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features and confirmed with serologic tests.[110] The most commonly used tests are the indirect fluorescent assay and the microimmunofluorescent test. Additional assays have also been developed. A latex agglutination test can detect IgM and IgG antibody against specific rickettsial antigens. Cross-reactions occur among rickettsial species. False-positive results with indirect fluorescent assays have been reported in users of illicit parenteral drugs.

The Weil-Felix assay is rarely used because of its lack of sensitivity and specificity.

IMMUNOSEROLOGY

Documentation of a specific immune response to a pathogenic microorganism provides proof of infection. Nonspecific indicators of infection, such as elevated C-reactive protein, Venereal Disease Research Laboratories (VDRL) antigen, and cytokine levels also assist in the diagnosis and management of serious infections. Methods used for the measurement of antibodies include hemagglutination, EIA, latex agglutination, complement fixation, immunofluorescence, and neutralization assays. Certain methods may be better for certain infections. Detailed assessment of assays is provided in a review by James.[111]

The VDRL and rapid plasma reagin continue to be the best screening tests for syphilis, as well as measures of the activity of infection. Observation of spirochetes in body fluids by dark-field examination (or by the direct fluorescent antibody test) or positive specific treponemal antibody tests such as the fluorescent treponemal antibody absorption test confirm infection.[112]

The immune response to specific pathogenic fungi can assist in diagnosis, as determined by immunodiffusion or completion fixation assays for *Aspergillus*, *Blastomyces*, *Histoplasma*, and *Coccidioides*. However, false-negative test results occur, especially early in the disease or in immunosuppressed hosts.

Febrile agglutinin tests have limited clinical utility in developed countries and should not be used.[113]

NEWER TECHNOLOGIES

Molecular biology has influenced the way in which infectious diseases are diagnosed.[25] DNA and RNA amplification by PCR has become an important resource in the diagnosis of infections. PCR has been shown to be more sensitive than in situ hybridization. It can detect as few as 10 to 100 copies of nucleic acid, whereas hybridization detects only 10 000 copies. PCR has been useful for identifying slowly growing organisms such as *Mycobacterium tuberculosis* and *Borrelia burgdorferi*; *Rickettsia*, viruses, potential agents of bioterrorism; viruses such as Epstein–Barr virus, polyomaviruses, human metapneumovirus, and multiple parasites. Quantitative PCRs (viral loads) have revolutionized the way we determine disease activity and response to therapy. It is becoming apparent that we can screen for multiple organisms from a single specimen, such as for respiratory pathogens in respiratory secretions.[114]

Molecular fingerprinting has become a useful resource in the investigation of nosocomial infections and has fostered the field of molecular epidemiology.[115]

CHAPTER 287

Laboratory Diagnosis of Infection Due to Viruses, *Chlamydia*, *Chlamydophila*, and *Mycoplasma*

Tony Mazzulli

VIRUSES

With the development of rapid viral diagnostic techniques and successful antiviral therapy, diagnostic virology has become as clinically important as diagnostic bacteriology. The availability of rapid and reliable viral diagnostic tests, particularly viral nucleic acid amplification tests (NATs), facilitates rational decision-making in the prevention and treatment of viral infections and the practice of effective infection control measures. Specific antiviral therapy is now available for a number of clinically relevant viruses, and thus a correct viral diagnosis is important for selecting proper therapy and limiting further diagnostic testing and unnecessary antibiotic therapy.[1,2]

Two major approaches can be used for the diagnosis of viral infection: virologic (detection of virus) and serologic (detection of antibody, antigen, or both). The virologic approach includes: (1) isolation of infectious virus in cell culture; (2) detection of viral antigen by immunologic methods such as fluorescent antibody (FA) testing or enzyme immunoassay (EIA); (3) identification of viral particles by electron microscopy (EM); and (4) detection of viral nucleic acid by direct hybridization or following an amplification step such as polymerase chain reaction (PCR). Cytologic examination of tissues and cells may identify viral effects prompting a need for further investigation. Occasionally, the cytologic changes can be sufficiently specific to suggest a particular viral agent (e.g., cytomegalovirus (CMV)).[3] The serologic approach to the diagnosis of viral infections includes a demonstration of: (1) immunoglobulin (Ig) G antibodies indicating recent, current (e.g., human immunodeficiency virus (HIV)), or past infection as well as immunity following recovery or vaccination; (2) a significant rise in virus-specific IgG antibody suggestive of acute or recent infection; (3) virus-specific antigens (e.g., hepatitis B surface antigen (HBsAg)); or (4) virus-specific IgM antibody in late acute- or early recovery-phase sera. EIAs capable of measuring the avidity of IgG antibodies to specific viruses have also been developed. Following a viral infection, as the immune response matures, low-avidity antibodies are replaced with high-avidity antibodies. These assays have been used to distinguish primary from secondary antibody responses to vaccination and to natural infection.[4,5]

Specimen Collection and Transport

The timing of specimen collection for the detection of viruses is crucial. For the detection of most viruses, it is important to obtain specimens soon after the onset of clinical symptoms (preferably within the first 3 to 4 days) when viral shedding is at its maximum. Optimal specimens for the diagnosis of viral infection vary depending on the site or sites of disease. In general, tissues, aspirates, and body fluids are superior to swabs for the detection of viruses. However, in many circumstances, swabs may be the only specimen available. Body sites or lesions that can easily be sampled with a swab include the pharynx or nasopharynx, conjunctiva, urethra, cervix, vagina, and vesicles or ulcers on the skin or mucous membranes. Many swab types are available for specimen collection,

including those made with a plastic, wooden, or flexible wire shaft and a tip made of cotton, Dacron, calcium alginate, or polyurethane.[6] However, different swab types may not be suitable for detection of some viruses. Swabs with a wooden shaft may contain toxic products that inactivate herpes simplex virus. Cotton-tipped swabs may contain fatty acids that can interfere with the survival of *Chlamydia* species, but are suitable for the collection of specimens from the vagina, cervix, or urethra for the detection of *Mycoplasma*. Calcium alginate-tipped swabs may be toxic for lipid-enveloped viruses such as herpesviridae and some cell cultures, but are useful for the collection of specimens for *Chlamydia*. Although swabs placed in viral transport media (VTM) can be used for molecular-based tests such as PCR, many commercial assays for detection of viruses by antigen detection or nucleic acid amplification techniques provide their own swab and transport media and these should be used for these tests.

Once collected, a swab for detection of viruses, *Mycoplasma,* and *Chlamydia* should be placed into VTM. A number of commercially prepared VTMs are available.[7] Tissues for virus detection may also be placed in VTM. VTM prevents drying, maintains viral viability during transport, and prevents the overgrowth of contaminating organisms.[6] Swabs collected for bacterial isolation that are placed in Amies or other bacterial transport medium are unacceptable for detection of virus.[6] The converse is also true; VTM contain antimicrobial agents that inhibit most bacteria and fungi. Specimens such as blood, bone marrow, cerebrospinal fluid (CSF), urine, and other body fluids should be placed in clean sterile containers without VTM.

Most respiratory viruses replicate preferentially in columnar epithelial cells located primarily in the posterior of the nasopharynx and the lower respiratory tract. For detection of most respiratory viruses, nasopharyngeal (NP) aspirates or washes, sputa, and bronchoalveolar lavage (BAL) specimens provide a better yield for detection of viruses than NP, nasal, or throat swabs.[7] Oropharyngeal and lower respiratory tract specimens may be superior to NP specimens for the detection of avian influenza A/H5N1 infections in humans. Multiple samples may need to be collected to maximize yield.

For detection of viruses in the gastrointestinal tract, freshly passed stool is superior to a rectal swab.[6]

Blood is an important specimen for isolation of certain viruses because viremia is a useful indicator of disease. Within blood, different viruses may be found in the cellular components, the plasma/serum, or both. For example, HIV is found in lymphocytes and macrophages, whereas CMV is associated with neutrophils and, to a lesser extent, mononuclear cells.[8] Enteroviruses can be isolated from plasma as well as from white blood cells (WBCs).[9] For the detection of viruses, blood should be collected into Vacutainer tubes containing an anticoagulant. Although the optimal anticoagulant is not known, ethylenediaminetetraacetic acid (EDTA) is recommended because recovery rates of HIV-1 from blood are higher with EDTA than with heparin[10] and heparin can inactivate herpesviruses in vitro.[11] In addition, heparin can inhibit some nucleic acid amplification assays, such as PCR.[12]

For tissue specimens or when the lability of particular viruses (e.g., respiratory syncytial virus (RSV) or varicella-zoster virus (VZV)) is a concern, commercially available vials with transport media containing albumin or serum as a stabilizer should be used.

The optimal temperature for transport and storage of specimens for viral culture is 4°C (refrigerator or wet ice temperature). Most viruses are stable for 2 to 3 days at this temperature.[6] Freezing at –20°C (ordinary freezer temperature) destroys or reduces the infectivity of most viruses. Freezing may also alter the ability to detect viral antigen with some commercially available kits. If specimens must be kept for longer than 2 to 3 days, they should be stored in an ultralow-temperature freezer (–70°C) and transported between laboratories on dry ice. For some molecular tests (e.g., detection of hepatitis C virus (HCV) RNA) in serum (or plasma), it is recommended that the serum/plasma be separated within 4 to 6 hours of collection and processed within 72 hours (if kept at 2°C to 8°C) or frozen at –70ºC until tested.[7]

For serologic detection of viral antibodies or antigen, blood can be transported to the laboratory at room temperature, but if delay is anticipated, the specimen should be kept refrigerated at 2°C to 8°C. Serum or plasma should be separated as soon as possible after the specimen is obtained. If an extended period will elapse before testing, the serum/plasma sample should be frozen at –20°C or lower. Repeated freeze/thaw cycles should be avoided. For viruses where a specific IgM assay is available (e.g., hepatitis A virus (HAV)), an acute-phase specimen may be sufficient for diagnosis. Otherwise, an acute-phase specimen collected within a few days of illness onset followed by a convalescent-phase specimen collected 2 to 4 weeks later should be obtained.

Virus Detection Methods

Virus Isolation

Parvovirus, human papillomavirus (HPV), hepatitis viruses, Epstein–Barr virus (EBV), rotaviruses, noroviruses, among others, are not cultivatable. Although it is possible to culture HIV, special containment facilities are required and other methods, including serologic tests, are recommended for routine diagnosis. The major viruses detected by isolation in cell culture include herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), CMV, VZV, RSV, influenza A and B viruses, parainfluenza viruses, respiratory adenoviruses, a number of enteroviruses (coxsackievirus, echovirus, poliovirus), and measles virus. Because not all cultivatable viruses replicate in a single cell line, several different cell lines are used for primary isolation from clinical specimens. Isolation of herpes group viruses such as HSV-1 and HSV-2, CMV, and VZV is usually performed with human fibroblast cell lines (e.g., human foreskin or lung fibroblasts). Respiratory viruses and enteroviruses grow best on primary rhesus monkey kidney cells. RSV grows on a continuous human epithelial cell line such as Hep-2 cells. The types of cell lines used in the diagnostic virology laboratory are determined by the specimen type, season, epidemiologic data, and clinical information provided. Many viruses cause morphologic changes, known as the cytopathic effect (CPE), when growing in cell culture. Some viruses cause CPE within 2 days (e.g., HSV) and others within a week (e.g., enteroviruses), whereas others do not cause CPE for several weeks (e.g., CMV). For viruses that do not cause typical CPE, detection is based on the adsorption of red cells to the surface of virus-infected cells in culture (e.g., influenza and parainfluenza viruses). Presumptive identification of a particular virus or virus group (e.g., HSV, RSV, or an enterovirus) in cell culture can be based on the cell type, the characteristic time of onset, and the appearance of CPE. Presumptive identification is facilitated if the laboratory personnel are informed of the source of the specimen and the suspected clinical diagnosis.

Confirmation of isolation of a particular virus requires immunologic methods using specific monoclonal or polyclonal antibodies. Fluorescein- or peroxidase-conjugated monoclonal antibodies are available commercially to enhance the speed and sensitivity of detection of viruses in cell culture. Antibodies to HSV, CMV, VZV, RSV, influenza A and B virus, parainfluenza virus, adenovirus, measles virus, and enterovirus antigens are available. To identify the specific serotype of influenza A or B virus, inhibition of hemagglutination by serotype-specific antiserum is used.

Centrifugation of specimens (also referred to as shell vial culture or spin-amplified culture) on to cell monolayers on coverslips placed in the bottom of small vials or in wells of flat-bottomed plates, followed by staining for viral antigen with monoclonal antibody after 1 to 3 days of incubation, has substantially reduced the time required to detect and confirm the presence of a number of viruses. The centrifugation step shortens the time needed for viral replication and production of viral antigen. For slowly growing viruses such as CMV, the use of monoclonal antibody against nonstructural proteins produced early in the replication cycle (i.e., immediate early antigen

or early antigen) allows detection of virus days to weeks before CPE can be observed by traditional cell culture techniques. The shell vial method is faster than conventional CPE detection for most viruses (Table 287-1) and has replaced conventional cultures in many laboratories. It is now routinely used in many laboratories for the detection of CMV, HSV, VZV, respiratory viruses, and the enteroviruses. Recently, the use of genetically altered cell lines such as the ELVIS (enzyme-linked virus-inducible system) test or mixtures of cells in a single culture such as R-Mix cells have been developed and have shown comparable sensitivity with standard culture and shell vial methods for some viruses.[13–15]

Antigen Detection

Antigen detection tests are performed directly on specimens from patients: nasal or NP secretions, BAL specimens, scrapings of vesicles or conjunctivae, swabs of the cervix or urethra, stool samples, or tissue biopsy samples. Because viral antigen is associated with cells, collection of an adequate number of infected cells is important (e.g., mucosal or skin epithelial cells are better specimens than purulent material).

Kits to perform EIA or the FA test are available commercially for the detection of: (1) rotavirus and enteric adenovirus in stool specimens; (2) RSV, influenza A and B viruses, parainfluenza viruses, and adenoviruses in respiratory tract specimens; (3) HBsAg and HIV p24 antigen in serum; (4) HSV and VZV in vesicle/ulcer swab specimens; and (5) CMV in BAL and blood specimens. The detection of CMV pp65 antigen in neutrophils is commonly used in the diagnosis and management of immunocompromised patients with new or reactivated CMV infection.[16] Overall, antigen detection tests are rapid, with results usually available within hours.[7] Viable virus is not required for detection.

Electron Microscopy

Although antigen detection kits and NAT have become increasingly popular in the clinical diagnostic virology laboratory because of their high throughput capabilities and increased sensitivities, EM continues to play a role for several reasons.[17] A large number of specimen types (if collected and processed properly) are suitable for use with EM. An experienced microscopist can morphologically identify a viral pathogen within 10 minutes of arrival of a specimen in the laboratory. The high specificity of reagents used in antigen detection and NAT may limit their ability to detect viruses with different antigenic determinants or nucleic acid sequences, respectively. Because EM detection of viruses is based on morphologic characteristics, it can be used broadly to detect members of different virus families as well as potential novel agents.

In the past, EM was mainly used to identify agents causing viral gastroenteritis. Although antigen detection tests are currently available for rotavirus[18] and enteric adenovirus,[19] EM is still required for detection of other viruses that cause gastroenteritis, including norovirus, astrovirus, other caliciviruses, or small round viruses.[19,20] EM can also be used to detect HSV or VZV in vesicle fluid,[21] or HSV, CMV and EBV in brain tissue,[22] but it cannot distinguish between them. Disadvantages of EM include the large number of viral particles (approximately 1×10^6 virus particles per milliliter of specimen) required for detection, the fact that it is not suitable for high-volume throughput, is expensive, and many centers lack availability and expertise.

Nucleic Acid Detection

Molecular probes directed at a unique, conserved portion of a viral genome are highly specific and bind only to complementary DNA or RNA sequences.[23] Probes are particularly useful for detecting and typing viruses for which reliable culture methods are not available, such as HPV.[24] Molecular probes are available in commercial kits for the detection of HIV,[25] HSV,[26] CMV,[27] hepatitis B virus (HBV),[28] and HCV.[29] However, for some viruses, the concentration of viral genomes in direct patient specimens may be too low to allow detection with adequate sensitivity. For example, commercially available probes for HSV and CMV detect only 70% to 90% of specimens positive by viral isolation.[26,27]

To increase the sensitivity for detection of viral genomes, NATs have been developed for many viruses. Three approaches have been taken: (1) target amplification such as PCR,[30] strand displacement amplification (SDA),[31] NASBA,[32] and transcription-mediated amplification (TMA) systems[33]; (2) probe amplification, including Q-beta replicase and ligase chain reaction (LCR)[34]; and (3) signal amplification, such as branched-chain DNA (bDNA) assay with a "tree" of enzymes attached to the probe[35] and hybrid capture using a chemiluminescence detection system.[36] These amplification technologies allow reliable detection of a number of viruses and several commercial and in-house ("home-brew") assays have been developed. The most common include detection of HIV in plasma

TABLE 287-1. Detection Rates[a] of Virus Detection Methods for Selected Viruses

	Shell Vial Culture + Stain		Conventional Tube Culture		Antigen Detection	IFA/DFA	PCR
Virus	**Days in Culture**	**% Detected**	**Days in Culture**	**% Detected**	**% Detected**	**% Detected**	**% Detected**
HSV	1	66–97	1	40–48	47–89	95	100
CMV	1	68	7	50	100 (Disease) 60–70 (Infection)	N/A	82-100
CMV	2	96					
VZV	2	70–90	5	50	N/Ab	77–97.5	84–100
Adenovirus (respiratory)	2	97	4	50	N/A	22–67	N/A
Influenza	2	60–100	4	50	39–100	40–90	95.8
RSV	2	95	6	98.2	70–100	80–90	98.6

CMV, cytomegalovirus; CPE, cytopathic effect; DFA, direct immunofluorescence; HSV, herpes simplex virus; IFA, indirect immunofluorescence; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.
[a]Detection rates will vary depending on the specimen type, stage of disease, length of incubation, cell line used for culture and shell vial, and definition of a true positive.
[b]N/A, not applicable or data sets include too few isolates for calculation.
[c]Data from references: 43, 52–57, 62, 66, 74, 75, 77, 79, 82–84, 90, 108–114, 137–144, 146, 147, 149–156, 158–160.

or WBCs,[25] CMV in WBCs, HSV in CSF,[37] enteroviruses in CSF and serum,[38] HBV or HCV in serum or plasma,[29] HPV in cervical cells, respiratory viruses (including influenza virus A/H5N1) in respiratory specimens, noroviruses in stool and parvovirus B19 in serum.[39] Quantification of viral genome in plasma or serum has been used to determine prognosis, select patients for antiviral therapy, and monitor response to treatment in patients with acquired immunodeficiency syndrome (AIDS),[25] chronic HBV and HCV infection,[29] and CMV infection in immunocompromised patients.[40] Further development of molecular-based assays is focusing on the use of multiplex tests capable of detecting a number of viruses in a single amplification reaction, particularly for herpes group viruses and respiratory viruses.[41–43] Another advance in molecular diagnostics has been the development of automated real-time PCR.[44] This method can produce a result faster (within 30 minutes in some cases) than conventional PCR by using fluorescence and continually analyzing the amplified product. As well, because it is a closed system, it is less prone to contamination by amplified product.

Choice of Virus Detection Method

Antigen detection methods provide results within hours and are preferred when sensitive and specific test kits and reagents are commercially available (e.g., rotovirus, influenza A and B viruses, RSV). Advantages of direct antigen detection include: (1) noncritical specimen collection and transport conditions; (2) the ability to detect viruses that cannot be cultivated (e.g., rotavirus, enteric adenovirus, HBV); (3) no need for cell culture equipment and highly trained personnel; (4) superior sensitivity compared with culture for certain viruses; and (5) the rapidity with which results are available (usually within hours). Disadvantages include: (1) lack of available test kits for many clinically important viruses such as EBV, HAV, HCV, enteroviruses, rubella, mumps, arboviruses, and parvovirus B19; and (2) inferior sensitivity compared with isolation for most viruses that can be cultivated, and inferior specificity.

Culture is preferred when results are available quickly with the shell vial centrifugation and staining methods (e.g., HSV, CMV, and VZV). Advantages of isolation include: (1) ability to recover a broad range of viruses; (2) availability of the infectious agent for further characterization; (3) 100% specificity; and (4) superior sensitivity in comparison to antigen detection. Disadvantages of isolation include: (1) a requirement for a laboratory with specialized equipment, supplies, and trained personnel; (2) a longer time to final results than with direct antigen detection; and (3) the lability of certain viruses under suboptimal collection and transport conditions.

The use of nucleic acid detection techniques is appropriate when the virus cannot be detected by rapid isolation or when antigen detection methods are not available or are insensitive. Molecular probe plus amplification technology is useful for detecting HSV in CSF from patients with encephalitis, parvovirus from serum in immunocompromised patients with chronic anemia, and enteroviruses in CSF from patients with suspected viral meningitis. Although commercial assays are available for some viruses (e.g., CMV, HIV, HPV), many of these assays are only performed in research or reference laboratories.

Serologic Methods

The major uses for viral serologic methods are to diagnose a current or recent acute infection and to determine specific susceptibility or immunity. Interpretation of serologic results is virus-specific. For example, the presence of HIV antibodies indicates current infection, whereas the presence of rubella IgG usually indicates immunity. Serologic diagnosis of acute infection is more useful when the incubation period is prolonged (e.g., 3 to 6 weeks) and antibody is present in serum concomitantly with signs of illness (e.g., EBV and CMV mononucleosis and viral hepatitis). Figure 287-1 shows a typical antibody response for an acute, moderate-incubation (several days to 2 weeks) viral illness such as measles, mumps, or rubella. At the onset of rash or other manifestations, antibody is undetectable

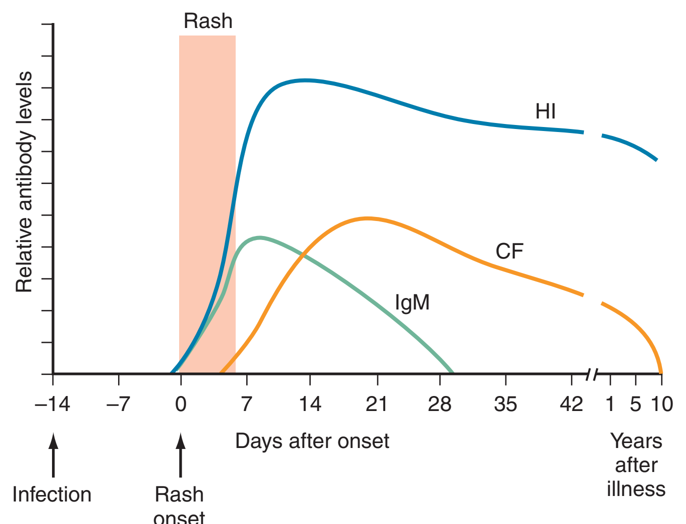

Figure 287-1. Antibody responses during acute measles. HI, hemagglutination inhibition antibody; CF, complement fixation antibody.

or is present at low titer. Within 10 to 14 days, appreciable titers of antibody are present. For short-incubation virus infections (e.g., respiratory viruses), a rise in antibody does not usually occur until the late recovery phase or during convalescence and is thus of little clinical value for acute diagnosis. With the use of older serologic assays such as hemagglutination inhibition (HAI) or complement fixation (CF), which detect IgG antibody, a greater than fourfold rise in titer between acute and convalescent sera when tested in parallel confirms a diagnosis. Acute seroconversion can also be used to diagnose an acute or recent infection.

A fourfold fall in titer is also presumptive evidence of a recent infection; unchanging low titers indicate past infection and immunity. The presence of antibody in high titer in a single serum specimen during convalescence does not usually permit a definitive diagnosis.

EIA kits and, to a lesser extent, latex agglutination and FA kits have replaced other antibody tests in many laboratories. Results are reported in optical density (OD) units rather than quantifiable dilutions of serum. Interpretation of OD units varies with the EIA kit used and the virus. For some assays, such as hepatitis B surface antibody (HBsAb) and rubella antibody, the result is converted to international units per milliliter, with a value > 10 IU/mL reflecting immunity with most kits.

The presence of virus-specific IgM antibody in serum obtained 1 to 2 weeks after the onset of illness permits a diagnosis of acute or recent infection for many viruses. Typically, IgM antibody disappears from serum within a few months after the acute illness, but it may persist for an extended time in some individuals and for some viruses. False-positive IgM results can occur through: (1) cross-reactivity (particularly among herpesviruses)[45]; (2) the presence of rheumatoid factor (IgM antibody that binds to the Fc portion of IgG)[46]; (3) persistence of IgM antibody for several months after the acute illness (e.g., EBV)[47]; (4) reactivation of latent viruses (e.g., HSV and CMV) resulting in the production of IgM antibody; and (5) inherent testing difficulties.[48]

False-negative IgM tests can result from: (1) an absent, low, or delayed IgM response, especially in immunologically immature hosts (e.g., infancy, congenital CMV or HIV infection) or in immunosuppressed patients (e.g., patients with AIDS)[49]; or (2) binding of all viral antigen sites in the test system by high titer of IgG antibody (precluding binding of IgM). Many commercially available kits contain reagents to adsorb IgG from the test serum, thus reducing the possibility of interference.

When using IgG antibody tests to determine susceptibility or immunity to a particular virus, the sensitivity of the method is

important. Generally, CF antibody titers are quantitatively lower than HAI titers and can disappear after several years. Therefore, CF should not be used for determining susceptibility or immunity.

Compared with detection of virus, the major advantages of serologic diagnosis of viral infection include noncritical specimen handling and wide availability. Disadvantages include: (1) a requirement for acute and convalescent sera for IgG antibody tests (for acute diagnosis); (2) false-positive and false-negative IgM antibody results; and (3) delay of 2 to 3 weeks before a diagnosis can be confirmed with short-incubation infections.

Optimal Tests for Specific Viruses

Table 287-2 lists the medically important viruses, major attributable diseases, optimal diagnostic specimen or specimens, available tests, and average time to a positive test result. For many tests, the average time to obtain a result may be a function of the test itself (e.g., culture), the logistics of routine laboratory testing schedules (e.g., serologic and molecular tests that are performed only on certain days) or the need to refer a sample to a reference lab for testing. The preferred test provides the most rapid result with acceptable sensitivity (> 90%) and specificity (> 95%). In general, detection of viral antigen and virus isolation is preferred to serologic tests because of the shorter time to a positive result. Serologic tests are used when isolation is not expedient or possible and antigen detection tests are not available. Nucleic acid detection tests are performed in some specific clinical situations (e.g., HSV encephalitis and enterovirus meningitis). A number of tests may be required to establish a specific diagnosis, particularly when different viruses can cause similar clinical syndromes. The preferred diagnostic test or tests may vary, depending on the patient population being tested (e.g., immunocompromised hosts).

Traditionally, calculation of the value of nonculture tests has been based on 100% sensitivity and 100% specificity of culture. However, isolation may be falsely negative and thus potentially obscure the true sensitivity of antigen or nucleic acid detection tests. In the results summarized herein for individual viruses, assessment is based on a definition of true-positive results by isolation of virus or positivity of two antigen or probe detection tests (e.g., direct fluorescent antibody (DFA) and EIA).

Herpes Simplex Virus

For diagnosis of suspected mucocutaneous lesions due to HSV, an aspirate or swab of the vesicular fluid or ulcer base placed in VTM is recommended. If possible, samples should be shipped cold on wet ice or with an ice pack. If a delay in processing is anticipated beyond 48 hours, samples should be frozen at –70°C until tested. Depending on the clinical situation, other potentially useful samples include blood in EDTA for PCR when viremia is suspected (e.g., neonates), CSF in a sterile container in suspected cases of HSV meningitis or encephalitis, and tissue biopsy in VTM or frozen for selected situations (e.g., disseminated HSV in neonates or immunocompromised patients).

The yield on culture varies depending on the stage of the clinical infection, the type of specimen, and the tissue culture cell type used in the laboratory.[50] In one study, the rate of recovery of HSV from genital herpes lesions was 94% during the vesicular stage but only 27% during the crusted stage.[51] Use of the shell vial method with mink lung cell cultures permits detection of HSV with 99% sensitivity and 100% specificity by 16 to 24 hours. Routine CPE in a sensitive cell line detects 50% of positive tests in 24 hours, 80% in 48 hours, and 95% in 72 hours.[52] Culture is not 100% sensitive. In a study of genital herpes lesions, culture and DFA on cells scraped from the base of lesions were equally (80%) sensitive, with 100% specificity.[53] Another study suggested shell vial was only 66% sensitive compared to conventional tube culture (sensitivity 97%).[54] The recovery rates of HSV in culture from CSF and blood specimens are relatively poor compared with recovery from the vesicular fluid obtained from skin lesions.

For direct antigen detection tests, samples should be collected in the same manner used for virus culture. A variety of assays (mainly EIA and DFA) are available with variable degrees of sensitivity and specificity. A direct comparison of three commercial EIAs for the detection of HSV reported sensitivities ranging from 47% to 89% and specificities of 85% to 100%.[55] None of these antigen detection assays is sufficiently sensitive to detect asymptomatic shedding reliably.[56] DFA is reliable for the detection of HSV in lesions. Using commercially available monoclonal antibodies, DFA detected 95% of HSV-positive samples compared with culture, which detected 92%.[57]

In the past, the definitive means for establishing a diagnosis of HSV encephalitis was brain biopsy.[22] Routine virologic studies of CSF have not been rewarding; HSV was isolated in < 5% of CSF specimens from biopsy-proven cases in one study.[22] Studies measuring high CSF-to-serum anti-HSV antibody ratios,[22,58] HSV-specific IgM in CSF,[58] antibody against HSV-1 glycoprotein B in CSF,[59] and HSV-1 antigen in CSF[60] have yielded results supporting the diagnosis of HSV infection of the central nervous system (CNS). However, CSF specimens obtained within 10 days of the onset of illness are unlikely to be positive.

HSV PCR on CSF is an excellent test for HSV encephalitis.[37] Based on a meta-analysis of HSV PCR performed on CSF that reported sensitivity of 96% and specificity of 99%,[61] HSV PCR on CSF has replaced brain biopsy as the diagnostic test of choice for HSV encephalitis.[62] PCR is positive at least through the first 6 to 7 days of illness, even in patients receiving acyclovir therapy.[37,63] However, negative results have been obtained in up to 25% of CSF samples from infants and children, and thus HSV PCR should not be used to rule out HSV encephalitis when clinical suspicion is high.[64] HSV PCR on CSF samples is also useful in cases of HSV meningitis.[65] PCR has also been used for detection of HSV in other clinical specimen types, with good results.[66] It can detect both HSV-1 and HSV-2 as well as allow for distinction between HSV-1 and HSV-2.

HSV-specific IgG and IgM antibody is detectable in serum 10 to 20 days after the onset of primary infection. IgG antibodies indicate past or current infection, but not necessarily active disease. The presence of HSV IgG antibody in organ transplant recipients is used as a risk factor for recurrences and has prompted the prophylactic use of acyclovir.[67] Because of fluctuations in HSV IgG antibody titers, serologic tests should not be used to diagnose recurrent HSV infections. IgM antibody is not a reliable indicator of primary infection in immunosuppressed individuals because reactivation can cause a rise in IgM levels.[67]

Older FA and EIA HSV antibody tests could not reliably distinguish between HSV-1 and HSV-2 IgG antibodies. However, commercially available EIA and immunoblot tests based on glycoprotein G antigen are now approved by the United States Food and Drug Administration (FDA) and are clinically available.[68] Western blotting (WB) can also distinguish the presence of HSV IgG type-specific antibodies, but it is not widely available. The use of HSV-2 type-specific assays has provided important information about the unreliability of clinical history and the epidemiology of genital HSV infection.[69] Recommendations have been proposed for the appropriate use of HSV-2 serologic tests.[70] No IgM test is commercially available that can distinguish between infection with HSV-1 and HSV-2.

Guidelines for standardization of in vitro susceptibility testing of HSV have been published.[71] Although most HSV isolates from drug-naive immunocompetent patients are susceptible to antiviral agents, resistance to acyclovir and other drugs has emerged as a clinical problem in immunocompromised patients receiving prolonged courses of continuous or intermittent suppressive therapy.[71–73] In vitro testing of HSV isolates for patients who fail to respond to therapy may be warranted.

Cytomegalovirus

CMV can be detected in a variety of clinical specimens by isolation,[74] antigen detection,[75] DNA probes,[76] or NATs.[77–79] Because CMV can

TABLE 287-2. Optimal Specimen, Preferred Test, and Performance in Confirmation of Specific Infections

Agent/Type or Site of Infection or Host	Major Diseases	Optimal Specimens	Available Tests[a]	Average Time to Positive Results[b]
ADENOVIRUS				
Respiratory	Pharyngitis, pneumonia, undifferentiated febrile illness	Throat swab, NP aspirate/wash Serum	Culture[c] Antigen detection/FA IgG antibody[d]	6 days 2 hours 1–5 days
Eye	Conjunctivitis	Eye swab Serum	Culture[c] Antigen detection IgG antibody[c]	7 days 2 hours 1–5 days
Intestinal (types 40/41)	Diarrhea	Stool	Antigen detection	2 hours
ARBOVIRUSES				
SLE, California, WEE, EEE, WNV	Fever, encephalitis	Serum, CSF	IgG and IgM antibody[d]	1–5 days
Colorado tick fever	Fever, malaise, neutropenia	Serum	IgG antibody	7 days
CHLAMYDIA/CHLAMYDOPHILA				
Chlamydia trachomatis Genital	Urethritis, proctitis, cervicitis, salpingitis, pelvic inflammatory disease	Urethral, cervical swab, first-void urine, rectal mucosal swab	NAT[c] Antigen detection DNA probe Culture	2–6 hours 4 hours 4 hours 48–72 hours
Neonatal	Conjunctivitis, pneumonitis	Eye swab, NP aspirate/wash	NAT[c] Antigen detection Culture	2–6 hours 4 hours 2 days
Sexual abuse, rape	Vaginitis, urethritis, proctitis	Cervical, urethral, rectal mucosal swab	Culture[e]	2 days
Chlamydophila pneumoniae (TWAR)	Pneumonia, pharyngitis, bronchitis	NP aspirate/swab, throat swab/wash Serum	Culture[c] Antigen detection IgG and IgM antibody	4 days 4 hours 1–5 days
Chlamydophila psittaci	Pneumonia	NP aspirate/wash, throat swab/wash Serum	Antigen detection Culture IgG antibody[d]	4 hours 2 days 1–5 days
CYTOMEGALOVIRUS				
Congenital	Hepatosplenomegaly, thrombocytopenia, microcephaly, hearing loss, chorioretinitis, amniotic fluid	Urine, throat swab, EDTA blood, serum	Shell vial culture with antigen stain[c] Culture NAT[f] IgG and IgM antibody[c]	2 days 3–4 weeks 2–5 hours 1–2 days
Postnatal infection	Heterophile-negative infectious mononucleosis	Throat swab, urine, EDTA blood, serum	Shell vial culture with antigen stain[c] Culture IgG and IgM antibody[d]	2 days 3–4 weeks 1–2 days
Immunosuppressed patients	Pneumonitis, colitis, retinitis	EDTA blood Bronchoalveolar lavage, rectal swab	Antigenemia assay,[c] NAT[c, f] Shell vial culture with antigen stain[c] Culture	4–6 hours 2–5 hours 2 days 3–4 weeks
Pretransplant screening/immune status	Past infection (donor and recipient)	Serum	IgG antibody	1–2 days
ENTEROVIRUSES				
Coxsackie A and B viruses, echovirus, poliovirus	Aseptic meningitis, fever and rash, herpangina, hand, foot, and mouth disease, myocarditis and pericarditis, paralytic disease	CSF, throat swab, stool, EDTA blood	Culture PCR[c, f] on CSF, serum	4–7 days 6 hours
Poliovirus	Paralytic disease	Serum	Neutralizing[d] antibody panel	5 days
Coxsackie B virus	Myocarditis and pericarditis	Serum EDTA blood, tissue	Neutralizing[d] antibody panel PCR[f]	5 days 6 hours
Echovirus	Any of the above	Serum	Neutralizing[g] antibody panel	5 days
EPSTEIN–BARR VIRUS				
Healthy individual	Mononucleosis syndrome	Serum	Slide agglutination test (monospot)[c] IgG and IgM antibody[d]	1–3 days 1–3 days

Continued

TABLE 287-2. Optimal Specimen, Preferred Test, and Performance in Confirmation of Specific Infections—*Continued*

Agent/Type or Site of Infection or Host	Major Diseases	Optimal Specimens	Available Tests[a]	Average Time to Positive Results[b]
Immunocompromised	Posttransplant lymphoproliferative disease (PTLD)	Serum, plasma, whole blood, leukocytes	PCR[f]	2–5 hours
GASTROINTESTINAL VIRUSES Rotaviruses, caliciviruses, enteric adenoviruses, astroviruses	Diarrhea	Stool	EM, antigen detection[c] PCR[f]	2 hours 6 hours
GENITAL *MYCOPLASMA* *Ureaplasma urealyticum*	Urethritis, cervicitis	Urethral, cervical swab; semen	Culture[c]	2 days
Mycoplasma hominis	Pneumonitis, meningitis in neonates	Tracheal aspirate, CSF in neonates		
HEPATITIS VIRUSES Hepatitis A	Acute	Serum	IgM antibody	2 days
	Immunity	Serum	Total antibody	2 days
Hepatitis B	Acute	Serum	HBsAg, anti-HBc IgM	1–2 days
	Chronic	Serum	HBsAg, anti-HBc total	1–2 days
		Serum/plasma	NAT for HBV DNA (quantitative)[f]	1 week
	Immunity	Serum	HBsAb	1–2 days
Hepatitis C	Acute/chronic	Serum	Anti-HCV EIA screen	1–2 days
		Serum	Anti-HCV RIBA supplementary	5 days
		Serum/plasma	NAT for HCV RNA (quantitative/qualitative)[f]	1 week
Hepatitis D (only occurs in patients with HBV coinfection/superinfection)	Acute Chronic	Serum Serum	HDV Ag, anti-HDV IgM HDV Ag, anti-HDV total	1–8 days 1–8 days
Hepatitis E	Acute	Serum	IgG and IgM antibody	1–8 days
HERPES SIMPLEX VIRUS Skin, mucous membranes	Oral, genital, cutaneous ulcers or vesicles, herpetic whitlow	Aspirate of vesicle fluid Swab of vesicle fluid or base of ulcer	Shell vial culture with antigen stain[c, h] Antigen detection (FA) NAT[f]	16–24 hours 2 hours 2–5 hours
Past infection	Recurrent genital symptoms but culture negative	Serum	IgG antibody[d]	1–2 days
Neonatal infection	Disseminated disease; hepatitis; pneumonitis; encephalitis; skin, eye, mouth ulcers or vesicles	Swab of lesion, EDTA blood, CSF, conjunctiva/nose/mouth swab Serum	Shell vial culture with antigen stain[c, h] Antigen detection (FA) IgG and IgM antibody[d]	16–24 hours 2 hours 1–2 days
Eye	Conjunctivitis, keratitis	Conjunctival or corneal swab or scraping	Shell vial culture with antigen stain[c, h] Antigen detection (FA)	16–24 hours 2 hours
Brain	Encephalitis[i]	CSF or brain biopsy[i] Serum	NAT[f] Shell vial culture with antigen stainc Antigen/antibody in CSF IgG and IgM antibody[d]	2–5 hours 16–24 hours 2 hours 1–2 days
Meninges	Aseptic meningitis	CSF Serum	Shell vial culture with antigen stain[c] IgG and IgM antibody[d]	16–24 hours 1–2 days
HUMAN HERPESVIRUS 6 Primary infection	Roseola (exanthem subitum)	Serum	IgG and IgM antibody[d]	1–3 days
Immunocompromised	Transplant recipients, AIDS	EDTA blood for PBMCs	PCR[f]	1–2 weeks

TABLE 287-2. Optimal Specimen, Preferred Test, and Performance in Confirmation of Specific Infections—*Continued*

Agent/Type or Site of Infection or Host	Major Diseases	Optimal Specimens	Available Tests[a]	Average Time to Positive Results[b]
HUMAN IMMUNODEFICIENCY VIRUS				
Suspected HIV infection in adult or older child	Symptomatic or asymptomatic	Serum	Screening HIV EIA[c] Confirmatory Western blot or IFA HIV p24 antigen, PCR[i]	1–2 days 1–3 days 2–4 days
Newborn	Suspected vertical or perinatal transmission	Serum EDTA blood	Screening HIV EIA Confirmatory Western blot or IFA Virus culture PCR[c,f]	1–2 days 1–3 days 2–3 weeks 1 week
OTHER VIRUSES				
Human papillomaviruses	Cervical dysplasia	Cervical swab	RNA probe, hybrid capture, PCR	1–4 days
Influenza viruses	"Flu" syndrome, pneumonia	NP aspirate/wash/swab, throat swab/wash, BAL Serum	Antigen detection for influenza A and B Culture[b] IgG antibody[d]	30 minutes–2 hours 7–9 days 1–5 days
Measles virus	Measles	NP aspirate/wash Serum	Culture[c] Antigen detection[c] IgG and IgM antibody[d]	5 days 2 hours 1–2 days
Mumps virus	Parotitis, aseptic meningitis, meningoencephalitis	Urine, throat swab Serum	Culture IgG and IgM antibody[d]	8 days 1–2 days
Parainfluenza viruses	Croup, pneumonitis, bronchiolitis	NP aspirate/wash Serum	Culture[c] Antigen detection using FA IgG antibody[d]	4–7 days 2 hours 1–5 days
Parvovirus B19	Erythema infectiosum Aplastic crisis, congenital, hydrops fetalis	Serum Blood, serum, bone marrow, amniotic fluid cells, placental tissue, cord	IgG and IgM antibody[d] IgG and IgM antibody[d] PCR	2 days 2 days 2 days
Respiratory Syncytial Virus	Bronchiolitis, pneumonia, croup	NP aspirate/wash Serum	Antigen detection[c] Shell vial with antigen staining Culture IgG and IgM antibody[d]	15 minutes–4 hours 16–48 hours 3–7 days 1–5 days
Rhinovirus	Common cold	NP aspirate/wash	Culture	7 days
Rubella	Acquired or congenital rubella	Serum Throat swab	IgG and IgM antibody[d] Culture	1–2 days 5–7 days
VARICELLA-ZOSTER VIRUS				
Skin, disseminated	Chickenpox, herpes zoster, occasional CNS complications	Vesicle fluid, scraping of base of vesicle Serum CSF	Antigen detection[c] Culture IgG and IgM antibody[d] PCR[f]	2 hours 3–7 days 1–2 days 1 week
Immune status	Past infection or vaccination	Serum	IgG antibody	1–2 days
Mycoplasma pneumoniae	Pneumonia, pharyngitis, Stevens–Johnson syndrome, meningoencephalitis	Throat swab CSF Serum	Culture PCR[f] IgG and IgM antibody[c]	3 weeks 4–6 days 1–5 days

Ag, antigen; AIDS, acquired immunodeficiency syndrome; BAL, bronchoalveolar lavage; CNS, central nervous system; CSF, cerebrospinal fluid; EBV, Epstein–Barr virus; EDTA, ethylenediaminetetraacetic acid; EEE, eastern equine encephalomyelitis; EIA, enzyme immunoassay; FA, fluorescence antigen detection; EM, electron microscopy; HAV, hepatitis A virus; HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IFA, indirect fluorescence assay; IgG, immunoglobulin G; NAT, nucleic acid amplification test (may include: LCR, ligase chain reaction; NASBA, nucleic acid sequence-based amplification; NP, nasopharyngeal; PCR, polymerase chain reaction); PBMCs, peripheral blood mononuclear cells; RIBA, recombinant immunoblot assay; SLE, St. Louis encephalitis; WEE, western equine encephalomyelitis; WNV, West Nile virus.

[a]Available tests may vary by laboratory. Samples may need to be sent to a reference lab for some tests. Not all tests need to be performed in all patients.

[b]The average time to a positive result may be as much a function of the test itself (e.g., culture) as it is the frequency with which the test is performed in the laboratory.

[c]Preferred test on the basis of sensitivity, specificity, and short time to a positive test result.

[d]Acute and convalescent (2 to 4 weeks after the onset of illness) serologic testing is recommended for most viruses. IgM antibody testing is available for CMV, EBV, HAV, HSV, measles, mumps, parvovirus B19, rubella, and varicella-zoster virus.

[e]In cases of sexual abuse or rape, culture is recommended because of concern about false-positive results with nonculture methods.

[f]PCR test times to a positive result vary.

[g]In the echovirus neutralizing antibody panel, four to five of the most prevalent recent serotypes are chosen for the panel.

[h]Serotyping of the isolate as HSV-1 or HSV-2 is available.

[i]Detection of proviral DNA after PCR amplification may be the preferred test in young infants, in adults with mononucleosis syndrome before seroconversion, and in adults with an indeterminate Western blot.

be shed asymptomatically for months to years after primary infection[80] and can be reactivated asymptomatically during periods of immunosuppression,[75] it is often difficult to distinguish between asymptomatic shedding and CMV disease. The major sites of asymptomatic shedding are urine, cervical secretions, semen, saliva, and respiratory tract secretions.[81] The preferred test for detection and diagnosis of CMV depends on the clinical syndrome and immune function of the patient (see Chapter 206, Cytomegalovirus).

For the isolation of CMV from clinical specimens, samples should be kept at 4ºC or refrigerated until processed. The shell vial method detects 68% of positive specimens in 24 hours and 96% by 48 hours. Although some studies suggest that the shell vial method can miss 25% to 30% of positive WBC cultures, the use of three centrifuged shell vials containing MRC-5 fibroblast cells (one vial stained at 24 hours, a second at 48 hours, and the third observed for CPE for 10 days) has detected all positive specimens regardless of the source.[82] In another study using blood samples, the shell vial method was more sensitive than conventional culture.[83]

Isolation of CMV from urine obtained during the first 3 weeks of life is diagnostic of congenital infection.[80] In all other situations, it is impossible to distinguish CMV viruria related to primary infection, reactivation or reinfection disease, or asymptomatic shedding. Interpretation of the presence of CMV in respiratory tract specimens is similarly confounded. In immunosuppressed patients with suspected CMV, testing of a BAL specimen may be useful. Compared with culture of lung biopsy specimens obtained from patients with CMV pneumonia, the sensitivity isolation from BAL fluid was 70% to 95% and the specificity was 50% to 100%.[84,85] The lower specificity probably represents contamination of the BAL specimen by asymptomatic respiratory tract shedding. Demonstration of CMV antigen in cells from BAL specimens by DFA staining may be more specific for CMV infection, but sensitivity is reduced to 60% to 100%.[84] Histologic examination of cells obtained by BAL for the presence of characteristic CMV infection (intranuclear inclusions with an "owl's eye" appearance) suggests a diagnosis of CMV pneumonia.

Detection of CMV in peripheral WBCs by culture techniques may be useful in the diagnosis of active CMV disease or as a predictor of future CMV pneumonia in transplant recipients.[81,86] CMV viremia is considered to be the best predictor of CMV disease, particularly in patients with severe immunosuppression.[87] However, the lack of sensitivity of culturing CMV from WBCs led to the development of CMV antigenemia assay (an immunocytochemical assay that detects the 65-kd lower-matrix phosphoprotein (pp65) of CMV directly in neutrophils) and a variety of NATs, including PCR, hybrid capture assay, NASBA, and bDNA assay of WBCs, plasma, serum, or whole blood.[77–79,88–90] These assays are most widely used in immunocompromised patients, including transplant and HIV-infected patients, and to a lesser extent in infants with congenital CMV infection. Some assays are quantitative or semiquantitative, and several studies support a relationship between the level of CMV in blood and the likelihood of active or emerging CMV disease in immunocompromised patients.[91–94] These assays are used in pre-emptive treatment strategies, as well as for monitoring response to anti-CMV therapy. Potential problems with these assays include the use of heparin as the anticoagulant for blood collection, which has been shown to inhibit PCR[12]; delay in processing of blood samples beyond 4 to 6 hours, which results in false-negative findings with the CMV antigenemia assay[95]; cost; the need for technical expertise; and labor intensity (e.g., CMV antigenemia). No one of these assays has been shown to be clearly superior.

For the diagnosis of CMV mononucleosis in otherwise healthy people, testing for CMV-specific IgM is the preferred test. False-positive CMV IgM results can occur in patients with acute EBV infection.[96] In immunologically immature hosts or in immunosuppressed patients, the CMV IgM response during acute infection can be delayed or absent. Because IgM antibodies do not cross the placenta, their detection in a newborn is diagnostic of congenital infection. However, production of IgM antibodies by the newborn may be delayed or absent and thus a negative result cannot be used to rule out congenital infection. False-positive IgM assays also occur.

The major diagnostic use of measuring CMV IgG in serum is to determine susceptibility to infection in healthcare or childcare workers[97] and to identify the CMV status of blood donors and organ donors and recipients.[98] Several of the commercially available EIAs, latex agglutination tests, or fluorescence-based IgG tests have acceptable sensitivity and specificity for these purposes.

Epstein–Barr Virus

In patients with suspected primary EBV infections, particularly infectious mononucleosis, testing of serum for the presence of heterophile antibodies remains the test of choice.[99] Using a simple spot agglutination assay (often referred to as a "monospot"), these IgM antibodies can be easily and rapidly detected.[100]

Heterophile antibodies develop in approximately 85% to 90% of adolescents and adults with EBV infectious mononucleosis[101] within 2 to 3 weeks after the onset of illness and their detection in typical cases is sufficient to confirm a primary EBV infection. They usually disappear within a few months. Responses can be delayed in some individuals, so repeat testing may be required. Because the heterophile test is negative in 70% to 80% of EBV infections in children younger than 4 years,[102] EBV-specific antibody assays are necessary for accurate diagnosis. Heterophile antibodies detected with the use of sheep or horse red blood cells can persist for more than a year after acute illness in 20% to 70% of patients[47]; persistence of heterophile antibodies should not be interpreted as recurrent or chronic infectious mononucleosis. Cases of heterophile-negative mononucleosis in school-aged children are due to CMV in 70% and EBV (proved by EBV-specific serology) in 16%.

EBV-specific antibody titers are indicated when the diagnosis of EBV infection is strongly suspected and the heterophile test is negative. The most useful test in the diagnosis of acute infectious mononucleosis is EBV viral capsid antigen (VCA) IgM; it appears within 1 to 2 weeks after the onset of symptoms, disappears within months, and is 91% to 98% sensitive and 99% specific for the diagnosis.[47,101] However, false-positive results can occur due to the presence of rheumatoid factor, other herpesvirus infections, and antinuclear factors in EIA test systems. False-negative results can occur if samples are collected late in the course of the illness and some children have low IgM titers. VCA IgG antibody titers are elevated when patients are manifesting signs and symptoms of illness; they persist for life and are thus less useful for the diagnosis of acute infection. Anti-early antigen (EA) antibody increases early and disappears in a few months, whereas anti-EBNA (Epstein–Barr nuclear antigen) antibody appears late and persists for life in individuals who recover. Several months after acute illness, an individual who recovers from infectious mononucleosis is expected to have antibody to VCA IgG and EBNA, but low or absent antibody against VCA IgM and EA[101] (see Figure 207-3). Both EIA and FA tests are available commercially for performing EBV-specific serology. FA tests have fairly uniform performance characteristics, whereas EIAs are more variable because of the wide variety of antigen preparations used in different kits.

Direct tests for EBV, such as cultivation in cord blood leukocytes,[103] direct detection by immunofluorescence staining with monoclonal antibodies, or detection of the genome by DNA probes,[104] are performed in research laboratories. Although EBV could be isolated from oropharyngeal washings or circulating lymphocytes of 80% to 90% of patients with infectious mononucleosis, such cultures are not routinely available in clinical laboratories. PCR detection of EBV DNA has been used on blood and CSF with good results.[104,105] Detection of EBV DNA in the CSF of patients with HIV infection is strongly associated with primary CNS lymphoma.[104] After organ and marrow transplantation, the use of quantitative EBV PCR may help to predict the development of posttransplant lymphoproliferative disease. Elevated levels of EBV DNA in peripheral blood may be an indication to decrease the dose of

immunosuppressive therapy, or consider therapies such as with CD20 monoclonal antibodies or EBV-specific cytotoxic T lymphocytes.[106]

Rarely, EBV infection is associated with an acute fulminant disease (e.g., X-linked lymphoproliferative syndrome and virus-associated hemophagocytic syndrome).[107] High-titer and persistent EBV antibodies, except against EBNA, are characteristic. Heterophile and EBV antibodies can be absent, however; the diagnosis depends on techniques to demonstrate virus or its genome.

Varicella-Zoster Virus

The diagnosis of chickenpox or herpes zoster (shingles) can usually be made clinically. In selected instances in which laboratory diagnosis is important, isolation of virus from vesicular fluid, demonstration of viral antigen in cells scraped from the base of lesions using FA staining, and detection of VZV DNA by PCR in vesicular fluid, skin scrapings, respiratory secretions, blood, or CSF[108–114] are available. VZV is extremely labile and therefore transport of samples to the laboratory should be as rapid as possible. Freezing of samples, particularly at –20°C, significantly decreases the recovery VZV in culture. Direct detection of VZV antigens by FA of smears prepared from cellular material collected from the base of fresh vesicular lesions is more sensitive than culture. Vigorous swabbing to retrieve cellular material from the base of the vesicular lesion optimizes the yield. Vesicular fluid, although good for culture, is inadequate for FA testing because of lack of cellular material. In one study involving 133 patients, the sensitivity of FA was 98% (77/79) compared with culture, which had a sensitivity of only 49%.[111] Superiority of FA staining has been shown in other studies; FA testing is the preferred method for diagnosis of VZV in skin lesions.[112]

The use of PCR for the detection of VZV DNA has several advantages. PCR is more sensitive than culture and FA, it can detect VZV in scrapings of older lesions when culture is usually negative and it can be used to distinguish between vaccine versus wild-type VZV. PCR analysis of CSF can confirm diagnosis of CNS syndromes associated with VZV that can occur as a complication of varicella or zoster, with or without cutaneous lesions. Detection of VZV DNA in CSF by PCR along with detection of VZV antibody in CSF are recommended to confirm VZV CNS infection.[115]

VZV IgG serologic tests are used primarily to assess susceptibility to infection in individuals with negative histories for chickenpox to determine the need for vaccination or risk of disease in an exposed immunosuppressed individual. Up to 75% of adults with no history of varicella and 90% of history-negative healthcare workers have antibodies to VZV and are therefore immune.[116] Serology can be used for the diagnosis of acute VZV infection. Antibodies to VZV appear within a few days after the onset of the acute varicella rash and peak by 2 to 3 weeks later. A greater than fourfold rise in IgG antibody titer between acute and convalescent serum collected 10 to 14 days apart or the detection of VZV-specific IgM antibodies in a single sample supports a diagnosis of acute varicella infection. However, the serologic diagnosis of acute VZV infection may be confounded by heterotypic HSV antibody increases that can occur in up to one-third of patients with primary HSV infection who have experienced a previous VZV infection.[117] Many assays are available for the detection of VZV antibodies, including the fluorescent antibody against membrane antigen (FAMA) test, EIAs, indirect fluorescent antibody (IFA) assays, anticomplement immunofluorescence (ACIF) assays, and latex agglutination assays.[118] Detection of antibody to VZV in healthy individuals by FAMA (considered the "gold standard") or latex agglutination correlates with protection in up to 96% of persons.[119] Occasionally, VZV infection has been reported to occur in patients with low levels of VZV antibodies detected by these assays.[120]

Human Herpesvirus Types 6, 7, and 8

Primary infection with human herpesvirus-6 (HHV-6) (roseola) occurs in most children before the age of 2 to 3 years. The following serologic criteria have been considered to be diagnostic of primary HHV-6 infection: (1) antibody seroconversion between acute- and convalescent-phase serum or plasma specimens collected 2 to 4 weeks apart; (2) detection of HHV-6-specific low-avidity antibodies in serum or plasma; (3) positive serum IgM in the absence of IgG antibodies; and (4) greater than fourfold rise in IgG antibody titer by immunofluorescence or ACIF assays.[121] Current commercial assays for HHV-6 IgG do not distinguish between variants A and B and may cross-react with HHV-7 and CMV.[122,123] Antibody avidity testing can be used to differentiate between primary HHV-6 and HHV-7 infections. HHV-6 IgM in serum alone is not a reliable indicator of acute or recent infection because low levels of IgM may also be found during reactivation or reinfection and approximately 5% of adults have detectable HHV-6 IgM at any time.[121] IgM may not appear until 5 to 7 days after the onset of illness and may not be detectable in culture-positive children.[122,124] During primary HHV-6 infection, virus can be recovered from cultures of peripheral blood mononuclear cells (PBMCs) in 100% of infants during the acute illness, but not after recovery.[125] HHV-6 DNA can be detected by PCR in PBMCs in infants both during acute illness and after recovery.[123,126] HHV-6 antibody titers and PCR tests are available in reference laboratories, but culture is only performed in research settings. Monoclonal antibodies are available for direct detection of HHV-6 antigen and have been used for confirming cell culture CPE and for immunohistochemical staining of tissues.

In immunosuppressed patients, HHV-6 infection has been associated with pneumonia,[127] rejection of a transplanted organ,[128] encephalitis,[129] and mononucleosis syndrome.[130] In these situations, proof of HHV-6 causation is difficult because specific antibodies can be absent and demonstration of viral DNA in peripheral leukocytes can represent latent infection. Although PCR detection of HHV-6 DNA in serum or plasma has low sensitivity, it may be a better marker for diagnosing active infection. PCR was negative in the serum or plasma of 57 healthy adults, but positive in 94% of 17 patients with exanthem subitum, 23% of 13 bone marrow transplant recipients, and 22% of 18 HIV-infected patients.[131,132]

Serologic response to HHV-7 can be measured with FA, EIA, and immunoblot assay, but these are not widely available. Some degree of cross-reaction between HHV-6 and HHV-7 antibodies occurs because of cross-reactive epitopes on the viruses but responses can be distinguished by antibody avidity testing.[122] A significant rise in HHV-7 antibodies with stable or absent antibodies to HHV-6 may indicate active infection with HHV-7. HHV-7 has been isolated from the saliva of 75% of healthy adults[133] as well as from ill individuals, making the value of such testing questionable. However, HHV-7 has been isolated only rarely from PBMCs of healthy asymptomatic individuals compared with those with active infections, suggesting that PBMC cultures may have some diagnostic value.[134] Specific primers for PCR amplification of HHV-7 have been developed that do not amplify the DNA from any of the other human herpesviruses, including HHV-6.[135,136]

Testing for HHV-8 is only available in research settings. PCR has been used for the detection of HHV-8 DNA in PBMCs and tissues. The use of plasma or serum for HHV-8 PCR has no value for identifying active infections.[121] Serologic assays based on IFA and EIA methods have been developed for the detection of HHV-8 IgG antibodies but not IgM. Apart from seroprevalence studies, the role of serologic tests in diagnosing and managing HHV-8 infections, whether in healthy individuals or immunocompromised patients, has not been established.

Respiratory Syncytial Virus

NP washes or aspirates are superior specimens to swab for identification of RSV infection. Samples for culture or FA testing should be transported on wet ice or refrigerated as there is substantial loss of cell culture infectivity at room temperature. Samples for antigen detection using EIAs can be transported at room temperature. Culture for RSV requires a mean of 3 to 7 days, and the sensitivity is less than that of

antigen detection techniques.[137,138] Use of the shell vial culture technique provides a more rapid result and appears to have a slightly greater sensitivity than standard culture methods.[139] However, culture has the advantage of detecting other respiratory viruses that are recovered from 5% to 10% of specimens submitted for diagnosis of RSV infection. Rapid detection of RSV antigen in respiratory secretions obtained by NP aspiration, washing, or swabbing is available commercially and includes EIA and FA, which have been evaluated extensively.[43,140–142] The average sensitivity of EIA microtiter plate kits is 85%, with an average specificity of 96%. The sensitivity of DFA is 96%.[137,138] Membrane filter EIA kits packaged as individual tests for processing small numbers of specimens have been reported to have an average sensitivity of 84% and specificity of 92% and can provide a result within 15 to 20 minutes.[137,138] Some assays can detect multiple respiratory viruses simultaneously, thus significantly simplifying laboratory testing.[143]

Several EIA and FA tests are available for the detection of RSV antibodies. In primary RSV infection, detectable IgM antibodies appear approximately 5 to 9 days after onset of symptoms and persist for several weeks. However, the antibody response may be poor or absent in very young infants, older individuals with repeat infections, and immunocompromised patients.[144] RSV antibody detection may be useful for epidemiologic purposes and for evaluating responses to candidate RSV vaccines. Molecular tests such as PCR improve detection of RSV in respiratory tract specimens. They have also been used to distinguish between RSV subgroups A and B during community and institutional outbreaks.[145] Multiplex PCR assays capable of detecting several different respiratory viruses in the same test have been evaluated.[146]

Influenza Viruses

Clinical samples for the detection and isolation of influenza viruses should be collected within 3 days of symptom onset when viral shedding is maximal. Transport to the lab should be as prompt as possible and specimens can be stored at 4ºC if processing will be delayed beyond 3 to 4 days. Standard tube culture for isolation of influenza viruses requires a mean time of 3 to 5 days. The shell vial method shortens the time for detection to 48 hours but has only 37% to 60% sensitivity compared with standard culture.[147] Several rapid antigen detection kits, including point-of-care tests, are available for the detection of influenza A only, influenza A and B together (without distinguishing between them), and influenza A or B.[148,149] Evaluations of rapid tests for the detection of influenza virus indicate an average sensitivity of 70% to 75% and a specificity of 90% to 95%.[147,149,150] These tests have not been fully evaluated for the detection of avian influenza A/H5N1. When good-quality respiratory specimens with well-preserved epithelial cells are used, DFA staining using monoclonal antibodies has sensitivity of 80% to 90% and specificity of > 90%.[147,149–153] The performance of all these direct detection tests may be affected by the type and quality of the specimen. NP aspirates are superior to NP swabs and throat swabs for the detection of influenza A in healthy volunteers.[154,155] A number of different PCR assays for the detection of influenza viruses have been evaluated in several studies and show at least 5% to 15% increased sensitivity compared with other methods, including culture.[156,157] Multiplex PCR assays capable of detecting influenza and other respiratory viruses are promising.[158] Eventually, PCR-based assays are likely to supplant other methods for the diagnosis of influenza virus infections. Serologic examination is available for influenza, but it has limited diagnostic utility because antibody is not detectable during illness; acute and convalescent sera at least 10 to 14 days apart are required to detect a greater than fourfold rise in titer. Serodiagnosis is utilized primarily for surveillance and epidemiology.

Other Respiratory Viruses

Numerous other viruses can infect the respiratory tract and cause clinical signs and symptoms indistinguishable from influenza and RSV. These include human parainfluenza viruses types 1, 2, and 3, adenoviruses (subtypes A to E), rhinoviruses, human coronaviruses 229E, OC43, severe acute respiratory syndrome (SARS), coronavirus, and human metapneumovirus (hMPV).[43] Despite the lack of proven effective antiviral therapy for these viruses, laboratory diagnosis may be important for epidemiologic purposes, for implementation of appropriate infection control measures, and for reducing empiric use of antibiotics. Culture for parainfluenza viruses and adenoviruses requires approximately 4 to 6 days for a positive result.[159] Although culture of rhinoviruses is possible, most laboratories do not routinely attempt isolation. No culture methods are available for isolation of coronaviruses or hMPV. DFA staining is available for parainfluenza viruses (sensitivity 50% to 70%) and for adenoviruses (sensitivity 10% to 30%).[153,160] Interpretation of the causal role of adenovirus is confounded by latency and reactivation. No antigen detection test is available for rhinoviruses, hMPV, or coronaviruses. Serology is of little value for rapid diagnosis of acute infection with these viruses. Several molecular assays for the detection of respiratory viruses have reported sensitivities and specificities approaching 100% compared with culture and antigen detection assays but are not available for routine diagnosis. Detection of many of these viruses is being incorporated into multiplex PCR assays that could provide relatively rapid and comprehensive results using a single patient sample.[158]

Hepatitis Viruses

Table 287-2 lists tests used to diagnose acute viral hepatitis. Diagnosis for all hepatitis viruses is based on serology; virus culture is not available. The diagnosis of acute hepatitis A is made by demonstration of anti-HAV IgM.[161] Immunity to hepatitis A, whether acquired after natural infection or after immunization with vaccine, is determined by measuring hepatitis A total IgG and IgM antibodies (frequently reported as "IgM/IgG" antibody and misinterpreted as a ratio).[161] In acute and chronic hepatitis B, both HBsAg and anti-hepatitis B core antibody (HBcAb) are present.[162] Anti-HBc IgM is generally present in acute hepatitis B infection and occasionally during a flare of inflammatory activity in chronic carriers. Thus, anti-HBc IgM does not always distinguish acute from chronic infection. A person with persistently positive HBsAg for > 6 months is a chronic carrier. In some patients, the only positive serologic marker is a positive HBcAb. There are many possible explanations for this, including: (1) "core window" period during acute infection between loss of detectable HBsAg and emergence of detectable HBsAb; (2) late chronic infection with HBsAg levels that have fallen below the level of detection of the assay; (3) co-infection with HCV or HIV that can result in suppression of HBsAg; (4) infection with a mutant HBV; or (5) a false-positive result. The presence of hepatitis B e antigen (HBeAg) and the absence of anti-HBe are markers of greater infectivity in chronic carriers and a poor prognosis with greater risk of progression to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[163] Conversely, the presence of anti-HBe is an indicator of likely recovery. The presence of HBsAb at a level > 10 IU/mL is considered to confer protection against acute infection.

Second- and third-generation antibody kits to diagnose acute and chronic hepatitis C contain structural proteins of the virus for screening EIA and supplementary recombinant immunoblot assay (RIBA).[164–166] Seroconversion occurs by 8 to 12 weeks after acute infection, with sensitivity of 94% to 100% (except in immunosuppressed individuals) and specificity of > 97% after the supplementary RIBA test.[29] HCV antibody is frequently negative at the onset of jaundice. No assay is currently available that can measure HCV IgM antibodies, and thus one cannot distinguish recent from past infection. The presence of HCV antibodies indicates current infection, not immunity, in most patients.

Molecular assays for the detection and quantification of viral nucleic acid in serum are available for both HBV and HCV.[167,168] These tests are useful for determining prognosis, selecting candidates for therapy, and monitoring response to therapy.[29] A lower concentration indicates a better prognosis and a greater likelihood of

response to treatment. Patients responding to antiviral treatment demonstrate a significant drop in HBV DNA or HCV RNA after the onset of therapy, whereas nonresponders do not.[29] Molecular assays are also available for HBV and HCV genotyping. HCV genotyping is useful for epidemiologic purposes and to identify patients most likely to respond to therapy.[169]

Serologic tests are also available for both hepatitis D (delta agent) and hepatitis E viruses.[170] Because infection with hepatitis D virus (HDV) occurs solely in conjunction with HBV infection, testing for HDV IgG should only be performed in patients acutely or chronically infected with HBV (i.e., HBsAg- and HBcAb-positive). Both hepatitis E virus (HEV) IgG and HEV IgM can be measured, although no licensed test is available in the United States. HEV IgM is positive in most patients 1 to 4 weeks after the onset of disease. By 3 months, HEV IgM is not detectable. HEV IgG levels typically decline after infection.[171]

Gastroenteritis Viruses

Stool samples for detection of viruses associated with gastrointestinal infections should be collected within the first 48 hours of illness. Specimens should be placed in a clean sterile container without VTM or preservative. Rectal swabs are poor specimens as they may not contain sufficient virus for detection using EM. Stool specimens are stable at 4ºC for up to a week. Although freezing at –70ºC may allow for prolonged storage, detection by EM may be reduced. None of the viruses that cause gastroenteritis can be cultivated in conventional cell culture systems, but all can be detected by EM. Commercial EIA and latex agglutination tests with > 95% sensitivity and specificity compared with EM are available for detection of rotaviruses, enteric adenoviruses, and astroviruses 40 and 41.[18–20] Occasionally, positive results for rotavirus are observed in asymptomatic neonates, probably representing false-positive results.[172] PCR-based assays for rotaviruses, caliciviruses, astroviruses, and enteric adenoviruses have been developed and are becoming available in many state health departments.[173,174] Because of their superior sensitivity and specificity, PCR-based assays are now the method of choice for diagnosing gastroenteritis viruses, particularly rotaviruses and caliciviruses. However, no commercial assays are currently available.

Enteroviruses

Because enteroviruses are generally stable and capable of surviving in the environment for weeks, rapid transport of clinical specimens to the laboratory is less critical than for other viruses. Appropriate specimens for the detection of enteroviruses include CSF, serum or whole blood, urine, and rectal, nasal, and throat swabs. Tissue biopsies can also be submitted depending on the clinical situation. Enterovirus viability decreases slowly over days to weeks at room temperature and is preserved for decades at –70ºC. Isolation of enterovirus requires a mean of 4 to 7 days.[175] Virus can be isolated more frequently from stool (80% to 85%) and throat swabs (50% to 60%) than from CSF (40% to 60%) and serum or peripheral leukocytes (40% to 50%). Some enteroviruses, particularly certain members of the coxsackievirus A group, do not grow in cell culture. Due to the lack of a common antigen among enteroviruses, immunoassays for the detection of these viruses are not available. Because of the low numbers of viruses in most clinical samples, EM is not useful for direct detection of enteroviruses.

The reverse transcriptase PCR (RT-PCR) technique has been used to test CSF of patients with aseptic meningitis. Compared with cell culture, RT-PCR has significantly improved the speed of detection of enteroviruses, with reported sensitivity from 86% to 100% and specificity from 92% to 100% in confirmed or probable cases of enteroviral meningitis.[176,177] In comparison, culture has sensitivity of only 40% to 60%.[175] Sensitivity and specificity of enteroviral RT-PCR on serum samples range from 81% to 92% and from 98 to 100%, respectively. However, detection in urine samples is poor, probably due to nonspecific inhibitors of PCR.[178] RT-PCR has also been used to detect enteroviruses in cardiac tissue from patients with suspected enteroviral myocarditis.[177]

Depending on the specimen type and clinical situation, the detection of enteroviruses, whether by culture or RT-PCR, must be interpreted cautiously. Asymptomatic shedding of wild enterovirus from the gastrointestinal tract can occur for weeks or months. Additionally, when relevant, oral polio vaccine virus can be shed in stool and, less commonly, the throat in young vaccinated children. Thus the detection of enteroviruses from these sites may be unrelated to the patient's clinical illness. Detection of virus in CSF, the genitourinary tract, or blood is proof of a causative role.

Antibody titers are not usually measured for enteroviruses. They are of limited value for prompt diagnosis, and a separate neutralization assay must be performed for each enterovirus type. If an isolate is obtained from the patient, a greater than fourfold rise in antibody titer in acute and convalescent sera to that particular enterovirus is diagnostic.

Measles, Mumps, and Rubella

The laboratory diagnosis of these viruses can be made by virus isolation, detection of antigen, the use of RT-PCR, or serologic testing. Suitable samples for isolation of these viruses or detection of viral antigen include whole blood, serum, throat and NP secretions, urine and, under appropriate clinical circumstances, CSF, brain and skin biopsies. As these are labile viruses, rapid transport to the laboratory is important. Specimens are best kept at 4ºC prior to processing, but may be frozen at –70ºC if a delay beyond 48 hours is anticipated. Samples for isolation of measles virus can be collected from 2 to 4 days before and up to 4 days after the onset of rash. Throat swabs for rubella virus isolation are usually positive if collected on the day of rash onset but rapidly become negative thereafter. Although mumps virus can be isolated from saliva 9 days before and up to 8 days after the onset of parotitis, specimens should be obtained early in the course of the illness. These viruses can be cultivated in conventional cell lines, but isolation requires 7 to 10 days for measles and mumps virus and > 3 weeks for rubella virus.[179,180] Use of the shell vial method for measles virus results in sensitivity of 78% at 1 to 2 days and 100% at 5 days compared with routine culture. Sensitivity of DFA staining for NP swab specimens for measles virus antigen is 100% compared with culture, but only 67% for throat swabs and 85% for urine specimens. The shell vial technique for detection of mumps virus has comparable sensitivity and specificity to traditional culture.

Molecular diagnosis using virus-specific RT-PCR has been used for detection of all of these viruses, but is not part of routine testing. It may be useful in special situations such as suspected measles-associated subacute sclerosing panencephalitis and congenital rubella syndrome.

The usual diagnostic method for measles, mumps, and rubella infection is serologic testing. Timing of specimen collection for serologic diagnosis of acute infection due to these viruses is critical. Many patients do not mount a sufficient IgM antibody response at the time of rash onset and thus a repeat sample, collected several days after rash onset, may be required for diagnosis. Most infants born with congenital rubella syndrome have detectable IgM antibodies at birth. Although the traditional serologic test is HAI for IgG antibody, a number of IFA and EIA IgG and IgM kits are available commercially.[181–183] Care must be exercised when interpreting positive IgM tests. Mumps IgM antibody can persist for months after acute illness.[184] Patients with infectious mononucleosis,[185] parvovirus B19 infection,[186] and CMV infection can have IgM antibodies that cross-react with rubella virus. False-positive rubella IgM tests are a particular concern in pregnant women.[187] It is therefore prudent to confirm critical IgM-positive test results, either with an IgM assay from another manufacturer or by a significant rise in IgG antibodies.[187] Recent developments have been the use of EIAs to measure the avidity of IgG antibodies to measles and rubella

viruses. These tests can distinguish between primary and secondary responses to vaccination and to natural infection. Measurement of virus-specific IgG antibodies can be used to determine immune status. For mumps virus, it should be noted that cross-reactions with other paramyxoviruses can occur. For rubella virus, an IgG level of > 10 IU/mL is thought to represent immunity in most cases.[179]

Human Immunodeficiency Virus

The major diagnostic tests for HIV are serologic (EIA and WB for HIV antibody, EIA for p24 antigen), and virologic (culture of PBMCs for infectious virus, and the use of molecular tests (e.g., PCR, NASBA, TMA, bDNA) to detect HIV RNA in plasma or proviral DNA in PBMCs). Standardized techniques for culturing HIV have been developed but are not generally used for routine diagnosis.[188] Molecular tests have been used for the diagnosis of infection in neonates with sensitivity equivalent to culture.[189,190] However, in other populations, the use of molecular tests such as PCR has yielded false-positive results, so they should be used cautiously.[191] The major use of quantitative molecular tests is to measure HIV viral load in plasma in persons already known to be HIV-seropositive. Molecular tests are used to monitor response to antiretroviral therapy routinely.[25,192] Because of the intra-assay and biologic variability in HIV RNA levels, greater than threefold change is required to suggest a clinically relevant change. Different molecular assays can also produce significant differences in HIV viral load, so baseline values should be repeated when the laboratory testing is changed from one assay to another.[192] Although each of the three currently available assays (PCR, bDNA, NASBA) has strengths and weaknesses, the bDNA assay requires 2 mL of plasma for testing, which may be difficult to obtain in children, whereas PCR requires 200 μL and NASBA requires 100 μL to 1 mL.[192]

The mainstay of diagnosis and screening for HIV remains HIV-specific serology using EIAs.[193] Early EIA antibody assays used partially purified viral antigens from HIV-infected cell lysates and had sensitivity and specificity exceeding 95% in the diagnosis of HIV infection in high-risk groups. However, in low-risk groups such as blood donors, who have an expected HIV infection prevalence of 0.3%, 90% of positive results could be false-positive.[193] The most common cause of false-positive results was cross-reacting antibodies in serum against human leukocyte antigens in the cell lysate. False-negative results were due to antigenic heterogeneity among HIV strains, particularly group O.[194] More recent EIA kits use more purified viral antigens from cell lysates, viral protein antigens derived from recombinant technology, and synthetic peptide antigens. These assays have increased sensitivity and specificity and fewer indeterminate results.[193] Moreover, most currently available assays detect antibodies to both HIV-1 and HIV-2.[195] Fourth-generation screening tests are now available that can detect both antibody and antigen. These assays have further reduced the seroconversion window period to approximately 16 days.[193]

A number of other assay formats have been developed, including IFA, radioimmunoprecipitation assay, screening latex agglutination, and dot immunoblot assay.[193] The IFA test can detect both IgG and IgM HIV antibody, is quite sensitive and specific, and can be used as an alternative to the technically more difficult and costly WB as a confirmatory test.[193] The latex agglutination and dot immunoblot assays require limited equipment and were developed to screen large populations, including those in developing countries. Rapid tests requiring minimal or no laboratory equipment have been developed that can yield a result within 30 minutes with comparable sensitivity and specificity to third-generation EIA-based tests. Testing systems for urine and saliva have been developed and approved by the FDA. These tests also have excellent sensitivity and specificity but their sensitivity in early seroconversion is not established.

Different laboratory diagnostic strategies are needed for the three most common situations in which HIV infection is considered: (1) an adult or older child who is suspected of having HIV infection; (2) an infant with suspected vertically acquired HIV infection; and (3) an individual in whom acute infection or seroconversion may develop because of exposure to someone infected with HIV.

An adult or older child who has been infected with HIV for weeks to months is expected to be antibody-positive. The standard approach in this situation is to perform: (1) screening EIA, with a repeat EIA if the test is positive; and (2) a confirmatory WB test if the repeat EIA is positive.[193,196]

WB remains the principal confirmatory test for HIV serology, despite the fact that its sensitivity in seroconversion panels is inferior to third- and fourth-generation screening tests. WB is also prone to give a high rate of indeterminate results due to detection of cross-reacting antibodies. WB measures the antibody response to 9 HIV proteins or glycoproteins: gp160, gp120, p66, p55, p51, gp41, p31, p24, and p17.[193] The Centers for Disease Control and Prevention (CDC) criterion for confirmation of HIV infection is presence of antibody to any two of the following: p24, gp41, or gp120/160.[197] No antibody response to HIV proteins represents a negative test, whereas the presence of some, but not all, antibodies required for a positive interpretation is an indeterminate result; repeat testing over the next 6 months is recommended in this situation. If WB results remain indeterminate over a 6-month period, persons are considered to be uninfected.[197] In low-risk populations, persons with a positive screening EIA test result and indeterminate WB are rarely, if ever, infected with HIV on follow-up serologic testing.[198,199]

If the results of serologic testing are not definitive, testing for surrogate markers or direct testing for virus should be considered. In a person with high-risk behavior (e.g., intravenous drug abuse, sexual contact with a known HIV-positive person) and clinical features strongly suggestive of HIV infection, the presence of surrogate laboratory markers such as a low $CD4^+$ T-lymphocyte count, neutropenia, or thrombocytopenia not explained on any other basis and hypergammaglobulinemia supports the diagnosis.[193,194] In addition, tests for p24 antigen, HIV DNA or RNA, or culture of PBMCs for the virus can be performed.[193,194] In the setting of high risk and clinical features of infection, p24 antigen test has a specificity of 99%.[193] The sensitivity of the antigen test varies according to clinical disease status: 4% in asymptomatically infected people, 56% in patients with AIDS-related complex, and 76% in patients with AIDS.[200]

Confirmation of vertical transmission of HIV is complicated by the presence of maternal antibodies transmitted transplacentally, which confounds interpretation of the screening EIA and WB for up to 15 months of age.[193,196,201] In a symptomatic infant > 4 to 6 months of age, detection of p24 antigen[202] or HIV genome by PCR[203] and culture of the virus from PBMCs[204] are reliable, definitive tests. The sensitivities of culture,[189,205] PCR,[189,203] p24 antigen,[189,205,206] and HIV-specific IgA[207,208] testing for the early diagnosis of HIV infection in young infants are shown in Table 287-3 and discussed further in Chapter 111, Diagnosis and Clinical Manifestation of HIV Infection. Although culture is considered the “gold standard” for pediatric HIV infection, PCR for viral DNA or RNA is more sensitive. In an older infant or child with clinical features suggestive of AIDS, and born to a seropositive mother, surrogate tests showing a low $CD4^+$ T-lymphocyte count, neutropenia, or thrombocytopenia without another explanation, and hypergammaglobulinemia support the diagnosis.[194]

In an individual with known HIV exposure, antibody to the virus can usually be detected within 2 to 8 weeks after infection. Based on the use of current third-generation antibody screening assays, HIV antibodies are detectable in 50% of infected individuals within 3 weeks after infection and in most of the remaining patients within 2 months.[193,209] Virtually all infected, immuno-competent individuals are seropositive 6 months after exposure.[209] A mononucleosis-like syndrome develops in some individuals 2 to 4 weeks after infection; p24 antigen can appear transiently during this period.[209]

TABLE 287-3. Sensitivity (%) of Diagnostic Tests for HIV in Infants According to Age

	Age				
Method	**1 week**	**2–4 weeks**	**1–2 months**	**3–6 months**	**> 6 months**
Culture	30–50	50	70–90	> 95	> 95
PCR	30–50	50	70–90	> 95	> 95
p24	1–25	20–50	30–60	30–50	20–40
IgA	< 10	10–30	20–50	50–80	70–90

HIV, human immunodeficiency virus; IgA, immunoglobulin A; PCR, polymerase chain reaction.
Adapted from Report of a Consensus Workshop, Siena, Italy. Early diagnosis of HIV infection in infants. J Acquir Immune Defic Syndr 1992;5:1169–1178.

Arboviruses

The major arboviruses causing encephalitis in the United States are St. Louis encephalitis virus, California (La Crosse) encephalitis virus, eastern (EEE) and western (WEE) equine encephalomyelitis viruses, and most recently, West Nile virus.[210,211] Because most arboviruses produce only a brief, low level of viremia which clears by the time the patient seeks medical evaluation, virus isolation and nucleic acid detection (e.g., PCR) from blood samples rarely yield positive results. Some arboviruses, including dengue, yellow fever, sandfly fever, Venezuelan encephalitis, and Colorado tick fever, produce a relatively high level of viremia that can persist for days or weeks. Therefore, for these agents, virus isolation or nucleic acid detection is possible. However, for most arbovirus infections, the diagnosis is established by IgG seroconversion or detection of specific IgM antibodies, or both.[212] Collection of paired acute and convalescent sera (the first collected during the first week of illness and the second 2 to 3 weeks later) is recommended. A single sample may be sufficient for diagnosis for some viruses for which an IgM test is available (e.g., EEE, WEE, California, SLE, West Nile virus). However, in some cases (e.g., West Nile virus), virus-specific IgM can be detected in serum for many months following infection, potentially confounding the interpretation of a positive result. Testing is usually referred to a reference or state public health laboratory. Both serum and CSF specimens (in cases of suspected CNS involvement) should be tested. The sensitivity of some of these tests approaches 100% by the 10th day of illness.[212] Traditional assays such as CF and HI tests have largely been replaced by FA and EIA tests.[213] The neutralization test remains the most specific test for serologic diagnosis of arbovirus infections and is mainly used to interpret results of other tests in which heterologous antibody reactions can yield a positive result among antigenically related viruses.

Parvovirus B19

Parvovirus cannot be cultivated in routine cell cultures. The diagnosis of acute infection in immunocompetent patients is made by demonstration of rising IgG titers or the presence of IgM antibody.[39,214–217] IgM antibodies are detectable in serum approximately 14 days after infection, when the rash or joint symptoms begin, and can persist for 4 months. The sensitivity of parvovirus-specific IgM exceeds 90% in the first month after the onset of symptoms. IgG antibodies appear within several days after IgM and persist for years in most cases. Current assays for the detection of IgG antibodies have a sensitivity of > 90% and their presence indicates past infection. IgG avidity assays have been used to help distinguish primary and secondary infections. Immunosuppressed or immunologically immature individuals may not produce antibody, and thus diagnosis is made by detection of viral DNA in serum using PCR.[39] Parvovirus-associated aplastic crisis, chronic infection, and congenital infection can be diagnosed by PCR analysis of serum.[60,215] PCR can also be used to detect parvovirus B19 DNA in bone marrow aspirates, cord blood samples, amniotic fluid cells, and biopsy specimens of the placenta and fetal tissues in cases of fetal hydrops.

Congenital and Perinatal Viral Infections

The major viruses infecting fetuses and newborn infants include CMV, VZV, HSV, rubella, parvovirus B19, HBV, HCV, HEV, enteroviruses, and HIV.[218] Negative maternal and neonatal serology for any of these viruses generally excludes the possibility of fetal infection.[218] Detection of virus (via culture, antigen detection, or molecular testing) may be required before a correct diagnosis can be made. Cord blood can yield false-positive and false-negative results and should not be relied upon for diagnosis.[218]

Congenital CMV infection is best diagnosed by isolating CMV from the urine of neonates within the first 3 weeks of life (see Chapter 206. Cytomegalovirus). Beyond 3 weeks of age, isolation of CMV from urine cannot distinguish congenital from perinatal or postnatal infection. CMV-specific IgM in a newborn is positive in only 50% to 70% of congenitally infected neonates and the test can yield false-positive results.[219]

Perinatal or postnatal infection with VZV, as well as with HSV and enteroviruses, can usually be diagnosed by conventional antigen detection or culture techniques. The use of IgM serology for rapid diagnosis of neonatal HSV infections is inappropriate because a response may not be detectable for 2 or 3 weeks after infection.[220]

Although rubella virus can be recovered from throat swabs and occasionally CSF of congenitally infected neonates, virus isolation is tedious and can require 3 to 4 weeks for confirmation.[221] Demonstration of rubella IgM in a neonate with features consistent with congenital rubella confirms the diagnosis.[218]

Parvovirus infection during pregnancy can be diagnosed in the mother by serologic examination. Detection of maternal IgM or a rising IgG antibody level is diagnostic, whereas a stable IgG titer reflects past infection. In neonates, positive parvovirus B19 antibody at 8 to 12 months suggests infection.[218] Parvovirus B19 infection of a fetus with hydrops can be confirmed using PCR and other molecular tests on fetal cord blood, amniotic fluid cells, or both.[215]

CHLAMYDIA AND *CHLAMYDOPHILA*

Chlamydia trachomatis, Chlamydophila pneumoniae, and *C. psittaci* cause disease in humans. Psittacosis, rare in children, is confirmed serologically (see Chapter 168, *Chlamydophila (Chlamydia) psittaci* (Psittacosis)).

Chlamydia trachomatis

Specimen Collection and Transport

Optimal specimens for the diagnosis of *C. trachomatis* are those that include mucosal epithelial cells rather than purulent material. In

postpubertal women, a cervical swab or Cytobrush specimen collected from the cervical os and containing columnar or squamocolumnar epithelial cells is recommended. For prepubertal girls, a vaginal swab is acceptable. The preferred specimen type for males is a urethral swab collected by inserting the swab 3 to 4 cm into the urethra and rotating. First-void urine (FVU) specimens from men and women and vaginal swab specimens are acceptable for use in a number of NATs.[222,223] Infants with suspected chlamydial conjunctival infection should have the purulent discharge removed, followed by swabbing or scraping of the palpebral conjunctiva. The yield of *C. trachomatis* is directly related to the quality of the specimen collected and the transport and storage conditions before testing.[224–226]

For culture, the swab used for collection of specimens is important because some types are toxic to cell cultures or inhibit growth of *Chlamydia* within cells. Swabs with wooden shafts should be avoided, whereas Dacron-, cotton-, rayon-, and calcium alginate-tipped swabs are acceptable.[227] In females, pooling of urethral and cervical swab specimens increases culture sensitivity by approximately 20%.[226] The yield on culture is optimized if specimens are placed immediately after collection at 2°C to 8°C and transported to the laboratory within 48 hours. Freezing at –70ºC is acceptable but may result in a 20% loss of viability.[227] Freezing at –20ºC should be avoided. Refrigeration of swab specimens during transport is not required for DFA testing. Swab specimens for amplification tests are stable at room temperature for up to 10 days. Swabs should be placed into appropriate culture transport media such as sucrose phosphate or sucrose phosphate glutamate supplemented with bovine serum and antimicrobial agents. Some transport media used for culture are also acceptable for use with molecular testing methods.

Collection of specimens for nonculture tests (e.g., EIA, DFA, or NAT) is generally similar to that for culture tests and should follow the instructions of the manufacturer. Unlike culture methods that require separate swabs for *C. trachomatis* and *Neisseria gonorrhoeae*, a single endocervical or urethral swab can be used for detection of both organisms when performing various NATs.[228,229] Some specimen types, such as vaginal, rectal, NP, or female urethral specimens, for which nonculture methods have not been fully developed, should be cultured. In cases of suspected sexual assault, only culture tests should be used, regardless of the site from which the specimen is collected.

Urine specimens are acceptable for molecular amplification tests.[228–233] Approximately 10 to 50 mL of first-catch urine should be collected into a clean sterile container without preservatives or additives. Urine specimens for NAT are stable for up to 24 hours at room temperature, after which they may be refrigerated for up to 4 days before processing. If a substantial delay between collection and testing will occur, urine specimens can be stored at –20ºC or lower for up to 2 months. Urine specimens should not be used for culture because of poor sensitivity.

Laboratory Test Methods

Tests for *C. trachomatis* can be grouped into four broad categories: serology, culture, direct detection, and molecular diagnosis.

1. Serologic tests for detecting *C. trachomatis* genital tract infections are not useful for diagnosis in individual patients. Antibodies to *C. trachomatis* persist for life and therefore cannot distinguish previous from current infection. In infants, however, detection of *C. trachomatis* IgM antibodies may be useful for diagnosing chlamydial pneumonia.[218] Detection of IgG antibodies is less useful in infants because of maternal transfer of IgG antibodies, which may persist for 6 to 9 months.[234] The microimmunofluorescence (MIF) test is the most sensitive serologic test for *Chlamydia* species and is the only serologic test that detects species- and serovar-specific responses.[226] Antigens for the MIF test consist of formalin-fixed elementary bodies grown in an egg yolk sac or cell culture. The MIF test for detection of IgM antibodies has been the diagnostic test of choice for *Chlamydia* pneumonia in infants. EIAs for the detection of IgM antibodies in infants have demonstrable variable performance compared with the MIF test.[234] EIAs detect antibodies to the genus-specific antigen, or lipopolysaccharide, of chlamydial elementary or reticulate bodies and thus detect antibodies to all chlamydial organisms and are not specific for antibodies to *C. trachomatis*. Interpretation of a single IgG antibody test result is difficult because 50% to 70% of people in the United States have antibodies to *Chlamydophila pneumoniae*.[235] CF tests have been widely used for the diagnosis of psittacosis and lymphogranuloma venereum, but they have no value in diagnosing genital tract or neonatal chlamydial infections.
2. Cell culture historically has been considered the gold standard for detection of *Chlamydia trachomatis* because of its specificity, which approaches 100%. However, relative insensitivity in comparison to NATs, requirement for cell culture facilities, and slow turnaround time (3 to 7 days) are disadvantages.[226,234] Because not all specimen types have been appropriately evaluated with other testing methods, the CDC continues to recommend that culture be performed on urethral specimens from women and asymptomatic men, on NP specimens from infants, on rectal specimens from all patients, and on vaginal specimens from prepubertal girls. Centrifugation of the inoculum on to a cell monolayer and the use of fluorescein-conjugated monoclonal antibodies (shell vial method) have improved the sensitivity and shortened the detection time (48 to 72 hours) of *C. trachomatis* inclusions.[227]
3. Direct diagnosis of *C. trachomatis* is most often accomplished by detection of antigens (EIA or DFA assays) or nucleic acid (hybridization assays) or by cytologic examination for the presence of intracellular chlamydial inclusions. EIAs use monoclonal or polyclonal antibodies to detect chlamydial lipopolysaccharide. The 4- to 5-hour automated EIAs are advantageous for processing large numbers of specimens, but their sensitivity is generally less than that of culture. Additionally, a positive EIA usually requires validation by a second nonculture method, especially in low-prevalence populations.[236–238] Two areas of interest with EIAs have been the use of urine specimens and the development of rapid "point-of-care" tests.[239–242] Both require further investigation, although EIAs have been less sensitive than NATs when urine specimens are used. Point-of-care tests can provide a result in < 30 minutes but generally have performed less well than tests performed in laboratories. DFA assays use monoclonal antibodies directed against the major outer-membrane protein of *C. trachomatis*. An advantage of DFA testing is direct visualization of the cellular material obtained, which allows assessment of the quality of the specimen. Both elementary bodies and intracellular inclusions can be detected with DFA tests, and results can be available within 30 minutes. However, DFA testing requires a skilled laboratory microscopist, and large numbers of specimens cannot be processed expediently.[243,244] Urine specimens should not be used with DFA because of poor sensitivity. Although DFA testing has been used to detect *C. trachomatis* in conjunctival[245,246] and respiratory specimens from infants,[247] its major use has been to test cervical and urethral specimens. Nucleic acid probes can be used to test a single specimen for *C. trachomatis* and *Neisseria gonorrhoeae*. These probes are similar in sensitivity to other antigen detection methods and they are relatively specific.[226,248] However, DNA probe tests (without previous amplification) require special transport media, thus precluding performance of another test on a single specimen to confirm a positive result. A confirmatory competitive DNA probe test is available, but it requires a second assay (usually performed the next day), which doubles the cost of the test.[249] Additionally, the DNA probe has a sensitivity for male genital secretions inferior to that of other methods. Cytologic examination of direct smears for the presence of intracellular inclusions has been shown to be useful for detection

of chlamydial conjunctivitis in neonates, but not for diagnosing conjunctivitis or genital infection in adults.[226]

4. NAT tests, including PCR, LCR, TMA test, and SDA, have been approved by the FDA for detection of *C. trachomatis*.[228,230,232,250,251] All can be performed in 2 to 5 hours and are the most sensitive and specific assays available. They require specialized equipment and a sophisticated laboratory to prevent false-positive results from cross-contamination. Despite using different molecular techniques to amplify *C. trachomatis* nucleic acid, all have been shown to have similar sensitivities and specificities. The presence of inhibitor substances in the specimen can interfere with these assays and result in false-negative results. The PCR assay appears to be more sensitive to inhibition than the others.

Comparison of Methods

Selection of a test or tests depends on the clinical setting, availability, and cost. The relative performance and usefulness of tests are summarized in Chapter 167, *Chlamydia trachomatis* (see Table 167-2). Culture was previously considered the gold standard because of 100% specificity and excellent sensitivity when optimal techniques were used.[226] However, for genital specimens, its sensitivity is approximately 90% compared with an expanded gold standard and only 70% compared with NATs.[252] For male urethral swab specimens, DFA and NATs provide the most accurate results. For cervical swab specimens, EIA and DFA are less sensitive than the DNA probe, PCR, and LCR tests, whereas PCR and LCR provide the best specificity and positive predictive value. Rapid EIA tests (performed in less than 30 minutes in an office setting) have a median sensitivity of only about 70% on urethral swabs from males and cervical swabs from females.

Noninvasive tests have been evaluated for screening of asymptomatically infected individuals. In asymptomatic males, screening of a FVU with a leukocyte esterase strip, followed by subsequent testing of positive specimens for *C. trachomatis* by EIA or another test, has reduced cost. Testing of FVU samples in men by PCR has a median sensitivity of 98%; LCR has a median sensitivity of 94%. Testing of FVU in women by LCR has a median sensitivity of 91%. The positive predictive value for PCR or LCR on FVU samples from both men and women is 100%. EIA has adequate sensitivity on FVU from males, but not on FVU from females.

In infants with conjunctivitis or pneumonitis, testing of conjunctival and NP specimens by culture, DFA, or EIA produces acceptable results.[245–247] In cases of suspected rape or sexual abuse, culture is required to avoid false-positive results.[253,254]

Chlamydophila pneumoniae

Accurate confirmation of acute infection with *C. pneumoniae* is difficult.[255,256] Laboratory diagnosis is most often based on serology. The most common test is the CF test, in which a greater than fourfold increase in titer will occur in approximately 50% of infected patients. However, it may take 4 to 6 weeks to detect seroconversion. Seroconversion may not occur at all in some individuals with clinically compatible respiratory disease documented by a positive culture or PCR test on NP specimens. The MIF test appears to be the most reliable serologic test for *C. pneumoniae,* and the following criteria for a positive test have been used: (1) greater than fourfold rise in titer; (2) IgM titer > 1:16; or (3) IgG titer > 1:512. IgG titers between 1:16 and 1:512 are considered evidence of previous, but not necessarily recent, infection.[257] EIA tests are available but have not been completely evaluated, and none is currently FDA-approved. Because EIA tests can detect antibodies to lipopolysaccharide, these tests detect antibodies to all *Chlamydia* species as well.

Isolation of *Chlamydophila pneumoniae* is difficult. The stability of *C. pneumoniae* in clinical specimens has not been well studied, although one study reported that 70% of organisms remain viable after 24 hours at 4°C.[258] Throat swabs, sputum, NP, BAL, and other respiratory tract specimens have been used with variable success. Detection of the organism in respiratory secretions does not prove causality because asymptomatic infections occur in children[256] and persistent shedding has been documented for months after acute disease in adults. Additional problems of confirmation by culture include: (1) small numbers of organisms present in respiratory secretions[256]; (2) poor recovery unless special chlamydial transport media and optimal transport and storage conditions are used[259]; and (3) limited availability.[260]

Molecular diagnosis with noncommercial PCR tests has been evaluated by a number of investigators.[261–263] Sensitivities appear to be as good as those of culture, but specificity is difficult to determine given the lack of a gold standard for comparison. An important issue that must be clarified is the clinical relevance of detecting *C. pneumoniae* by NATs in asymptomatic or prolonged carriage states, particularly in the absence of any corresponding serologic response.

MYCOPLASMA

The major mycoplasmal organisms causing disease in children are *Mycoplasma pneumoniae* and the genital mycoplasmas, *Ureaplasma urealyticum* and *M. hominis*.

Mycoplasma pneumoniae

Rapid and accurate diagnosis of *M. pneumoniae* infection is problematic. Methods for direct detection of the organism in respiratory tract secretions are not widely available, and positive test results can reflect persistent shedding in healthy individuals.[264,265] Significant rises in antibody during infection require 3 to 4 weeks in otherwise healthy individuals[266] and may not occur in immunocompromised patients or young infants.[267]

Culture is the most widely accepted method for detecting *M. pneumoniae* in respiratory tract secretions, but: (1) availability is limited; (2) solid agar and diphasic media (agar plus broth)[268] are required for optimal results; and (3) only 60% to 70% of positive specimens are detected at 3 weeks, and 97% to 100% are not detected until 6 weeks.[268] For optimal isolation of *M. pneumoniae*, specimens should be inoculated into appropriate media, at the bedside if possible. Specimens should be refrigerated if not processed within 24 hours. Because *M. pneumoniae* is relatively slow-growing, cultures should be maintained for 4 weeks before being reported as negative.

In a large study of over 3500 patients with pneumonia seen over a 12-year period in a community setting, culture was 64% sensitive and 97% specific for the diagnosis of *M. pneumoniae* pneumonia.[268] Shedding of *M. pneumoniae* is persistent for several weeks after the onset of illness, particularly in children.[268] Positive cultures have been demonstrated in 5% of healthy individuals during nonepidemic periods and in 14% during a community outbreak.[264]

Antigen detection tests on respiratory tract secretions perform well in research settings, but they are not available commercially.[269,270] Persistent shedding and detection of antigen in asymptomatic individuals confound interpretation of positive results.

PCR amplification of the conserved P1 gene of *M. pneumoniae* on respiratory secretions has been evaluated.[271] PCR is more sensitive than culture and antigen detection but commercial PCR kits are not available. When performed on CSF, PCR can be useful for the diagnosis of *M. pneumoniae*-associated meningoencephalitis.[272–274]

CF assay using a chloroform-methanol glycolipid extract of organisms is the best validated test and has often been used as the reference method for serologic diagnosis.[275] A greater than fourfold rise in titer between acute and convalescent sera or a single titer > 1:32 is 86% to 90% sensitive and 87% to 94% specific for the diagnosis of disease by *M. pneumoniae*.[268,276] However, increases in antibody titer do not occur for 3 to 4 weeks after the onset of pneumonia in normal hosts[256] and can be diminished or absent in immuno-

suppressed patients and infants. In adults older than 40 years, the IgM response may be minimal or absent despite *M. pneumoniae* disease proved by CF antibody titers, culture, or both, presumably as a result of reinfection. It is not clear how long the IgM response persists after infection. *M. pneumoniae*-specific IgM or IgA antibody assays can increase the diagnostic sensitivity to 99%.[276]

Measurement of *M. pneumoniae*-specific IgG, IgM, and IgA antibody titers can be performed with commercially available EIA, FA, and latex agglutination kits.[277–279] These assays are more sensitive and specific than CF and have replaced CF in many diagnostic laboratories. Their usefulness is limited by time to seroconversion (2 to 4 weeks). Some of these tests can provide a result within 10 to 15 minutes. In children, adolescents, and young adults, a single positive IgM result with appropriate immunoglobulin class-specific reagents may be considered diagnostic, although false-positive test results can occur.

Cold agglutinin antibody titers are simple to perform and widely available. A positive bedside screening test (not recommended because of quality control issues) indicates a titer of 1:64 or higher.[265,280] The sensitivity of this test for *M. pneumoniae* infection is only 50% to 90%, and the specificity is approximately 75%.[266,275,280,281] The height of the antibody titer and the specificity of the test increase with increasing severity of pneumonia.

Direct antigen tests (EIA, DFA, immunoblotting) for the detection of *M. pneumoniae* have been evaluated.[282,283] They have been hampered by variable sensitivity and cross-reactivity with other *Mycoplasma* species found in the respiratory tract. One study reported a sensitivity of 91% when the assay was used on sputum and NP aspirates from patients with *M. pneumoniae* infection documented by culture or serology.[284]

The diagnosis of *M. pneumoniae* infection in ambulatory patients rests, for practical purposes, on epidemiologic and clinical features. However, because *M. pneumoniae* can cause fulminant pulmonary disease[285] and complications in multiple extrapulmonary sites,[281,286,287] hospitalized children with suspected *Mycoplasma* infection should have specimens collected at the time of admission and submitted to appropriate reference laboratories.

Genital Mycoplasmas

The major means for laboratory diagnosis of *U. urealyticum* and *M. hominis* infections is culture of the organism from infected body sites. Commercial culture kits are available, with positive results available within 2 to 3 days.[288] Serologic testing has little utility except potentially as an epidemiologic tool. Patients with invasive *M. hominis* infection almost always have seroconversion or a significant rise in antibody titer.[289] Serologic tests for genital mycoplasmas have not been standardized, and none is available commercially. Other diagnostic modalities such as PCR are under development.

CHAPTER 288

Laboratory Manifestations of Infectious Diseases

Sarah S. Long and Ann-Christine Nyquist

ACUTE-PHASE RESPONSE

The acute-phase response is the term given to the coordinated series of events that occur nonspecifically in response to infection, inflammation, or trauma. This response can be seen as the host's means of creating an inhospitable environment for the invading microbe. The cytokines that are produced during and participate in the inflammatory processes are the chief stimulators of the production of acute-phase proteins (see Chapter 11, Fever and the Inflammatory Response). The most important sources of acute-phase proteins are macrophages and monocytes. As with all inflammation-associated phenomena, the acute-phase response is not uniformly beneficial. Extreme cytokine-induced changes associated with the acute-phase response can be fatal, as in septic shock[1–4] (see Chapter 12, The Systemic Inflammatory Response Syndrome (SIRS), Sepsis, and Septic Shock). Expected metabolic changes in the acute-phase response and changes in soluble defense molecules, trace elements, and inflammatory cells of the acute-phase response are listed in Box 288-1. The magnitude, type, and duration of these acute-phase responses provide a guide to the intensity of inflammation or the extent of tissue involvement, although the predictive value of biomarkers for diagnosis or prognosis is variable.

BOX 288-1. Expected Manifestations of the Acute-Phase Response

BRAIN
- Increased release of corticotropin, endorphin, prolactin, neuropeptides, and neurotransmitters
- Increased production of thyroid-stimulating hormone, vasopressin, insulin, and glucagon
- Decreased production of insulin-like growth factor I
- Fever, diminished appetite, and somnolence

BLOOD CELLS
- Neutrophilia, increased neutrophil activation, and demargination
- Thrombocytosis
- Reticulocytopenia, eosinopenia
- Activation of B lymphocytes (antibody production) and T lymphocytes (lymphokine production)
- Redistribution lymphopenia
- Anemia of chronic disease

TISSUE
- Collagen proliferation by fibroblasts
- Demineralization of bone
- Proteolysis and amino acid release from muscle

LIVER
- Increased synthesis/release of complement components and expression of receptors, fibronectin, fibrinogen, mannose-binding protein, lipopolysaccharide-binding protein, transferrin, glycoproteins, C-reactive protein, α_1-antitrypsin, α_2-macroglobulin, lipids and lipoproteins, and ceruloplasmin
- Increased synthesis of serum amyloid A, haptoglobin, immunoglobulins, and serum copper
- Decreased free and total serum iron, zinc, and retinol
- Decreased synthesis of albumin and cytochromes
- Decreased gluconeogenesis

ERYTHROCYTE SEDIMENTATION RATE

Physiology and Measurement

The erythrocyte sedimentation rate (ESR) can be helpful for evaluating a specific disease or monitoring a disease process or response to therapy. Erythrocyte sedimentation in plasma depends on red blood cell (RBC) mass, volume, and shape; RBC–RBC forces; and the protein constitution of plasma. Electrostatic forces normally cause RBCs to repel each other and inhibit their aggregation. An increased amount of plasma fibrinogen or globulins coat the RBCs, foster their aggregation, and hasten settling, consequently elevating the ESR. Large-molecular-weight, needle-shaped fibrinogen has the greatest effect, followed by β-globulins and distantly by α_2-globulins, γ-globulins, and albumin.[5]

The Westergren method of measuring ESR is the most familiar and also the most discriminating at high and low values. It measures