For some specimen types, the presence of inflammatory and epithelial cells is used to judge the suitability of a specimen for culture. For example, the presence of more than 10 epithelial cells per low-power field in a sputum specimen is highly suggestive of a specimen contaminated with oral secretions. In addition, a preliminary assessment of the etiologic agent can be made based upon the morphology (e.g., cocci vs rods) and stain reaction (e.g., Gram-positive isolates are purple; Gram-negative are red) of the microorganisms. However, a negative Gram stain does not rule out infection as $10^4$ to $10^5$ microorganisms per mL in the specimen are required for detection by this method.

In addition to the Gram stain, many other stains are used in microbiology, both to detect organisms and to help infer their identity. Table 170-1 provides an overview of the most commonly used stains.

**Isolation and Identification**

The approach to isolation of microorganisms in a clinical specimen will vary depending on the body site and pathogen suspected. For body sites that are usually sterile, such as cerebrospinal fluid, nutrient-rich media such as sheep blood agar and chocolate agar are used to aid in the recovery of fastidious pathogens. In contrast, stool specimens contain abundant amounts of commensal bacteria and thus to isolate pathogens, selective and differential media must be used. Selective media will inhibit the growth of some organisms to aid in isolation of suspect pathogens; differential media rely on growth characteristics or carbohydrate assimilation characteristics to impart a growth pattern that differentiates organisms. MacConkey agar supports growth of Gram-negative rods while suppressing Gram-positive organisms, and a color change in the media from clear to pink distinguishes lactose-fermenting organisms from other Gram-negative rods. Special media, such as Sabouraud dextrose agar and inhibitory mold agar, are used to recover fungi in clinical specimens. Many pathogens, including *Bar- tonella, Bordetella pertussis, Legionella, Mycoplasma*, and certain fungal pathogens such as *Malassezia furfur*, require specialized growth media or incubation conditions. Consultation with the laboratory is advised when these pathogens are suspected.

Once an organism is recovered in culture, additional testing will be performed to identify the isolate. Confirmation of microbial identity has classically been performed using phenotypic tests that rely on the phenotypic properties of an isolate. Some examples include carbohydrate assimilation patterns, indole production, and motility. However, these methods are not able to resolve all organisms to species level and require incubation time. In some instances, sequence based identification, for bacteria usually based on sequence analysis of the bacterial 16S rRNA gene, is used for organism identification (particularly organisms that are difficult to culture).

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and accurate technique that is based on generating a protein fingerprint of an organism and comparing that fingerprint to a library of known organisms to produce an identification. This method can identify bacteria or yeast growing in culture in a matter of minutes, and the consumable costs for these analyses are minimal.

**Blood Culture**

The performance of blood cultures is one of the most important functions of the clinical microbiology laboratory. Most blood cultures are performed by collecting blood into bottles of nutrient-rich broth to facilitate the growth of bacteria and yeast. Some blood culture media contain resins or other agents to help neutralize antibiotics that may be present in the patients’ blood. Blood culture bottles are then incubated on an automated blood culture incubator that will monitor the blood culture bottle at regular intervals for evidence of growth. Once the instrument detects evidence of microbial growth, it will alarm to...
alert the laboratory. Approximately 80% of blood cultures that will ultimately be positive are identified within the 1st 24 hr of incubation. A portion of broth from a blood culture bottle that has signaled positive is then Gram-stained and cultured onto appropriate growth media so that the organism can be isolated and identified. There are numerous pre-analytical variables that can influence the accuracy of blood culture results. In order to facilitate accurate interpretation of a positive blood culture, a minimum of 2 blood cultures drawn from different sites should be collected whenever possible. Growth of an organism that is part of the normal skin flora from a single blood culture raises concern that the isolate resulted from contamination of the culture. Proper skin antisepsis prior to collection of blood through an indwelling line, proper antisepsis prior to collection of blood cultures should be discouraged because it is difficult to determine the significance of coagulase-negative staphylococci and other skin flora or environmental organisms isolated from blood obtained from line cultures. Differential time to positivity of 2 hr or more between paired blood cultures drawn simultaneously from a catheter and peripheral vein is a useful indicator of catheter-related bloodstream infection. The volume of blood collected is also an important factor in the recovery of bloodstream pathogens, especially as the number of organisms per milliliter of blood in sepsis may be low. The optimal amount of blood to collect from a pediatric patient varies depending on the weight of the child. There are a number of rapid diagnostic assays that can be used directly on positive blood culture broth to identify pathogens commonly associated with bacteremia and some antimicrobial resistance determinants. Most of these rapid diagnostic assays are based on nucleic acid detection techniques. An example of this is the Verigene system, which can identify a number of streptococcal and enterococcal species, as well as mecA and vanA genes, in positive blood culture broth, in approximately 2 hr. MALDI-TOF MS can also be performed on blood culture broth that is positive for growth of microorganisms. These assays can help shorten the interval between a positive blood culture and definitive organism identification, with the goal of early optimization of antimicrobial therapy.

Detection of mycobacteria and some filamentous fungi (such as Histoplasma capsulatum and Fusarium) from the bloodstream is maximized using lysis-centrifugation techniques, such as the Isolator system (Wampole, Cranbury, NJ).

### Cerebrospinal Fluid Culture

Cerebrospinal fluid (CSF) should be transported quickly to the laboratory and then cytocentrifuged to concentrate organisms for microscopic examination. CSF is routinely cultured on blood agar and chocolate agar, which support the growth of common pathogens causing meningitis. If tuberculosis is suspected, cultures for mycobacteria should be specifically requested. Culture of larger volumes of CSF (>5 mL) significantly improves yield of mycobacteria.

Historically, rapid antigen detection tests for bacterial pathogens such as *Haemophilus influenzae* type b and *Streptococcus pneumoniae* were used to attempt to detect organisms in CSF without the need for culture. These techniques have now been proven to lack sensitivity and, in some cases, specificity. It has been demonstrated that a cyto-spin Gram stain is as sensitive as bacterial antigen tests for detection of microorganisms in CSF. In contrast, the cryptococcal antigen test can be useful when cryptococcal meningitis is suspected. Historically, India Ink preparations were used to detect *Cryptococcus* in CSF.
but this method is insensitive compared to the antigen detection assay.

In the postvaccine era, the epidemiology of infectious meningitis is rapidly changing, and acute bacterial meningitis is now a relatively infrequent event in North America. Many CSF infections are associated with shunts or other hardware, and Propionibacterium and coagulase-negative staphylococci are the organisms most frequently isolated from shunt infections. The laboratory should include media to facilitate the growth of Propionibacterium in CSF specimens received from neurosurgery patients.

Urine Culture
Urine for culture (including colony count) can be obtained by collecting clean-voided midstream specimens, by catheterization, or by suprapubic aspiration. Urine samples collected by placing bags on the perineum are unacceptable for culture because samples are often contaminated. Rapid transport of urine to the laboratory (<2 hr) is imperative, and delay in transport or plating of specimens renders colony counts unreliable. Refrigeration or urine transport devices with boric acid preservative may be used when delay is unavoidable.

The specific colony counts used to define growth in a urine culture as “significant” are somewhat controversial and vary somewhat by laboratory. Urine obtained by suprapubic aspirate is normally sterile, and thus any organism growth is typically considered significant. Urine collected by catheterization is likely to reflect infection if there are ≥10^4 to 10^5 organisms/mL. Clean-voided urine is considered abnormal if ≥10^3 to 10^4 organisms/mL are present.

Genital Culture
*N. gonorrhoeae* is a fragile organism, and collection and transport in special medium is essential for efficient recovery. Selective agar, such as modified Thayer-Martin medium, should be used to enhance recovery of *N. gonorrhoeae* in clinical specimens, such as genital, anorectal, and pharyngeal swabs. Antimicrobial resistance is increasing in *N. gonorrhoeae*, although few clinical laboratories have the ability to perform antimicrobial susceptibility testing for this organism. In pediatric patients, the identification of an organism as *N. gonorrhoeae* should be confirmed using 2 independent methods.

Specimens for *Chlamydia trachomatis* culture are obtained by cotton-tipped, aluminum-shafted urethral swabs. Endocervical specimens, using swabs with aluminum or plastic shafts, should be collected by rubbing the swab vigorously against the endocervical wall to obtain as much cellular material as possible. *C. trachomatis* is an obligate intracellular organism and is cultured by inoculation into cell culture systems, followed by immunofluorescent staining with monoclonal antibody against the organism. Nonculture methods such as enzyme immunoassay (EIA) tests, direct immunofluorescent staining by monoclonal antibodies, and DNA amplification methods are widely used and are more cost-effective than culture.

Although nucleic acid amplification assays (NAAT assays) for *N. gonorrhoeae* and *C. trachomatis* are not FDA cleared for use in children, these assays are frequently used in this population to detect these organisms in urine specimens, endocervical and vaginal swabs, and penile swabs. The NAAT assays exhibit superior sensitivity compared to culture-based techniques. Some laboratories take the approach of confirming all NAAT-positive specimens with an alternative NAAT test that detects an alternative genetic target.

Throat and Respiratory Culture
Streptococcal pharyngitis and tonsillitis is a common diagnosis in pediatric patients; vigorous swabbing of the tonsillar area and posterior oropharynx can be done to obtain a specimen for detection of group A streptococcus (**Streptococcus pyogenes**). Rapid antigen detection assays are frequently used when group A streptococcus pharyngitis is suspected. Negative rapid antigen assays should be confirmed using culture based techniques. NAAT assays for detection of group A streptococcus are also being used with increasing frequency. Most laboratories screen throat cultures exclusively for the presence of group A streptococci. However, large colony variants of group C and group G streptococci also cause pharyngitis, but are not associated with the same postinfectious sequelae attributed to group A streptococcus; laboratory practices for detecting and reporting group C and group G streptococci are variable and an area of controversy.

In addition to the detection of pathogenic streptococci, the clinical laboratory may query for diphtheria, gonococcal pharyngitis, or infection with *Arcanobacterium haemolyticum* in throat specimens. The laboratory should be notified if any of these pathogens are suspected to ensure that appropriate methods are used to recover these organisms if present.

Cultures for *Bordetella pertussis* can be obtained by aspiration or swabbing of the nasopharynx using a Dacron or calcium alginate swab. The aspirate or swab is inoculated onto special charcoal-blood (Regan-Lowe) or Bordet-Gengou media, although molecular assays are now frequently used for detection of *B. pertussis* in these specimens.

The cause of lower-respiratory-tract disease in children is frequently difficult to confirm microbiologically because of the challenge of obtaining adequate sputum specimens. Gram-stained smears of specimens should be performed to assess the adequacy of sputum samples; specimens with large numbers of epithelial cells (>10 per high-powered field) or with few neutrophils are unsuitable for culture, as there is a lack of correlation between upper respiratory tract flora and organisms causing lower-respiratory tract disease. For patients with cystic fibrosis, special media should be used to detect pathogens important in cystic fibrosis, such as *Burkholderia cepacia*.

Endotracheal aspirates from intubated patients may be useful if the Gram stain shows abundant neutrophils and bacteria, although pathogens recovered from such specimens might still reflect only contamination from the endotracheal tube or upper airway. Quantitative cultures of bronchoalveolar lavage fluid may be valuable for distinguishing upper respiratory tract contamination from lower tract disease.

If infection with *Legionella* is suspected, the laboratory should be alerted so that the specimen can be inoculated to special media (such as buffered charcoal yeast extract agar) to facilitate the recovery of this pathogen. The *Legionella* urinary antigen test is a sensitive and specific, noninvasive method for rapid detection of *Legionella pneumophila* serogroup 1.

The diagnosis of pulmonary tuberculosis in young children is best made by culture of early-morning gastric aspirates, obtained on 3 consecutive days. Sputum induction for obtaining specimens for mycobacterial culture has also proved useful in young children but requires skilled personnel and containment facilities to prevent exposure of healthcare workers. Cultures for *Mycobacterium tuberculosis* should be processed only in laboratories equipped with appropriate biologic safety cabinets and containment facilities. NAAT tests for detection of *M. tuberculosis* in smear-positive respiratory specimens are becoming more widely available.

Detection of Enteric Pathogens
In pediatric patients with diarrheal illnesses, culture of stool for enteric pathogens may be requested. A fresh stool specimen is preferred, but is not always possible to obtain. If there is an unavoidable delay in specimen transport, the specimens should be placed into an appropriate transport medium, such as Cary-Blair. Rectal swabs for enteric culture are also acceptable specimens if the swab is visibly soiled. In general, enteric cultures should be performed on specimens from outpatient or patients who have been hospitalized for fewer than 3 days, as nosocomial acquisition of an enteric pathogen is very unusual.

Stool specimens are typically plated on a series of selective and differential media to decrease the overgrowth of normal flora and recover pathogenic organisms if present. The specific pathogens queried vary by laboratory. Most laboratories in North America will routinely culture for *Salmonella*, *Shigella*, *Campylobacter*, and Shiga toxin-producing strains of *Escherichia coli*. The CDC recommends that all laboratories use an agar-based medium for recovery of *E. coli* O157 in addition to an assay for detection of Shiga toxin production for all specimens submitted for enteric culture. Practices surrounding the routine culture for *Yersinia enterocolitica*, *Vibrio cholerae*, *Edwardsiella*, *Aeromonas*, and *Plesiomonas* will vary with local epidemiology, and the
laboratory should always be notified if one of these pathogens is specifically suspected.

Clostridium difficile is an important cause of antibiotic-associated diarrhea. C. difficile was long characterized as a nosocomial pathogen of older adults, but community-associated disease is emerging and the incidence and severity of C. difficile infection in children is increasing. Although for many years laboratories relied on EIAs for detection of C. difficile toxins, these assays lack adequate sensitivity. Laboratories use nucleic acid detection methods to aid in the diagnosis of C. difficile. Testing for C. difficile in children <1 yr of age should be discouraged as a result of the high incidence of colonization in this patient population.

Viruses are an important cause of gastroenteritis in pediatric patients. Methods for viral detection will vary but may include antigen detection (e.g., for rotavirus or adenovirus 40/41) or nucleic acid detection methods (such as for norovirus).

In North America, the burden of parasitic gastroenteritis is low. Complete microscopic exams for ova and parasite detection in stool samples is usually of low yield, and antigen detection assays for Cryptosporidium and Giardia, the most commonly encountered agents, are a sensitive and cost-effective method for detection of these pathogens.

Multiplex nucleic acid detection tests for simultaneous detection of a dozen or more enteric pathogens, including bacteria, viruses, and parasites are emerging. It is not completely clear how these assays will be deployed by clinical laboratories.

Culture of Other Fluids and Tissues

Abscesses, wounds, pleural fluid, peritoneal fluid, joint fluid, and other purulent fluids are cultured onto solid agar and, in some cases, broth media. Whenever possible, fluid rather than swabs from infected sites should be sent to the laboratory, because culture of a larger volume of fluid can detect organisms present in low concentration. Anaerobic organisms are involved in many abdominal and wound abscesses. These specimens should be collected and transported to the laboratory rapidly in anaerobic transport tubes.

Although Staphylococcus aureus is the most common cause of bone and joint infections, Kingella kingae is an important cause of septic arthritis in children, especially in children <4 yr of age. The detection of K. kingae is maximized by inoculation of synovial fluid into blood culture broth in addition to plating on solid medium, as well as by molecular detection of K. kingae in specimens from young patients with suspected septic arthritis.

Screening Cultures

Clinical laboratories may perform surveillance cultures for specific pathogens either to assist infection control in identifying patients requiring contact isolation or for outbreak investigation. Screening cultures for detection of methicillin-resistant S. aureus or vancomycin-resistant enterococci may be routinely performed in certain patient populations. In addition, hospitals with carbapenem-resistant Enterobacteriaceae may screen patients for rectal carriage of these organisms. Chromogenic media are frequently used for this purpose. These media contain proprietary compounds to select for the agent of interest and result in growth of colored colonies to identify pathogens of interest.

**ANTIMICROBIAL SUSCEPTIBILITY TESTING**

Antimicrobial susceptibility testing is generally performed on organisms of clinical significance for which standards and interpretive criteria for susceptibility testing exist. In North America, most laboratories use commercial, automated systems for susceptibility testing. The output from these systems is a minimum inhibitory concentration (MIC) value and interpretation of that value as susceptible, intermediate, or resistant. The next most common technique is Kirby-Bauer disk diffusion, in which a standardized inoculum of the organism is seeded onto an agar plate. Antibiotic-impregnated filter paper disks are then placed on the agar surface. After overnight incubation, the zone of inhibition of bacterial growth around each disk is measured and compared with nationally determined standards for susceptibility or resistance.

A less-commonly used technique is broth or microbroth dilution testing. A standard concentration of a microorganism is inoculated into serially diluted concentrations of antibiotic, and the MIC in µg/mL, the lowest concentration of antibiotic required to inhibit growth of the microorganism, is determined. The E-test is a hybrid of disk diffusion and broth dilution and can be used to determine the MIC of individual antibiotics on an agar plate. It uses a paper strip impregnated with a known continuous concentration gradient of antibiotic that diffuses across the agar surface, inhibiting microbial growth in an elliptic zone. The MIC is read off the printed strip at the point at which the zone intersects the strip. Major advantages of the E-test are reliable interpretation, reproducibility, and applicability to organisms that require special media or growth conditions.

In addition to providing data to guide the treatment of individual patients, laboratories use aggregate susceptibility testing data to generate institution specific antibiogram reports. These reports summarize susceptibility trends for common organisms and can be used to guide empirical therapy prior to the availability of specific susceptibility testing results.

Antimicrobial susceptibility patterns are rapidly changing as microbes evolve new resistance mechanisms. Recommendations for performance standards for antimicrobial susceptibility tests and their interpretation are regularly updated by the Clinical and Laboratory Standards Institute.

**Fungal Cultures**

Special growth media is used to recover fungi, both yeasts and molds, in clinical specimens. As most fungi prefer reduced growth temperatures, and some species grow slowly, fungal cultures are incubated at 30°C (86°F) for 4 wk.

Most yeasts are identified using methods similar to those used for bacteria. In contrast, the identification of filamentous fungi has not changed in nearly a century. The laboratory takes into consideration the growth rate, color, and colony characteristics of an isolate and then prepares the specimen in lactophenol alanine blue for microscopic evaluation. These features in aggregate are used to identify the isolate. In some cases, DNA sequencing is used for fungal identification and MALDI-TOF MS is also emerging for identification of filamentous fungi. All manipulations of filamentous fungi should take place in the biologic safety cabinet to avoid infecting laboratory personnel and prevent laboratory contamination.

Antigen detection assays are also available for some fungal pathogens such as Cryptococcus neoformans and H. capsulatum. Assays to detect galactomannan, a molecule found in the cell wall of Aspergillus, are commercially available and increasingly used to assist in making the diagnosis of invasive aspergillosis in immunocompromised populations.

**POINT-OF-CARE DIAGNOSTICS**

Some assays to detect infections may be performed in the office setting, provided the site is certified as meeting appropriate quality-assurance standards specified by the Clinical Laboratory Improvement Amendments (CLIA) of 1988. These include procedures listed under the category of “provider-performed microscopy” such as wet mounts, potassium hydroxide preparations, pinworm examinations, and urinalysis.

Many pediatric offices perform rapid antigen testing for detection of group A streptococcal pharyngitis. The sensitivity of point of care testing is dependent upon specimen collection technique, the type of kit used and on the concentration of streptococci present in the sample. However, in light of the fact that up to 30% of group A streptococcal rapid antigen tests are falsely negative, it is recommended that all negative results should be confirmed by culture.

Office laboratories licensed to perform waived tests are limited to performing these tests and avoid having to undergo inspections and proficiency testing, although they are still subject to CLIA certification requirements specific to these tests. Gram staining, culture inoculation, and isolation of bacteria are considered moderately to highly complex under CLIA specifications. Any office laboratory performing
Gram stains or cultures must comply with the same requirements and inspections for quality assurance, proficiency testing, and personnel requirements as fully licensed microbiology laboratories.

**LABORATORY DETECTION OF PARASITIC INFECTIONS**

Most parasites are detected by microscopic examination of clinical specimens. *Plasmodium* and *Babesia* can be detected in stained blood smears, *Leishmania* can be detected in stained bone marrow smears, and helminth eggs, *Entamoeba histolytica*, and *Giardia lamblia* can be detected in stained fecal smears (see Table 170-1). Serologic tests are important in documenting exposure to certain parasites that are not typically found in stool or blood, and thus are difficult to demonstrate in clinical specimens, such as *Trichinella*.

Pinworm is a relatively common parasitic infection in pediatric patients. A diagnosis of pinworm can be made by evaluating a “pinworm prep.” The best time to obtain this specimen is first thing in the morning, before the patient has bathed or had a bowel movement. A piece of clear scotch tape is pressed onto the perianal region of the patient and then the tape is applied to a clear microscope slide. The slide is then examined for recovery of pinworm eggs or worms.

Fecal specimens should not be contaminated with water or urine, because water can contain free-living organisms that can be confused with human parasites, and urine can destroy motile organisms. Mineral oil, barium, and bismuth interfere with the detection of parasites, and specimen collection should be delayed for 7-10 days after ingestion of these substances. Because *Giardia* and many worm eggs are shed intermittently into feces, a minimum of 3 specimens on nonconsecutive days are required to adequately exclude the diagnosis of an enteric parasite. Because many protozoan parasites are easily destroyed, collection kits with appropriate stool preservatives (commonly a 2-rial system with formalin and polyvinyl alcohol fixatives) should be used if delay between time of specimen collection and transport to the laboratory is anticipated.

Ova and parasite examination of fecal specimens includes a wet mount (to detect motile organisms if fresh stool is received), concentration (to improve yield), and permanent staining, such as trichrome, for microscopic examination. *Cryptosporidium*, *Cyclospora*, and *Isospora* are detected by modified acid-fast stain, and microsporidia by a modification of the trichrome stain. In addition, *Cyclospora* and *Isospora* autofluoresce under UV microscopy. The laboratory should be alerted if these parasites are suspected. Detection of certain intestinal parasites, especially *Giardia* and *Cryptosporidium*, can be simplified by using antigen detection tests.

Amebic encephalitis, caused by *Acanthamoeba*, *Balamuthia*, or *Naegleria*, is a rare but devastating and rapidly progressive disease. Special laboratory stains and procedures are required to detect these organisms. The laboratory should be notified if this infection is suspected. Rapid antigen detection tests for *Plasmodium* species are available. The sensitivity and specificity of these tests vary depending on the burden of parasite in the sample, and the specific *Plasmodium* species. In general, these tests are most sensitive for detecting *Plasmodium falciparum* and least sensitive for detecting *Plasmodium malariae*. These tests are particularly useful for laboratories lacking personnel trained in evaluation of thick and thin smears for malaria, or to provide a rapid preliminary result while awaiting microscopy. All positive and negative rapid malaria assays should be confirmed with blood smear analysis.

*Trichomonas vaginalis* is a sexually transmitted protozoan parasite that can also be transmitted on household fomites. Infected individuals may be asymptomatic or may have mild inflammation or severe inflammation and discomfort. *Trichomonas* may be detected using a wet mount, but this method is insensitive. Rapid antigen assays are available. Culture-based detection or nucleic acid amplification techniques are the most sensitive way to make the diagnosis.

**SEROLOGIC DIAGNOSIS**

Serologic tests are primarily used in the diagnosis of infectious agents that are difficult to culture in vitro or detect by direct examination, such as *Bartonella*, *Francisella*, *Legionella*, *Borreilia* (Lyme disease), *Treponema pallidum*, *Mycoplasma*, *Rickettsia*, some viruses (HIV, Epstein-Barr virus [EBV], hepatitis A virus), and parasites (*Toxoplasma*, *Trichinella*).

Antibody tests may be specific for immunoglobulin (Ig) G or IgM or can measure antibody response regardless of immunoglobulin class. In very general terms, the IgM response occurs earlier in the illness, generally peaking at 7-10 days after infection, and usually disappears within a few weeks, but for some infections (e.g., hepatitis A, West Nile Virus) it can persist for months. The IgG response peaks at 4-6 wk and often persists for life. Because the IgM response is transient, the presence of IgM antibody in most cases correlates with recent infection. Methods for IgM antibody detection are difficult to standardize, however, and false-positive results commonly occur with some tests. The presence of IgG antibody can indicate new seroconversion or past exposure to the pathogen. To confirm a new infection using IgG testing, it is essential to demonstrate either seroconversion or a rising IgG titer. A 4-fold increase in a convalescent titer obtained 3-4 wk following the acute titer is considered diagnostic in most situations. In neonates, interpretation of serologic tests is very difficult because of passive transfer of maternal IgG that can persist for 6-18 mo after birth.

Context is extremely important in the interpretation of serologic findings. Important considerations are the ability of the host to mount an immune response, the background rate of seropositivity (especially for IgG detection assays), and, for some diseases, the antibody titer. In addition, interpretation of some serologic assays, such as those used to diagnose Lyme disease, are problematic because of lack of specificity of the immunoassays. A confirmatory immunoblot (Western blot) is required for all positive and equivocal EIA results for Lyme disease.

**LABORATORY DIAGNOSIS OF VIRAL INFECTIONS**

Viral diseases are extremely important in pediatrics, and diagnostic virology has long been important to pediatric practice, especially in the inpatient setting.

**Specimens**

Specimens for viral diagnosis are selected on the basis of knowledge of the site that is most likely to yield the suspected pathogen. When evaluating patients with acute viral infections, specimens should be collected early in the course of infection when viral shedding tends to be maximal. Swabs should be rubbed vigorously against mucosal or skin surfaces to obtain as much cellular material as possible and sent in viral transport media that contain antibiotics to inhibit bacterial growth. Rectal swabs should contain visible fecal material. “Flocked” swabs have been shown to provide more material for the laboratory with consequent improvement in the performance of diagnostic tests. Fluids and respiratory secretions should be collected in sterile containers and promptly delivered to the laboratory. All specimens should be transported on ice if delay is anticipated. Freezing specimens, especially at −20°C (−4°F), can result in a significant decrease in culture sensitivity. Consultation with the laboratory is recommended, because some commercial diagnostic test kits used by laboratories may require specific collection devices.

Laboratory diagnosis of viral infections may be by electron microscopy, antigen detection, virus isolation in culture, serologic testing, or molecular techniques to detect viral nucleic acids. In the past few years, molecular tests have emerged as the primary means for detecting viral infections, with some virology laboratories abandoning the use of viral culture altogether. Serologic testing still has a role, especially for arboviral infections such as West Nile, acute EBV infections, HIV, hepatitides A to C, and diseases of childhood such as measles, rubella, and mumps. Serology is also uniquely useful for defining immunity to specific viral infections.

**Antigen Detection Tests**

Immunofluorescent-antibody (IFA) techniques or other methods, such as EIA, that use antibodies to detect viral antigens directly in clinical specimens to permit rapid identification of viruses, were the mainstay...
of the diagnosis of respiratory viral infections but are now being replaced by molecular tests. Smears of cellular material from respiratory secretions stained by immunologic reagents can identify the antigens of respiratory syncytial virus (RSV), adenovirus, influenza A and B viruses, parainfluenza virus types 1-3, and human metapneumovirus within 2-3 hr after the specimen is received. The sensitivity of IFA staining for RSV exceeds that of culture in many laboratories. For influenza A and B, IFA sensitivity approaches that of culture, whereas for parainfluenza viruses and adenoviruses, sensitivity of IFA is lower. Novel influenza strains, such as the one responsible for H1N1 pandemic influenza, may be poorly detected by IFA and other antigen detection techniques and require molecular tests for optimal sensitivity.

Sensitive IFA staining techniques are also commercially available for identifying varicella-zoster virus and herpes simplex virus (HSV). These specific methods have supplanted the Tzanck smear for multi-nucleated giant cells characteristic of varicella-zoster virus or HSV infections. A method for detecting cytomegalovirus (CMV) pp65 antigen in blood of immunocompromised patients is also available but is being replaced by molecular testing. IFA is not useful for detecting viruses in specimens that do not contain an adequate number of infected cells.

Rapid antigen tests usually based on lateral flow immunochromatography have been approved by the FDA for detection of influenza A and B and RSV. Some of these tests have "waived" status under CLIA, meaning that they can be performed by personnel who are not trained laboratory technologists, with relatively little formal quality control other than controls that are incorporated into the test devices. Some require as little as 10 min to perform. Consequently, these tests can be performed in a doctor’s office or an emergency unit. Sensitivity in children is higher than in adults and is in the range of 50-80%. Rapid antigen tests can be useful in managing patients with acute respiratory infections, provided the caregiver keeps in mind that a negative test does not rule out the presence of a concomitant bacterial infection.

In addition to their role in respiratory virus infections, antigen-detection EIA tests are commonly used for the diagnosis of viruses that are difficult to culture, such as rotavirus, enteric adenovirus, and hepatitis B virus. The detection of the p24 antigen of HIV along with HIV antibodies is included in "fourth-generation" EIA tests used for the diagnosis of HIV.

Viral Culture
Viruses require living cells for propagation; the cells used most often are human- or animal-derived tissue culture monolayers, such as human embryonic lung fibroblasts or monkey kidney cells. Historically, in vivo methods such as inoculation of suckling mice were also used, but are rarely used today. Viral growth in susceptible cell culture is usually accomplished by detecting characteristic cytopathic effect that is visible by light microscopy under low magnification in the cultured cells. For some viruses (e.g., influenza, parainfluenza, and mumps viruses), this method is supplemented by hemadsorption, based on the production of virally encoded hemagglutinins on infected cell membranes that cause adherence of erythrocytes to infected cells. The most reliable confirmatory method for viral detection in cell culture involves fluorescein- or enzyme-labeled monoclonal antibody staining of infected cell monolayers. An important technical improvement in respiratory viral cultures is the development of cell culture systems that include more than 1 type of cell (R-Mix, Diagnostic Hybrids/Quidel, San Diego, CA) and employ IFA staining for virus detection. This system provides results in 16-48 hr from the time the specimen is received in the laboratory, compared to 2-10 days for conventional cultures. Cell culture methods are now being steadily replaced by molecular tests, which are faster, may be more sensitive, and have the potential to detect viruses that do not grow readily in cell cultures.

Molecular Diagnostics
Most molecular tests to detect viruses use the polymerase chain reaction (PCR) and other nucleic acid amplification tests. The first application of PCR to become widely accepted was a test to detect HSV DNA in CSF in patients with possible HSV encephalitis. The first FDA-cleared test for this purpose was approved in 2014. Many laboratories still use laboratory-developed tests, whose performance characteristics must be validated as specified by CLIA. The consequence of this situation is that testing is not standardized and the performance characteristics of this testing (sensitivity and specificity) may vary from laboratory to laboratory. At its best, PCR has sensitivity and specificity greater than 95% for HSV encephalitis. PCR is also increasingly used to diagnose mucocutaneous HSV and varicella-zoster virus infections. This testing is more sensitive than virus culture and provides a more rapid turnaround time.

An FDA-cleared test for enterovirus in CSF (GeneXpert, Cepheid, Sunnyvale, CA) provides sensitive detection of enteroviruses with a performance time of approximately 3 hr. Because this testing is simple to perform, some hospital laboratories are able to offer testing at all times, thus maximizing the clinical utility of the test. The parechoviruses, which may cause illnesses similar to those caused by enteroviruses, especially in infants <6 mo of age, must be detected by separate molecular assays. No parechovirus assays are currently approved by the FDA.

FDA-cleared molecular tests for respiratory viruses are increasingly replacing antigen detection and culture. Several FDA-cleared multiplex molecular tests are available for detection of influenza A and B and RSV. As of 2014, 4 multiplex tests that detect larger numbers of respiratory viruses are also available (Table 170-2). Viruses detected by these tests include influenza A and B, RSV, parainfluenza 1-4, human metapneumovirus, adenovirus, rhinovirus/enterovirus, and coronaviruses OC43, 229E, NL63, and OC43. The performance of each test for each of the viral targets must be approved or cleared by the FDA, so the tests vary among one another in the specific virus targets for which they have achieved FDA approval/clearance (Table 170-2). In addition, 1 of the tests (FilmArray) is also cleared for the detection of the bacterial agents B. pertussis, Mycoplasma pneumoniae, and Chlamydia pneumoniae. This test is also notable because the performance time is only

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<th>Table 170-2</th>
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<td>eSensor</td>
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* Cleared by the FDA as of July, 2013. Other versions that detect additional viruses are available outside of the United States.

¹Detects rhinoviruses and enteroviruses but does not distinguish between them.

Ad, adenovirus; AH1, influenza A, hemagglutinin type 1; AH3, influenza A, hemagglutinin type 3; Flu a, influenza A; Flu b, influenza B; HMPV, human metapneumovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.
approximately 1 hr, permitting very rapid turnaround time. A multiplex assay for the detection of viruses (norovirus and rotavirus), as well as important bacterial and parasitic pathogens (xTAG Gastrointestinal Panel, Luminex, Austin, TX), has been cleared by the FDA and similar tests are being developed by numerous other companies.

Another important area of application of molecular testing is the detection of viruses in the blood. FDA-approved assays to detect HIV and hepatitis C RNAs are essential for the management of these infections, including the prevention of transmission from mother to infant. Hepatitis B molecular testing is also increasingly used. In addition, molecular testing is now widely used for viruses that cause systemic disease in immunocompromised patients, especially CMV, EBV, HSV, the BK polyomavirus, and adenosivirus. For these viruses, as well as for HIV and the hepativiruses, quantitative testing is required. An FDA-approved PCR assay for the quantitative measurement of CMV DNA in plasma is now available. In addition, international standards for CMV have been developed. This is important because it makes possible better comparability among different quantitative CMV assays if they are each referenced to the international standard. Testing for the other viruses must be carried out using laboratory-developed tests, sometimes with the use of analyte-specific reagents, a class of reagents that are regulated by the FDA although not incorporated into complete diagnostic test kits.

Laboratory-developed PCR and other molecular assays are used by some laboratories for numerous other viruses, including parvovirus B19; human herpesvirus 6; mumps, measles, and rubella viruses; and the JC polyomavirus.

Host gene expression patterns in whole blood have been used to differentiate viral from bacterial infections. This microarray-based assay may rapidly identify a viral or bacterial profile of host gene expression reprise, thus greatly shortening the time to diagnosis and potentially avoiding inappropriate treatment while suggesting indicated therapies.

Bibliography is available at Expert Consult.

Figure 171-1 Common molecular methodologies for identifying the components and the functions of complex microbial communities.
Bibliography


