rapid antigen assays used for enteric adenoviruses. NAA tests used to monitor viral load in compromised hosts.
Arboviruses	C	B	C	C	A	Serology is primary diagnostic method. Most arboviruses are readily cultured; isolation of some agents may require BSL-3 or BSL-4 facilities.
Coronaviruses	C	C	B	A	B	Positive SARS CoV NAA tests and antibody results require confirmation by a reference laboratory. NAA tests becoming more widely used for non-SARS CoV.
Cytomegalovirus	B	A	A	A	B	IHC for tissue specimens; shell vial culture rapid and sensitive for nonblood specimens. Detection of inclusions in infected cells may be useful. Quantitative NAA tests and pp65 antigenemia used to assess risk of disease and response to therapy. Serology primarily used to determine prior infection.
Enteroviruses and parechovirus	C	A	C	A	C	Enterovirus RNA detection preferred for CNS infection. Parechovirus detected primarily through virus isolation or NAA tests.
Epstein-Barr virus	C	C	B	A	A	Serology test of choice for routine diagnosis. NAA tests useful for virus-related tumors. Quantitative NAA tests useful for monitoring viral load in blood of transplant recipients. IHC or ISH can be used on tumor biopsy specimens.
Filoviruses and arenaviruses	B	B	B	B	A	Testing confined to specialized laboratories. Antigen and NAA tests key to rapid diagnosis. BSL-4 facility needed for culture, except for LCM. Patients with severe disease may die without developing antibody. LCM diagnosed primarily by serology.
Hantaviruses	C	C	B	B	A	Testing confined to specialized laboratories. Serology and NAA tests equally useful for diagnosis. IHC used in fatal cases. BSL-4 facility needed for culture. Isolation difficult.
Hendra and Nipah viruses	C	B	B	B	A	Testing confined to specialized laboratories. Serology and NAA tests equally useful for diagnosis. IHC used in fatal cases. BSL-4 facility needed for culture. Patients with severe disease may die without developing antibody.
Hepatitis A virus	C	C	C	C	A	Serology is the standard diagnostic test. False-positive IgM problematic in low-prevalence areas.
Hepatitis B virus	C	C	A	A	A	Detection of specific viral antigens and antibodies allows for diagnosis and for monitoring the course of infection. NAA tests used to monitor therapy.
Hepatitis C and G viruses	C	C	C	A	A	Serology is used for diagnosis. Qualitative NAA tests used to confirm active infection. Quantitative NAA tests used to monitor response to therapy. Genotyping helps determine duration of therapy.
Hepatitis D virus	C	C	B	B	A	Testing confined to reference laboratories. Diagnosis is relevant only in the presence of hepatitis B infection. IHC of biopsy tissue useful for diagnosis.
Hepatitis E virus	C	C	C	B	A	Serology is the standard diagnostic test. False-positive IgM problematic in low-prevalence areas. NAA test is specific for acute infection but lacks sensitivity.
Herpes simplex virus	B	A	A	A	B	Shell vial used for rapid determination of viral replication. IFA and IHC used for rapid detection in skin or mucous membrane lesions or tissue specimens. NAA test is test of choice for CNS infection. Serology used to determine prior infection.
Herpesviruses 6 and 7	C	C	C	A	B	NAA test is test of choice for diagnosis. MAbs available to differentiate virus isolates and for IHC. Serology can document primary infection in children.
Herpesvirus 8	C	C	B	B	A	Serology used to identify infected persons; sensitivity and specificity hampered by difficulty in setting cutoff values. NAA tests of blood may be useful for monitoring KS risk. IHC more specific than NAA tests for KS.
Human immunodeficiency virus	C	C	B	A	A	Serology is primary diagnostic method; rapid antibody testing becoming more widely available. Proviral DNA and plasma RNA tests used to diagnose neonatal infection. Quantitative RNA tests used to guide therapy and monitor response.
Human metapneumovirus	C	B	A	A	C	NAA is the test of choice for diagnosis. Shell vial culture and antigen detection reagents not yet widely available. Conventional culture difficult.

Continued

TABLE 16-16 Methods for Detection and Identification of Viruses*—cont'd

VIRUS	DETECTION METHOD†					COMMENTS
	Electron Microscopy	Culture	Antigen Detection	Nucleic Acid Detection	Antibody Detection	
Human T-cell lymphotropic virus	C	C	C	B	A	Serology is primary diagnostic method. NAA test useful for virus identification in HTLV Western blot-positive but untypeable specimens.
Influenza viruses	C	A	A	A	B	Rapid antigen tests widely used but some are suboptimal in sensitivity and specificity; IFA and rapid culture more sensitive. NAA test most sensitive. Serology useful for epidemiologic studies or retrospective diagnosis.
Measles viruses	B	B	C	B	A	Serology most useful for diagnosis and determination of immunity. Isolation useful only if attempted early (prodromal period to 4 days after rash develops). Detection of viral inclusions (Warthin-Finkeldey giant cells) may be useful.
Mumps virus	C	B	C	C	A	Serology used most commonly for diagnosis and determination of immunity.
Noroviruses	C	C	B	A	C	NAA test of choice. Antigen testing also sensitive.
Parainfluenza viruses	C	A	A	A	C	IFA most common rapid detection method. NAA test more sensitive than culture.
Papillomaviruses	C	C	C	A	C	NAA is test of choice for detection and genotype differentiation. Cytopathology useful for diagnosis. Serologic diagnosis of exposure not available.
Parvoviruses	C	C	C	A	A	Serology used to diagnose parvovirus B19 in immunocompetent individuals. NAA is test of choice for exposed fetuses and immunocompromised hosts. NAA test can be used to detect human bocavirus.
Polyomaviruses	C	C	B	A	C	JC virus DNA detection in cerebrospinal fluid useful for presumptive diagnosis of PML. BK virus DNA quantification in plasma or urine used for preemptive diagnosis of PVAN. IHC and EM useful for biopsy tissues.
Poxviruses	A	B	C	A	A	EM is the most useful routine test. NAA tests are also useful. Smallpox isolation requires BSL-3 or BSL-4 and should be attempted only in WHO Collaborating Centers. Vaccinia virus requires BSL-2 and grows readily in cell culture.
Rabies virus	B	C	B	A	B	IFA performed on fresh and frozen tissue at the CDC for detection of inclusions in infected cells (Negri bodies). Serology used to confirm immunization status and confirm exposure in absence of RIG. NAA test is most useful for non-CNS samples when fresh CNS tissue not available.
Respiratory syncytial virus	C	A	A	A	C	Rapid antigen tests, especially IFA, more sensitive than culture. NAA test is most sensitive. Serology useful only for epidemiologic studies.
Rhinoviruses	C	A	C	A	C	Culture still most common test in clinical laboratories. NAA tests are most sensitive.
Rotaviruses	B	C	A	A	C	Direct antigen detection or NAA tests are tests of choice for diagnosis. EM useful in suitably equipped laboratories.
Rubella virus	C	B	C	C	A	Serology most useful for diagnosis and determination of immunity. Isolation useful for postnatal rubella if attempted early (prodromal period to 4 days postrash). In CRS, virus can be isolated for weeks to months after birth.
Transmissible spongiform encephalopathy genetic agents	C	C	C	C	C	Histology most useful diagnostic test. Surrogate markers popular but lack specificity. Western blot for PrP performed in specialized laboratories. Human genome sequencing useful for diagnosis of disorders.
Varicella-zoster virus	A	A	A	A	B	IFA and NAA tests are most commonly used rapid tests. Culture less sensitive than IFA or IHC. Serology most useful for determination of immunity.

*Virus isolation includes conventional cell culture with detection of viral growth by cytopathic effects or hemadsorption; shell vial centrifugation culture uses immunostaining for detection of viral antigens. Viral antigens can be detected by a variety of immunoassays, such as enzyme-linked immunosorbent assays, agglutination assays, immunofluorescence or immunoperoxidase techniques, and immunochromatography. Viral nucleic acids (DNA or RNA) can be detected and quantified by direct hybridization or by the performance of amplification methods, such as polymerase chain reaction. Electron microscopy involves the visualization of viral particles by negative staining or immunoelectron microscopy or by thin-section techniques. Antibody detection involves measurement of total or class-specific immunoglobulins directed at specific viral antigens.

†A, Test is generally useful for the indicated diagnosis; B, test is useful under certain circumstances or for the diagnosis of specific forms of infection; C, test is seldom useful for general diagnostic purposes.

BSL, biosafety level; CDC, Centers for Disease Control and Prevention; CNS, central nervous system; CoV, coronavirus; CRS, congenital rubella syndrome; DNA, deoxyribonucleic acid; EM, electron microscopy; HTLV, human T-cell lymphotropic virus; IFA, immunofluorescence assay; IgM, immunoglobulin M; IHC, immunohistochemistry; ISH, in situ hybridization; KS, Kaposi's sarcoma; LCM, lymphocytic choriomeningitis virus; MAb, monoclonal antibody; NAA, nucleic acid amplification; PML, progressive multifocal leukoencephalopathy; PrP, prion protein; PVAN, polyomavirus-associated nephropathy; RIG, rabies immune globulin; RNA, ribonucleic acid; SARS, severe acute respiratory syndrome; WHO, World Health Organization.

Modified from Murray PR, Baron EJ, Jorgensen JH, et al, eds. Manual of Clinical Microbiology. *9th ed. Washington, DC: American Society for Microbiology Press; 2007:1304. With permission from ASM Press.*

minimally trained technologists as specimens are received in the laboratory.

Commercial quantitative assays are available for HIV-1 and HIV-2, HCV, and CMV. All of these assays are important for monitoring the progression of disease and response to antiviral therapy. Quantitative home-brew assays have also been developed for other viruses, such as Epstein-Barr virus (EBV), BK virus, and adenovirus. These home-brew assays are used to differentiate between asymptomatic shedding of low levels of virus and high viral titers associated with disease. Quantitative assays, referred to as "viral load" can be used to assist in making decisions for initiation of therapy as well as to follow the effects of antiviral therapy.

Serology

Before the development of viral antigen tests and NAA tests, serology was the diagnostic test used by most clinical laboratories and remains a commonly used test for many viruses. The sensitivity and specificity of viral serology is determined by the assay format, target viral antigens, immune competence of the host, and timing of the specimen collection. A variety of assay formats have been used historically, with the most common current assays including immunoglobulin M (IgM) capture ELISA, membrane EIA, indirect fluorescent antibody (IFA), and immunochromatographic assays. Replacement of whole-virus lysates with purified viral antigens or recombinant antigens has improved the assay specificity and in some cases sensitivity. Overall test sensitivity is determined by the timing of the sample collection—a test will have poor sensitivity if the blood sample is collected before antibodies are detectable reliably. Likewise, if an infection is defined by documenting a significant rise in specific antibodies, then the collection of the acute and convalescent samples must be sufficiently timed to document the increase. Finally, the patient must be able to mount an antibody response to infection for serologic testing to be useful.

Currently, serology may play no more important role than in the detection of HIV infection. The initial diagnosis of HIV-1 or HIV-2 infection is by detection of antibodies in the patient serum by a screening test, followed by confirmation with a supplementary test. The screening tests have undergone a series of developments, moving from use of HIV viral protein lysates, to HIV recombinant antigens bound to a solid phase, to combination assays for the detection of both HIV antigens and antibodies. The succeeding generations of assays have improved specificity; increased sensitivity, particularly early after infection; and the ability to detect both HIV-1 and HIV-2. Rapid point-of-care serologic assays have also been developed by using membrane EIAs or immunochromatographic assay methods. The immunochromatographic assays are particularly easy to perform because the target antigens are immobilized on a nitrocellulose membrane and then interact with antibodies in the patient sample as it migrates up the membrane. These point-of-care screening assays have performance characteristics comparable with laboratory-based testing.[70] Serologic screening of blood donors is also important, with testing performed to detect infections with HIV-1 and HIV-2, human T-cell lymphotropic virus (HTLV-1 and HTLV-2), HBV, and HCV.

Serologic testing is used to measure immunity to mumps virus, measles virus, rubella virus, and VZV, as well as acute infections with these viruses. Immunized patients will have persistent immunoglobulin G (IgG) antibodies, so detectable antibody levels indicate the patient is immune to infection. Patients with acute infections will develop detectable IgM antibodies within the first week after the onset of symptoms and then persist for the next few months. IgG antibodies will develop after the first week of symptoms, increase in titer over a 1- to 2-month period, and then persist for years. Thus, confirmation of an acute infection is made by detection of IgM antibodies or a significant increase in IgG antibodies. If IgM antibodies are absent during the acute phase of infection, a second IgM level should be measured 1 week after the onset of symptoms because many patients will not have detectable antibodies at the time of onset.

In many laboratories, serology is the only way to document an arbovirus infection. Commercial tests are available for many arboviruses, including West Nile virus, Western equine encephalitis (WEE) virus, St. Louis encephalitis (SLE) virus, Eastern equine encephalitis (EEE) virus, Dengue virus, and La Crosse virus. However, care must be used in the interpretation of the serologic results because cross-reactions between these viruses are common. Serology is also helpful for documenting infections with hantavirus, arenaviruses, and filoviruses, although acutely ill patients may fail to develop antibodies.

Serology is the foundation for assessing the immune status of immunocompetent individuals to parvovirus B19 infection, in whom both IgM antibodies and total antibodies can be measured using commercially available assays. In contrast, serology is not useful for neonates or immunocompromised individuals, and NAA tests should be performed to determine if infection exists.[71]

Detection of antibodies to CMV in serum from blood or organ donors and recipients is important for the prevention of CMV disease in immunocompromised patients at high risk for developing severe, life-threatening disease and is performed with ELISA or latex agglutination tests. The detection of IgM antibodies to CMV can also be used to diagnose acute infection in neonates and pregnant women, although NAA tests have replaced serology for this application in most laboratories. In this setting, assessment of IgG antibodies in neonates is not useful because maternal antibodies may be present.

Serologic methods are used for the diagnosis of primary EBV infection and for screening and monitoring of nasopharyngeal carcinoma, whereas molecular methods are used to diagnose EBV-associated lymphoma of the brain and EBV involvement in other organ lesions.[72] The most common test for the diagnosis of primary EBV infection—most frequently, infectious mononucleosis—is a heterophil antibody test. Heterophil antibodies are IgM antibodies that are reactive to antigens on heterologous erythrocytes. The antibodies are detected in a variety of assay formats, including detection of lysis of bovine erythrocytes in the presence of complement, agglutination reactions with horse or sheep erythrocytes or antigen-coated latex agglutination particles, and ELISA formats that use purified heterophil antigens. A negative heterophil antibody test in the presence of a clinical mononucleosis syndrome should prompt the physician to consider tests for EBV-specific antibodies, CMV, or toxoplasmosis.[72] Specific antibodies used to diagnose EBV infection commonly include IgG and IgM to the viral capsid antigen, antibody to early antigen, and antibody to Epstein-Barr nuclear antigen.

The hepatitis viruses are a group of unrelated DNA and RNA viruses that cause liver disease as their major clinical manifestation. Laboratory diagnosis of these infections is usually made by serologic testing for a number of different antigenic markers associated with the viruses and the patient's antibody response to these antigens. Laboratories have tried to make hepatitis diagnosis easier by offering panel or profile testing that varies according to patient characteristics: exposure but asymptomatic, symptomatic with acute disease (<6 months), or symptomatic with chronic disease (>6 months). The most commonly used methods for serologic diagnosis are ELISA and immunofluorescence assays. The first generation of ELISA tests for hepatitis C lacked sensitivity and specificity, but second- and third-generation tests have shown greatly improved performance.[73] Similar to HIV testing, immunoblots performed with recombinant antigens (recombinant immunoblot assays [RIBAs]) are used to confirm positive sera detected by ELISA. Third-generation RIBA tests were approved by the FDA in 1999; these use synthetic c100 and c22 peptides, with a significant reduction in the number of indeterminant RIBA results.[73] Details concerning the interpretation of results for hepatitis tests can be found in the chapters in which acute and chronic hepatitis are discussed and in individual chapters for each virus.

Antiviral Susceptibility Testing

Antiviral drugs are available for the management of viral infections, including those caused by HIV-1, HSV types 1 and 2, CMV, VZV, influenza A and B virus, RSV, HBV, and HCV. The emergence of resistant viruses is inevitable in that antiviral agents are used frequently and widely.[74] Antiviral resistance is defined as a decrease in susceptibility to an antiviral agent established by in vitro testing (phenotypic testing) and confirmed by genetic analysis of the viral genome (genotypic testing) and biochemical analysis of the altered enzymes. Clinical failures may not always result from the presence of a drug-resistant virus. Factors to consider in a patient with a poor clinical response to an

TABLE 16-17 Classification of Parasites

PROTOZOA				HELMINTHS			ARTHROPODS
Amoebae	**Flagellates**	**Sporozoa**	**Ciliates**	**Cestodes**	**Nematodes**	**Trematodes**	
Acanthamoeba *Balamuthia* *Entamoeba*	*Dientamoeba* *Giardia* *Leishmania* *Naegleria* *Trichomonas* *Trypanosoma*	*Babesia* *Cryptosporidium* *Cyclospora* *Plasmodium* *Toxoplasma*	*Balantidium*	*Diphyllobothrium* *Echinococcus* *Hymenolepis* *Taenia*	*Ancylostoma* *Ascaris* *Brugia* *Dirofilaria* *Dracunculus* *Enterobius* *Gnathostoma* *Loa* *Mansonella* *Necator* *Onchocerca* *Strongyloides* *Toxocara* *Trichinella* *Trichuris* *Wuchereria*	*Clonorchis* *Fasciola* *Fasciolopsis* *Heterophyes* *Opisthorchis* *Paragonimus* *Schistosoma*	Acarines: ticks and mites Anoplura: lice Diptera: flies and mosquitoes Hemiptera: kissing bugs Siphonaptera: fleas

antiviral agent include the patient's immunologic status and the pharmacokinetics of the drug in the individual patient (e.g., dose or route of administration). The development of resistance is favored by long-term suppressive therapy, recurrent intermittent therapy, and the use of suboptimal doses of an antiviral agent. Antiviral susceptibility testing is warranted for the following clinical situations: failure of HSV or VZV lesions to resolve or the appearance of new lesions during treatment with acyclovir, progression of CMV disease during ganciclovir therapy, continued shedding or transmission of influenza A virus during treatment or prophylaxis with amantadine or rimantadine, and increased HIV-1 RNA plasma levels or decreased CD4 cell counts in HIV-infected patients during antiretroviral therapy.[75] Susceptibility tests can also be used to guide treatment of HCV infections.

Laboratory assays for antiviral susceptibility testing include phenotypic and genotypic assays. Phenotypic assays require growth of the virus in vitro; therefore, viruses for which in vitro culture systems are not available, such as hepatitis B virus, cannot be tested with these assays. Results from phenotypic assays may not be available within a time frame that is clinically relevant because they all require initial propagation of the virus to attain a standard inoculum concentration, followed by growth of the virus in the assay to obtain results. After up to a week of propagation to prepare inoculum, once the assay has been set up, results for HSV may be obtained in 48 hours. For CMV, results may take 8 to 14 days after several weeks of propagation. Results of phenotypic assays are reported as the drug concentration that causes a 50% inhibition (inhibition concentration $[IC]_{50}$) or a 95% inhibition (IC_{95}) in growth of the virus. Comparison of the results of phenotypic assays from different laboratories is difficult. The most commonly used phenotypic antiviral susceptibility assay is the plaque reduction assay (PRA), which measures the ability of varying concentrations of an antiviral agent to inhibit the viral cytopathic effect as the end point. The CLSI offers a proposed standard in an effort to standardize the PRA for HSV.[76] Other end points for phenotypic viral assays that have been developed in an effort to make phenotypic testing more rapid and sensitive include dye uptake by viable (noninfected) cells, DNA hybridization, ELISA, plaque autoradiography, and flow cytometry.[77]

Genotypic assays have been developed for the rapid detection of mutations that confer antiviral-drug resistance. Results from these assays simply report the presence or absence of a mutation known to confer resistance to a specific antiviral agent. Most genotypic assays use PCR to amplify specific viral genes, followed by direct sequencing of the amplified products or hybridization to specific probes on membrane strips or gene chips to detect changes in the sequence known to be associated with resistance to antiviral agents. Genotypic assays for the detection of drug resistance in HSV and VZV reveal thymidine kinase mutations that confer resistance to acyclovir, famciclovir, penciclovir, and valacyclovir. Genotypic assays used for determining resistance to ganciclovir, foscarnet, and cidofovir in CMV detect mutations in the phosphotransferase and DNA polymerase genes.[77]

The major drawback of genotypic assays is that they can detect only resistance caused by known mutations. Both in-house and commercial assays must be continually updated as new mutations or resistance mechanisms are identified. If resistance to an antiviral agent can be caused by different gene mutations, multiple assays might be needed for optimal detection. Strains with new or different resistance genes would go undetected, so phenotypic assays will still be required to identify new mutations responsible for antiviral resistance.

PARASITES

Eukaryotic organisms that are classified as parasites are organized into three broad groups: protozoa, helminths, and arthropods. Within the protozoa are placed the amebae, flagellates, sporozoa, and ciliates; the helminths include cestodes (tapeworms), nematodes (roundworms), and trematodes (flatworms); arthropods are a collection of bugs including ticks, mites, lice, fleas, flies, mosquitoes, and so forth (Table 16-17). One group that has been omitted by this classification scheme is the microsporidia, which is a collection of more than 100 species. Historically, this group was placed with the protozoa, but more recent genomic analysis demonstrates these "parasites" belong with the fungi.[78] For convenience and historical purposes, we will discuss the microsporidia with the parasites in this section of the chapter.

Parasitology Specimen Collection and Transport

The most common specimens submitted for parasitic studies are blood and feces. Other specimens that may also be collected are other sterile body fluids and tissues, respiratory and urogenital specimens, and skin biopsies. Table 16-18 provides guidelines for specimen collection for parasites associated with human infections.

Blood

Microscopic examination for parasites can be done by using whole blood, buffy coat preparations, or concentrated blood. Blood films can be prepared typically from fresh blood with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Smears should be prepared within 1 hour after collection of the blood, or some morphologic features of the parasites (e.g., stippling in malarial smears) may be lost. Timing of the collection should be noted so that this can be correlated with the fever pattern of the patient. In addition, some parasites have a circadian rhythm and are present in the blood only during specific periods during the day (i.e., *Loa,* diurnal; *Wuchereria* and *Brugia,* nocturnal). Blood collected in EDTA tubes should be used for antigen and NAA tests, and serology tests can use either serum or plasma. Blood for serologic testing should be collected at the onset of symptoms (acute phase) and then 2 to 4 weeks later (convalescent phase).

Stool

Most stool specimens will be for microscopic examination. The presence of barium will obscure intestinal protozoa for up to 10 days, so it should be avoided until after three specimens have been submitted for ova and parasite examination.[79] In addition, other substances can

TABLE 16-18 Guidelines for Specimen Collection and Diagnostic Tests for Detection of Parasites Associated with Human Infections

BODY SITE/SPECIMEN	POTENTIAL PARASITES	LABORATORY TEST
Amniotic fluid	*Toxoplasma*	NAA test: aspirated fluid for *Toxoplasma*
Blood	*Babesia, Brugia, Leishmania, Loa, Mansonella, Plasmodium, Trypanosoma, Wuchereria*	Microscopy: fresh whole blood for Giemsa-stained thick and thin smears for all blood parasites; Giemsa- or hematoxylin-stained smears for microfilariae; buffy coat for detection of motile microfilariae and trypanosomes Antigen detection: EDTA blood for detection of malarial and microfilarial antigens NAA tests: EDTA blood for malaria and *Leishmania* Antibody detection: Serum or plasma for detection of antibodies to parasites
Bone marrow	*Leishmania, Plasmodium, Toxoplasma, Trypanosoma*	Microscopy: EDTA blood for Giemsa-stained thick and thin smears for all parasites Culture: EDTA blood for *Leishmania* and *Trypanosoma cruzi* NAA tests: EDTA blood for *Leishmania* and *Toxoplasma*
Cerebrospinal fluid and brain biopsy	*Acanthamoeba, Balamuthia, Echinococcus,* microsporidia, *Naegleria, Taenia* (cysticerci), *Trypanosoma*	Microscopy: CSF stained with Giemsa (*Trypanosoma*, *Toxoplasma*, amebae), calcofluor white (amebae), acid-fast (microsporidia), trichrome (amebae), or modified trichrome (microsporidia); tissue stained with Gram stain (microsporidia) or H&E (larval cestodes, *Taenia solium* cysticerci, *Echinococcus*) Culture: fluid or tissue for *Acanthamoeba* NAA tests: fluids or tissue for amebae, *Toxoplasma*, and helminths
Eye	*Acanthamoeba, Loa,* microsporidia, *Toxoplasma*	Microscopy: Scrapings or biopsy stained with Giemsa or calcofluor white (amebae) or modified trichrome (microsporidia) Culture: Scrapings or biopsy for amoebae and *Toxoplasma* NAA tests: scrapings or biopsy for microsporidia and *Toxoplasma*
Intestinal tract	*Ascaris, Balantidium, Blastocystis, Cryptosporidium, Cyclospora, Diphyllobothrium, Entamoeba, Enterobius, Giardia,* hookworm, *Hymenolepis, Isospora,* microsporidia, *Schistosoma, Strongyloides, Taenia, Toxocara, Trichuris*	Microscopy: stool or duodenal aspirates examined by direct wet smear (detection of motile protozoa), iodine staining (protozoa, helminth eggs), or concentrated and stained with trichrome or iron hematoxylin (protozoa), modified trichrome (microsporidia), or modified acid-fast stains (*Cryptosporidium, Cyclospora, Isospora*); unstained anal impression smears used to detect *Enterobius* (pinworm eggs) Antigen detection: freshly collected, nonpreserved stool used for detection of *Entamoeba histolytica, Giardia, Cryptosporidium* NAA tests: in-house assays but no commercial assays available
Liver and spleen	*Capillaria, Clonorchis, Echinococcus, Entamoeba, Fasciola, Fasciolopsis, Heterophyes, Leishmania, Metagonimus,* microsporidia, *Opisthorchis, Toxoplasma*	Microscopy: biopsy or aspirate for *E. histolytica, Echinococcus*, or *Capillaria* (wet mount) or *Leishmania* (Giemsa) Culture: biopsy or aspirate for *Leishmania* NAA tests: In-house assays but no commercial assays available
Muscle	*Ascaris, Cryptosporidium, Echinococcus,* hookworm, microsporidia, *Paragonimus, Strongyloides, Toxoplasma, Trypanosoma*	Microscopy: biopsy for tissue stains (H&E, methenamine silver, PAS, Gram) for *Trichinella* and *Echinococcus* NAA tests: in-house assays but no commercial assays available
Respiratory tract	*Ancylostoma, Echinococcus, Gnathostoma,* microsporidia, *Multiceps, Onchocerca, Taenia* (cysticerci), *Trichinella, Trypanosoma*	Microscopy: sputum (expectorated, induced), nasal discharge, BAL (aspirate, brushing), and biopsy for *Ascaris, Strongyloides, Paragonimus, Capillaria*, and *Echinococcus* (unstained preparation), Giemsa (*Toxoplasma*), modified acid-fast (*Cryptosporidium*), modified trichrome (microsporidia), and tissue stains (H&E, methenamine silver, PAS, Gram) for helminths, protozoa, and microsporidia NAA tests: in-house assays but no commercial assays available
Skin and cutaneous ulcers	*Acanthamoeba, Leishmania,* microsporidia, *Naegleria*	Microscopy: aspirates, skin snips, scrapings, and biopsy—Giemsa or H&E for *Leishmania, E. histolytica, Acanthamoeba*, and *Onchocerca*; direct visualization for arthropods (e.g., *Demodex*, botflies, ticks, lice) Culture: biopsy for *Leishmania* NAA tests: in-house assays but no commercial assays available
Urogenital tract	*Acanthamoeba, Leishmania, Onchocerca,* microsporidia, *Schistosoma, Trichomonas*	Microscopy: vaginal discharge, urethral discharge, and prostatic secretion for *Trichomonas* (Giemsa), microfilariae (hematoxylin), and microsporidia (modified trichrome, acid fast); urine sediment for *Schistosoma* or microfilariae (wet mount) Culture: vaginal or urethral discharge for *Trichomonas* NAA tests: in-house assays but no commercial assays available

BAL, bronchial alveolar lavage; CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; H&E, hematoxylin and eosin; NAA, nucleic acid amplification; PAS, periodic acid–Schiff.

interfere with detection of protozoa, including mineral oil, bismuth, metronidazole, tetracyclines, and antimalarial agents. If the specimen must be examined within 2 weeks of administering these agents, then the report, if negative, should indicate the specimen was suboptimal, and additional specimens should be submitted at a later time. Stool specimens may be examined for the presence of motile parasites. In this case, the specimen should be promptly transported to the laboratory immediately after collection and not placed in preservatives. If a delay in transport is anticipated, then the specimen should be placed in preservatives at the time of collection because protozoan trophozoites are fragile and will rapidly deteriorate. This is not a problem with protozoan cysts or helminth eggs and larvae, coccidia, or microsporidia. A variety of fixatives are available for preserving stool specimens (e.g., formalin, sodium acetate-acetic acid-formalin, Schaudinn's fluid, polyvinyl alcohol). The selection of preservatives will be determined by the methods used in the microbiology laboratory for examining the specimen and use of additional tests (e.g., antigen or NAA tests). Regardless of the preservative used, care must be used to add the appropriate amount of specimen to the container and ensure complete mixing of the stool specimen with the preservative.[80] It is recommended that three specimens should be collected, to exclude reliably a parasitic infection. Examination of a single specimen may have a sensitivity of 50% to 60%.[81] The specimens should be collected on separate days and, if possible, spread over a week or more.

Other Specimens

Normally, sterile body fluids and tissues submitted for culture should be promptly transported to the laboratory in a sterile container. Specimens collected for microscopic examination should not be placed in preservative; therefore, it is critical to have the laboratory examine the specimen promptly. Specimens for NAA tests should be refrigerated if immediate transport is not feasible.

Detection and Identification Methods for Parasites

As with the other microorganisms discussed in this chapter, five approaches exist for the detection of parasites: microscopy, culture, antigen or nucleic acid detection, and antibody detection. In contrast with the other groups of organisms, microscopy is the primary detection and identification procedure for most parasites. Culture is used for only a few parasites and, when available, is not the primary diagnostic method. Antigen testing is used for a limited number of parasites, and nucleic acid–based tests are primarily restricted to reference or research laboratories. Serology is useful for populations where endemic infections are uncommon but has little value in countries with a high incidence of infection.

Microscopy

Microscopy is the definitive method for the detection and identification of most parasites. Many parasites can be readily detected by examining the collected specimen directly or following staining with iodine; however, maximum sensitivity requires concentration of the specimen and examination with specific stains to allow differentiation of the internal structures (e.g., trichrome stain, iron hematoxylin stain, Giemsa stain). Identification of the parasite is determined by the morphologic features of the protozoa and the characteristics of the eggs, larvae, or adult forms of cestodes, nematodes, and trematodes. Details of these features are discussed in the individual chapters in this book and in the cited reference texts.[8,79]

Culture

Culture is primarily performed in research laboratories with one exception: *Trichomonas vaginalis* infections. Although trichomonal infections can be readily detected by microscopic inspection of vaginal secretions, commercial kits permit the growth of *T. vaginalis* directly by culturing the vaginal exudate. These systems are more sensitive than microscopic examination of the exudate, although this may increase detection of patients with asymptomatic colonization.

Antigen Detection

Antigen tests are commercially available for *Entamoeba histolytica*, *Cryptosporidium* spp., *Giardia lamblia*, *Plasmodium* spp., and *T. vaginalis*. The tests are generally more sensitive than microscopic examination and less subjective (better specificity). In geographic areas where parasitic infections are uncommon, some laboratories restrict their routine testing to immunoassays for *E. histolytica*, *Cryptosporidium,* and *Giardia*.

Nucleic Acid–Based Tests

NAA tests are primarily restricted to reference and research laboratories, with the exception of commercial tests developed for the detection of *Trichomonas*. Although home-brew assays have been developed for most parasites, there is little need for these tests for routine diagnostic purposes. The assays are useful for the diagnosis of infections with *Toxoplasma*, *Leishmania*, and *Trypanosoma,* for which relatively few parasites may be present in a sample.

Serology

Serologic testing is rarely performed except to document infection in a patient from a nonendemic country who has been exposed to a particular parasite or patients suspected to be infected with *Toxoplasma gondii*.[82] The presence of IgG antibodies to a parasite is consistent with past exposure, and evidence of a recent seroconversion is consistent with an acute infection. However, if seroconversion cannot be demonstrated, then a careful travel and exposure history is required to interpret the significance of seropositive results. For *Toxoplasma* infections, IgM and IgG antibody titers are determined. A negative IgM titer excludes acute infection because all patients with active disease have IgM antibodies at the time symptoms develop, which decrease to nondetectable levels by 6 to 9 months. IgG antibody levels will persist for years.

References

1. Peterson LR, Hamilton JD, Baron EJ, et al. Role of clinical microbiology laboratories in the management and control of infectious diseases and the delivery of health care. *Clin Infect Dis*. 2001;32:605-611.
2. Hindler JF, Barton M, Callihan DR, et al. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline*. 3rd ed. CLSI document M39-MA3. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
3. Medicare, Medicaid, and CLIA Programs. Laboratory requirements relating to quality systems and certain personnel qualifications. Final rule. *Fed Regist*. 2003;68:3639-3714.
4. Elder BL, Hansen SA, Kellogg JA, et al. *Verification and Validation of Procedures in the Clinical Microbiology Laboratory*. Cumitech 31. Washington, DC: American Society for Microbiology; 1997.
5. Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology Press; 2011.
6. Zadroga R, Williams DM, Gottschall R, et al. Comparson of two blood culture media shows significant differences in bacterial recovery for patients on antimicrobial therapy. *Clin Infect Dis*. 2013;56:790-797.
7. Bouza E, Sousa D, Rodriquez-Creixems M, et al. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? *J Clin Microbiol*. 2007; 45:2765-2769.
8. Little JR, Murray PR, Traynor PS, Spitznagel E. A randomized trial of povidone-iodine compared with iodine tincture for venipuncture site disinfection: effects on rates of blood culture contamination. *Am J Med*. 1999;107:119-125.
9. Leisure M, Moore D, Schwartzman J, et al. Changing the needle when inoculating blood cultures. A no-benefit and high-risk procedure. *JAMA*. 1990;264:2111-2112.
10. Martinez J, Pozo L, Almela M, et al. Microbial and clinical determinants of time-to-positivity in patients with bacteremia. *Clin Microbiol Infect*. 2007;13:709-716.
11. Baron EJ, Scott JD, Tompkins LS. Prolonged incubation and extensive subculturing do not increase recovery of clinically significant microorganisms from standard automated blood cultures. *Clin Infect Dis*. 2005;41:1677-1680.
12. American Association of Blood Banks. *Comprehensive Report on Blood Collection and Transfusion in the United States in 2001*. Bethesda, MD: National Blood Data Resource Center; 2003.
13. Khuu H, Patel N, Carter C, et al. Sterility testing of cell therapy products: parallel comparison of automated methods with a CFR-compliant method. *Transfusion*. 2006; 46:2071-2082.
14. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter related infection. *N Engl J Med*. 1977;296:1305-1309.
15. Collignon PJ, Soni N, Pearson IY, et al. Is semiquantitative culture of central vein catheter tips useful in the diagnosis of catheter-associated bacteremia? *J Clin Microbiol*. 1986;24: 532-535.
16. Siegman-Igra Y, Anglim AM, Shapiro DE, et al. Diagnosis of vascular catheter-related bloodstream infection: a meta-analysis. *J Clin Microbiol*. 1997;35:928-936.
17. O'Grady NP, Alexander M, Dellinger EP, et al. Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis*. 2002;35:1281-1307.
18. Bisno AL, Gerber MA, Gwaltney JM, et al. Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. *Clin Infect Dis*. 2002;35: 113-125.
19. Cloud JL, Hymas W, Carroll KC. Impact of nasopharyngeal swab types on detection of *Bordetella pertussis* by PCR and culture. *J Clin Microbiol*. 2002;40:3838-3840.
20. Rossney A, Herra C, Brennan G, et al. Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert real-time PCR platform for rapid detection of MRSA from screening specimens. *J Clin Microbiol*. 2008;46:3285-3290.
21. Dalpke AH, Hofko M, Zimmermann S. Comparison of the BD MAX MRSA assay and the BD GeneOhm MRSA achromopeptidase assay with direct and enriched culture techniques using clinical specimens for detection of MRSA. *J Clin Microbiol*. 2012;50:3365-3367.
22. Cantral DE, Tape TG, Reed EC, et al. Quantitative culture of bronchoalveolar lavage fluid for the diagnosis of bacterial pneumonia. *Am J Med*. 1993;95:601-607.
23. Roberts A, Robinson R, Beard R. Some factors affecting bacterial colony counts in urinary infection. *Br Med J*. 1967;1:400-403.
24. Hines J, Nachamkin I. Effective use of the clinical microbiology laboratory for diagnosing diarrheal diseases. *Clin Infect Dis*. 1996;23(suppl 1):S97-S101.
25. Kuijper EJ, Coignard B, Tull P. Emergence of *Clostridium difficile*-associated disease in North America and Europe: review. *Clin Microbiol Infect*. 2006;12:2-18.
26. Novak-Weekley SM, Marlowe EM, Miller JM, et al. *Clostridium difficile* testing in the clinical laboratory by use of multiple testing algorithms. *J Clin Microbiol*. 2010;48:889-893.
27. Chapin KC, Dickenson RA, Wu F, Andrea SB. Comparison of five assays for detection of *Clostridium difficile* toxin. *J Mol Diag*. 2011;11:395-400.
28. LeGuern R, Herwegh S, Grandbastien B, et al. Evaluation of a new molecular test, the BD Max Cdiff, for detection of toxigenic *Clostridium difficile* in fecal samples. *J Clin Microbiol*. 2012;50:3089-3090.
29. Amsel R, Totten PA, Spiegel CA, et al. Nonspecific vaginitis: diagnostic criteria and microbial epidemiologic association. *Am J Med*. 1983;74:14-22.
30. She RC, Billetdeaux E, Rhansalkar AR, Petti CA. Limited applicability of direct fluorescent-antibody testing for *Bordetella* sp. and *Legionella* sp. specimens for the clinical microbiology laboratory. *J Clin Microbiol*. 2007;45:2212-2214.
31. Crobach MJT, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: data review and recommendations for diagnosing *Clostridium difficile* infection. *Clin Microbiol Infect*. 2009;15: 1053-1066.
32. National Committee for Clinical Laboratory Standards (NCCLS). *Abbreviated Identification of Bacteria and Yeast: Approved Guideline*. NCCLS document M35-A. Wayne, PA: NCCLS; 2002.
33. Maiden MCJ, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A*. 1998;95:3140-3145.
34. Murray P. What is new in clinical microbiology: microbial identification by MALDI-TOF. *J Mol Diagn*. 2012;14: 419-423.
35. Wieser A, Schneider L, Jung J, Schubert S. MALDI-TOF MS in microbiological diagnostics—identification of microorganisms and beyond (mini review). *Appl Microbiol Biotechnol*. 2012;93:965-974.
36. Kok J, Thomas LC, Olma T, et al. Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper and time of flight mass spectrometry. *PLoS One*. 2011;6:e23285.
37. National Committee for Clinical Laboratory Standards (NCCLS). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 6th ed. Approved Standard. NCCLS document M7-A8. Wayne, PA: NCCLS; 2009.

38. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing: 18th Informational Supplement*. CLSI document M100-MS23. Wayne, PA: CLSI; 2013.
39. National Committee for Clinical Laboratory Standards (NCCLS). *Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard*. 8th ed. NCCLS document M2-A10. Wayne, PA: NCCLS; 2009.
40. Chosewood LC, Wilson DE, eds. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: U.S. Government Printing Office; 2007.
41. Pfyffer GE, Palicova F. *Mycobacterium*: general characteristics, laboratory detection, and staining procedures. In: Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: ASM Press; 2011:472-502.
42. Ascioglu S, Rex JH, dePauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis*. 2002;34: 7-14.
43. O'Sullivan CE, Miller DR, Schneider PS, Roberts GD. Evaluation of Gen-Probe amplified *Mycobacterium tuberculosis* direct test by using respiratory and nonrespiratory specimens in a tertiary care center laboratory. *J Clin Microbiol*. 2002;40:1723-1727.
44. Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, et al. *Aspergillus* section Fumigati: antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Chemother*. 2008;52:1244-1251.
45. Savic B, Sjöbring U, Alugupalli S, et al. Evaluation of polymerase chain reaction, tuberculostearic acid analysis, and direct microscopy for the detection of *Mycobacterium tuberculosis* in sputum. *J Infect Dis*. 1992;166:1177-1180.
46. Tenover FC, Crawford JT, Huebner RE, et al. The resurgence of tuberculosis: is your laboratory ready? *J Clin Microbiol*. 1993;31:767-770.
47. American Thoracic Society. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175:367-416.
48. Jasmer RM, Roemer M, Hamilton J, et al. A prospective, multicenter study of laboratory cross-contamination of *Mycobacterium tuberculosis* cultures. *Emerg Infect Dis*. 2002; 8:1260-1263.
49. Centers for Disease Control and Prevention (CDC). Multiple misdiagnoses of tuberculosis resulting from laboratory error—Wisconsin, 1996. *MMWR Morb Mortal Wkly Rep*. 1997;46:797-801.
50. Clinical and Laboratory Standards Institute (CLSI). *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard*. CLSI document M24-A2. Wayne, PA: CLSI; 2011.
51. van Embden JDA, Cave MD, Crawford JT, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol*. 1993;31:406-409.
52. Mazurek GH, Jereb J, Vernon A, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010. *MMWR Morb Mortal Wkly Rep*. 2010;59(RR-5):1-25.
53. Conville PS, Witebsky FG. *Nocardia, Rhodococcus, Gordonia, Actinomadura, Streptomyces*, and other aerobic actinomycetes. In: Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology Press; 2011:443-471.
54. Hibbett DS, Binder J, Bischoff M, et al. A higher level phylogenetic classification of the Fungi. *Mycol Res*. 2007;111: 509-547.
55. Fisher MC, Koenig GL, White TJ, Taylor JW. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia*. 2002;94:73-84.
56. Noble MA. Prevention and control of laboratory-acquired infections. In: Murray PR, Baron EJ, Jorgensen J, et al, eds. *Manual of Clinical Microbiology*. 9th ed. Washington, DC: American Society for Microbiology Press; 2007:97-106.
57. McGowan KL. Specimen collection, transport, and processing: mycology. In: Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology Press; 2011:1756-1766.
58. Morris AJ, Byrne TC, Madden JF, et al. Duration of incubation of fungal cultures. *J Clin Microbiol*. 1996;34:1583-1585.
59. Larsen HH, Masur H, Kovacs JA, et al. Development and evaluation of a quantitative touch-down, real-time PCR assay for diagnosing *Pneumocystis carinii* pneumonia. *J Clin Microbiol*. 2002;40:490-494.
60. Stevenson LG, Drake SK, Shea YR, et al. Evaluation of matirix-assisted laser desorption ionization time of flight mass spectrometry for identification of clinically important yeast species. *J Clin Microbiol*. 2010;48:3482-3486.
61. de Hoog GS, Guarro J, Gene J, Figueras MJ. *Atlas of Clinical Fungi*. 2nd ed. Utrecht, The Netherlands: Centraalbureau voor Schmmelcultures; 2000.
62. Lau AF, Drake SK, Calhoun LB, et al. Development of a clinically comprehensive database and simple procedure for the identification of molds from solid media by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *J Clin Microbiol*. 2013;51:828-834.
63. Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*. 3rd ed. CLSI document M27-S3. Wayne, PA: CLSI; 2008.
64. Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard*. 2nd ed. CLSI document M38-A2. Wayne, PA: CLSI; 2008.
65. Wang J-T, Wang T-H, Sheu J-C, et al. Effects of anticoagulants and storage of blood samples on efficacy of the polymerase chain reaction assay for hepatitis C virus. *J Clin Microbiol*. 1992;30:750-753.
66. Forman MS, Valsamakis A. Specimen collection, transport, and processing: virology. In: Murray PR, Baron EJ, Jorgensen J, et al, eds. *Manual of Clinical Microbiology*. 9th ed. Washington, DC: American Society for Microbiology Press; 2007:1284-1296.
67. Coffin S, Hodinka R. Utility of direct immunofluorescence and virus culture for detection of varicella-zoster virus in skin lesions. *J Clin Microbiol*. 1995;33:2792-2795.
68. Lakeman FD, Whitley RJ, National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. *J Infect Dis*. 1995;171:857-863.
69. Yan SS, Fedorko DP. Recent advances in laboratory diagnosis of human cytomegalovirus infection. *Clin Applied Immunol Rev*. 2002;2:155-167.
70. Bulterys M, Jamieson D, O'Sullivan M, et al. Mother-Infant Rapid Intervention at Delivery (MIRAD) Study Group. Rapid HIV-1 testing during labor: a multicenter study. *JAMA*. 2004;292:219-223.
71. Brown KE, Young NS. Parvovirus B19 in human disease. *Annu Rev Med*. 1997;48:59-67.
72. Gartner BC. Epstein-Barr virus. In: Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology Press; 2011:1575-1584.
73. Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. *J Clin Microbiol*. 2002;40: 4407-4412.
74. Shafer RW, Najera I, Chou S. Mechanisms of resistance to antiviral agents. In: Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology Press; 2011:1710-1728.
75. Hodinka RL. What clinicians need to know about antiviral drugs and viral resistance. *Infect Dis Clin North Am*. 1997; 11:945-967.
76. National Committee for Clinical Laboratory Standards (NCCLS). *Antiviral Susceptibility Testing; Proposed Standard*. NCCLS document M33-P. Wayne, PA: NCCLS; 2000.
77. Smith TF. Susceptibility testing. Viral pathogens. *Infect Dis Clin North Am*. 2001;15:1263-1294.
78. Hirt RP, Logsdon JM, Healey B, et al. Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. *Proc Natl Acad Sci U S A*. 1999;96:580-585.
79. Garcia LS. *Diagnostic Parasitology*. 5th ed. Washington DC: American Society for Microbiology Press; 2007.
80. Clinical Laboratory Standards Institute (CLSI). *Procedures for the Recovery and Identification of Parasites from the Intestinal Tract; Approved Guideline*. 2nd ed. CLSI document M28-MA2. Wayne, PA: CLSI; 2005.
81. Marti H, Koella JC. Multiple stool examinations for ova and parasites and rate of false-negative results. *J Clin Microbiol*. 1993;31:3044-3045.
82. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J Infect Dis*. 2002;185: S73-S82.