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

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VIRUSES

The availability of rapid and reliable viral diagnostic tests, particularly nucleic acid amplification tests (NAATs), facilitates decision-making in the prevention and treatment of viral infections and the practice of effective infection control measures. With specific antiviral therapy now available for many clinically relevant viruses, a correct viral diagnosis is important and also limits further diagnostic testing and unnecessary antibiotic therapy.[1,2]

Two major approaches to diagnosis of viral infection are 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 NAATs such as polymerase chain reaction (PCR) which may be qualitative or quantitative.

Cytologic examination of tissues and cells can identify viral effects, prompting further investigation. Occasionally, cytologic changes can be sufficiently specific to suggest a particular 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, 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. As the immune response matures following a viral infection, low-avidity IgG antibodies are replaced with high-avidity antibodies. EIAs capable of measuring the avidity of IgG antibodies to specific viruses have been used to distinguish primary from secondary antibody responses to vaccination or natural infection.[4,5]

In the clinical setting, laboratory tests for the detection of virus infection can be divided into three specific categories: those used to (1) make a specific viral diagnosis; (2) measure viral activity in patients known to be infected (e.g., viral load testing for HIV); and (3) screen for infection (e.g., pretransplant or blood donation).

Specimen Collection and Transport

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 maximal. Optimal specimens vary depending on the site(s) of disease. In general, tissues, aspirates, and body fluids are superior to swabs. However, in many circumstances, swabs may be the only specimen available. Body sites or lesions that can be sampled easily with a swab include the pharynx or nasopharynx, conjunctiva, urethra, cervix, vagina, and vesicles or ulcers on the skin or mucous membranes. Many types of swabs are available for specimen collection, including plastic, wooden, or those with a flexible wire shaft and a tip made of cotton, Dacron, calcium alginate, or polyurethane.[6] However, certain swabs may not be suitable for detection of some viruses. Swabs with a wooden shaft can contain toxic products that inactivate herpes simplex virus (HSV). Cotton-tipped swabs can 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 can be toxic for lipid-enveloped viruses such as herpesviruses 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 NAATs, many commercial assays for detection of viruses and *Chlamydia* by antigen detection or molecular techniques provide their own swab and transport media, which should be used.

Swabs and tissues for detection of viruses should be placed into VTM to prevent drying, maintain viral viability during transport, and prevent the overgrowth of contaminating organisms.[6] A number of commercially prepared VTMs are available.[7] Swabs collected for bacterial isolation that are placed in bacterial transport medium are unacceptable for detection of viruses.[6] Conversely, VTM contains 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.

For detection of most respiratory viruses, nasopharyngeal (NP) aspirate or wash, sputum, or bronchoalveolar lavage (BAL) specimen provides a better yield for detection of viruses than NP, nasal, or throat swabs.[7] Multiple samples may be required to maximize yield. Freshly passed stool is superior to a rectal swab for detection of gastrointestinal viruses.[6]

Specific viruses can be found in different blood cells, the plasma/serum, or both (e.g., HIV in lymphocytes and macrophages; CMV in neutrophils and, to a lesser extent, mononuclear cells; enteroviruses in plasma and white blood cells (WBCs)).[8,9] Blood should be collected into Vacutainer tubes containing an anticoagulant such as ethylenediaminetetraacetic acid (EDTA). Recovery rates are higher with EDTA than with heparin.[10] Heparin can inactivate herpesviruses and can inhibit some NAATs;[11,12] this may be less of a concern for real-time PCR and may be related to the type of heparin (sodium versus lithium) used.[13,14]

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 VTM containing albumin or serum as a stabilizer should be used.

Most viruses are stable for 2 to 3 days at 4°C (refrigerator or wet ice temperature).[6] Freezing at −20°C (ordinary freezer temperature) destroys or reduces the infectivity of most viruses and can alter the ability to detect viral antigen using some commercially available kits. Beyond 2 to 3 days, specimens should be stored in an ultra-low-temperature freezer (−70°C) and transported on dry ice. For some NAATs (e.g., detection of hepatitis C virus (HCV) RNA in serum/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 −70°C until tested.[7]

For serologic detection of viral antibodies or antigen, blood can be transported at room temperature. If a delay is anticipated, the specimen should be kept refrigerated at 2°C to 8°C. Serum/plasma should be separated as soon as possible after specimen collection. 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 for which an IgM assay is available (e.g., hepatitis A virus (HAV)), an acute-phase specimen can 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

Monolayer cell culture techniques are used in most laboratories for virus isolation. However, many clinically relevant viruses, such as parvovirus, human papillomavirus (HPV), hepatitis viruses, Epstein–Barr virus (EBV), rotaviruses, noroviruses and others, are not cultivatable in the routine diagnostic laboratory; laboratory diagnosis is based on other methods. Although it is possible to cultivate HIV using suspension cultures of lymphocytes, special containment facilities are required; alternative methods are used for routine diagnosis. The major viruses detected by isolation in monolayer cell culture include 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. Examples are isolation diploid cell lines (e.g., human foreskin or lung fibroblasts for herpesviruses), primary cell lines such as primary rhesus monkey kidney cells for respiratory viruses and enteroviruses, and heteroploid or continuous human epithelial cell line such as Hep-2 cells for RSV. The types of cell lines used in the diagnostic laboratory are determined by the specimen type, season, epidemiologic data, and clinical information provided. Many viruses cause morphologic changes, i.e., cytopathic effect (CPE), in the cell monolayer. Some viruses cause CPE within 2 days (e.g., HSV), others within a week (e.g., enteroviruses), and others after several weeks (e.g., CMV). For viruses that do not cause typical CPE, detection can be based on the adsorption of red cells to the surface of virus-infected cells in culture (e.g., influenza and parainfluenza viruses) or by the use of interference assays (e.g., rubella virus). Presumptive identification of a particular virus or virus group in cell culture is based on the cell type, the characteristic time of onset, and the appearance of CPE, and is facilitated if the laboratory personnel are informed of the source of the specimen and the suspected clinical diagnosis.

Confirmation of the virus isolated requires immunologic methods such as fluorescein- or peroxidase-conjugated virus-specific monoclonal and polyclonal antibodies. Antibodies to HSV, CMV, VZV, RSV, influenza A and B virus, parainfluenza virus,

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	2	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/A[b]	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.*

Data from references: 42, 56, 57–60, 65, 76–78, 82–84, 90, 109–114, 137–143, 146, 147, 149–160.

adenovirus, measles virus, and enterovirus antigens are available readily.

Centrifugation of specimens (also referred to as shell vial culture or spin-amplified culture) onto cell monolayers on coverslips placed in the bottom of small vials or in wells, followed by incubation and staining for viral antigen using monoclonal antibody after 1 to 3 days, substantially reduces the time required to detect and confirm the presence of many viruses. 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. Because of its speed, the shell vial method has replaced conventional cultures in many laboratories (Table 287-1) and is used routinely for the detection of CMV, HSV, VZV, respiratory viruses, and the enteroviruses.

Two techniques for isolation of some viruses have been developed with comparable sensitivity to standard culture and shell vial methods.[15–17] The use of genetically engineered cell lines such as the ELVIS (enzyme-linked virus-inducible system) was introduced first for the isolation of HSV. A baby hamster kidney cell line has been transformed using an HSV-inducible promoter (UL39 gene) and an *E. coli* β-galactosidase gene. The addition of a substrate for the β-galactosidase enzyme results in formation of a color reaction in the HSV-infected cells. This technique has been adapted for performing rapid HSV antiviral susceptibility testing. Mixing multiple cell types in a single shell vial culture can provide rapid detection of respiratory viruses (R-Mix), HSV, CMV and VZV (H and V Mix), and enteroviruses (E-Mix).

Antigen Detection

Antigen detection tests can be performed directly on a variety of specimen types and are highly specific and rapid.[7] Viable virus is not required for detection. Because virus antigen is cell-associated, collection of an adequate number of infected cells is important. A number of commercial kits (EIA, latex agglutination, FA) are available 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 FA technique has been used for the detection of rabies virus in brain tissue, mumps virus in throat and urine sediment, and measles virus in conjunctival cells. The detection of CMV pp65 antigen in neutrophils is used commonly in the diagnosis and management of immunocompromised patients with new or reactivated CMV infection.[18]

Electron Microscopy

A variety of specimen types (if collected and processed properly) are suitable for EM.[19,20] An experienced microscopist can identify a viral pathogen morphologically within 10 minutes of arrival of a specimen in the laboratory. Unlike antigen detection and NAATs, which are limited in ability to detect viruses with different antigenic determinants or nucleic acid sequences, respectively, because of the high specificity of reagents used, EM detection is based on morphologic characteristics and can be used broadly to detect members of different virus families as well as potential novel agents.[19]

EM continues to be used for the detection of gastrointestinal pathogens such as rotavirus, enteric adenoviruses, norovirus, and others as well as non-enteric viruses.[21–25] Disadvantages of EM include the large number of virus particles (approximately 1×10^6 per mL of specimen) required for detection, limited throughput, expense, and lack of availability and expertise in many centers.[25]

Nucleic Acid Detection

Molecular hybridization techniques using probes directed at a unique, conserved portion of a viral genome are highly specific and bind only to complementary DNA or RNA sequences.[26] Probes are particularly useful for detecting and typing viruses for which reliable culture methods are not available. Molecular probes are available as commercial kits for the detection of HPV,[27] HIV,[28] HSV,[29] CMV,[30] hepatitis B virus (HBV),[31] and HCV.[32] For some viruses, the concentration of viral genomes in direct patient specimens may be too low to permit detection with adequate sensitivity (e.g., commercially available probes for HSV and CMV detect only 70% to 90% of specimens positive by isolation).[29,30]

The increased sensitivity of NAATs has revolutionized testing in the clinical virology laboratory.[20,33–35] Three approaches have been taken: (1) *target amplification* such as PCR, strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), and transcription-mediated amplification (TMA) systems; (2) *probe amplification*, including Q-beta replicase and ligase chain reaction (LCR); and (3) *signal amplification*, such as branched-chain DNA (bDNA) assay and hybrid capture assay.[20,36–38] Several commercial and in-house ("home-brew")

assays have been developed. Quantification of viral genome in plasma or serum can be used to determine prognosis, select patients for antiviral therapy, and monitor response to treatment in a variety of patient populations.[34] Multiplex assays capable of detecting a number of viruses in a single amplification reaction have been developed, e.g., for herpesviruses, enteric, bloodborne, and respiratory viruses.[39–42] The development of automated real-time PCR using fluorescence techniques and continuous detection of amplified product has shortened detection times significantly relative to conventional PCR assays.[43] Because these assays use a closed system (i.e., amplification and detection occur in a single tube that need not be opened once the reaction is completed), they also are less prone to contamination. NAAT has been applied to genotyping of viruses (e.g., HIV, HBV, and HCV) as well as for the detection of mutations that confer resistance to antiviral agents.[34]

Choice of Virus Detection Method

Choosing optimal test(s) depends on the virus being sought, the clinical setting, specimen type, availability of kits, reagents and equipment, experience of laboratory personnel, and cost. Antigen detection methods offer the following advantages: (1) noncritical specimen collection and transport conditions; (2) ability to detect viruses that cannot be cultivated easily; (3) no need for cell culture equipment and highly trained personnel; (4) superior sensitivity compared with culture for certain viruses; and (5) rapid turnaround time (usually within hours). Disadvantages include: (1) lack of available test kits for many clinically important viruses; (2) inferior sensitivity compared with isolation for many cultivatable viruses; and (3) inferior specificity due to nonspecific/cross-reactions particularly with the use of polyclonal antibodies.

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 compared with antigen detection for some viruses. Disadvantages include: (1) requirement for specialized equipment, supplies, and trained personnel; (2) longer turnaround time; (3) the lability of certain viruses under suboptimal collection and transport conditions; and (4) the inability to culture many clinically relevant viruses.

The use of NAATs is rapidly replacing older viral diagnostic methods due to their rapid turnaround time, superior sensitivity, and the ability to quantify virus density. A number of relatively simple home-brew and commercially available NAATs (including analyte-specific and for research use only) are available for a wide variety of viruses.[34,35]

Serologic Methods

Serologic methods can be used to diagnose a current or recent acute infection, to determine specific susceptibility or immunity, and for epidemiologic and surveillance purposes. Interpretation of serologic results is virus-specific (e.g., the presence of HIV antibodies indicates current infection, whereas the presence of IgG anti-rubella indicates immunity as a result of immunization or recovery from natural infection). 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). Figure 287-1 shows a typical antibody response for an acute, moderate-incubation (several days to 2 weeks) viral illness such as measles. At the onset of rash or other manifestations, antibody is undetectable 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 usually does not occur until the late recovery phase or during convalescence and has no value for acute diagnosis. With the use of older serologic methods such as hemagglutination inhibition (HAI) and complement fixation (CF) that detect IgG antibody, a >4-fold rise in titer based on serial dilution endpoints between acute and

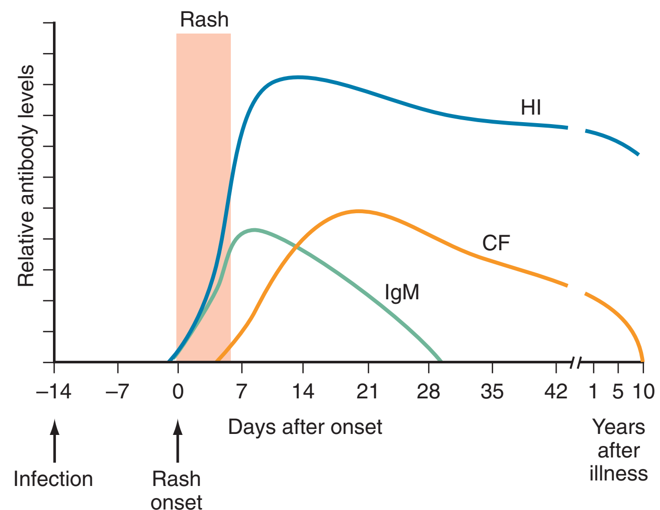

Figure 287-1. Antibody responses during acute measles.
HI, hemagglutination inhibition antibody; CF, complement fixation antibody.

convalescent sera tested in parallel confirms a diagnosis. A 4-fold fall in titer also is presumptive evidence of a recent infection whereas unchanging low titers indicate past infection and immunity. The presence of antibody in high titer in a single serum specimen during convalescence usually does not permit a definitive diagnosis. Acute seroconversion also can be used to diagnose an acute or recent infection.

EIA kits and, to a lesser extent, latex agglutination and FA kits have replaced other antibody tests in many laboratories. EIA/ELISA results usually are measured in optical density (OD) units and results reported in international units (IU) or index values. Interpretation of OD units varies with the EIA/ELISA kit used and the virus antibodies being detected. One must refer to the specific kit manufacturer for the criteria defining a significant difference in antibody levels between acute and convalescent sera.

The presence of virus-specific IgM antibody in serum obtained 1 to 2 weeks after the onset of illness permits a diagnosis of acute/recent infection for many viruses. Typically, IgM antibody disappears from serum within a few months after the acute illness, but can persist for an extended time in some individuals and for some viruses.[44] False-positive IgM results can occur through: (1) cross-reactivity (e.g., among herpesviruses or due to polyclonal stimulation secondary to EBV infection);[45] (2) the presence of rheumatoid factor (IgM antibody that binds to the Fc portion of IgG);[46] and (3) inherent testing difficulties.[47] Misinterpretation of IgM antibodies as indicative of an acute/recent infection can occur as a result of: (1) persistence of IgM antibody for several months after the acute illness (e.g., EBV, West Nile virus);[48] or (2) reactivation of latent or chronic viruses (e.g., HSV, HBV).

False-negative IgM tests can result from: (1) an absent, low, or delayed IgM response, especially in immunologically immature hosts (e.g., during infancy, congenital CMV or HIV infection) or in immunosuppressed patients (e.g., patients with AIDS);[48,49] or (2) presence of high-titer IgG antibody (precluding binding of IgM).[20] Many commercially available kits contain reagents to adsorb IgG from the test serum or use a background substraction step, thus reducing the possibility of interference.[50,51]

When using IgG antibody tests to determine susceptibility or immunity to a particular virus, the sensitivity of the method is important. Generally, complement fixation (CF) antibody titers quantitatively are lower than hemagglutination inhibition (HAI) titers and can disappear after several years. Therefore, CF should not be used for determining susceptibility or immunity.

The major advantages of serologic diagnosis of acute viral infection include noncritical specimen handling and wide availability. Disadvantages include: (1) requirement for acute and convalescent sera for IgG antibody tests; (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. Because of the many confounding factors (e.g., passive transfer of antibodies from mother to infant, receipt of immunoglobulin, immunocompromised) serologic results always should be interpreted within the context of the clinical situation. Whenever possible, serologic diagnosis should be confirmed with the use of viral isolation or direct detection of virus antigens or nucleic acids.

Depending on the serologic assay, either serum and/or plasma can be used. The use of other specimen types has not been well validated for most viruses. Some exceptions include the use of saliva for the detection of HIV antibodies and CSF in patients with viral central nervous system (CNS) disease.[52,53]

Optimal Tests for Specific Viruses

Table 287-2 lists the medically important viruses, major attributable diseases, optimal diagnostic specimen(s), available tests, and average time to a positive test result. For many tests, the time to obtain a result may be a function of the test itself (e.g., culture), the logistics of laboratory testing schedules, or the need to refer a sample to a reference lab. The preferred test provides the most rapid result with acceptable sensitivity (>90%) and specificity (>95%). Serologic tests remain the mainstay for diagnosis of certain virus infections such as the hepatitis viruses. The preferred diagnostic test or tests can vary, depending on the patient population being tested (e.g., immunocompromised hosts).

In the results summarized herein for individual viruses, assessment of sensitivity and specificity of different tests may be based on a variety of parameters and not simply comparison to culture alone.

Herpes Simplex Virus

For diagnosis of suspected mucocutaneous lesions due to HSV, an aspirate or swab (Dacron, rayon, or cotton on aluminum shafts but not calcium alginate or swabs on wood shafts) of the vesicular fluid or ulcer base placed in VTM is recommended. Other potentially useful samples include blood in EDTA for PCR when viremia is suspected (e.g., neonates), CSF in a sterile container when HSV meningitis or encephalitis is suspected, conjunctival swab or corneal scrapings in VTM in suspected cases of herpes keratitis, and tissue biopsy in VTM or frozen (e.g., disseminated HSV in neonates or immunocompromised patients). In infants, duodenal aspirates may also be collected.

The yield on culture varies depending on the tissue culture cell type used in the laboratory,[54] the stage of the clinical infection (greater during vesicular stage than crusted stage),[55] and the type of specimen (including transportation time and conditions). CPE in a sensitive cell line detects 50% of positives in 24 hours, 80% in 48 hours, and 95% in 72 hours.[56] The shell vial method permits detection of HSV with 66% to 99% sensitivity and 100% specificity by 16 to 24 hours.[54,57] ELVIS has sensitivity similar to both standard and shell vial culture.[15] The use of type-specific monoclonal antibodies distinguishes HSV-1 from HSV-2 in culture.

Direct antigen detection tests for HSV have variable sensitivity (47% to 95%) and specificity (85% to 100%).[58,59] None is sufficiently sensitive to reliably detect asymptomatic shedding.[60] Assays using monoclonal antibodies can distinguish HSV-1 from HSV-2.

In CNS HSV infection, the yield of CSF culture is <5% compared with biopsy-proven cases.[61] HSV PCR performed on CSF (sensitivity of 96% and specificity of 99%)[62] is the diagnostic test of choice for HSV encephalitis and meningitis.[61] PCR is positive at least through the first 6 to 7 days of illness, even in patients receiving acyclovir therapy.[61,63] Conversely, because negative results have been obtained in up to 25% of CSF samples from infants and children, HSV PCR alone cannot exclude HSV encephalitis.[64] HSV PCR also is useful with other clinical specimen types,[65] and can distinguish between HSV-1 and HSV-2. The role of quantitative HSV PCR remains unclear, with conflicting results for amount of HSV in CSF and prognostic value.[66,67] However, successful antiviral therapy is associated with a decline in HSV viral load in CSF.[61]

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.[68] 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 because reactivation can cause a rise in IgM levels.[68]

Older HSV antibody tests used crude antigen mixtures and could not reliably distinguish between HSV-1 and HSV-2 IgG antibodies. However, commercially available EIA, Western blot (WB), and immunoblot tests based on glycoprotein G antigen now reliably distinguish type-specific HSV antibodies.[69] The use of HSV-2 type-specific assays has provided important information about the insensitivity of clinical history and the epidemiology of genital HSV infection.[70] Recommendations have been proposed for the appropriate use of HSV-2 serologic tests.[71] No IgM test is commercially available that can distinguish HSV-1 and HSV-2 infection.

Guidelines for standardization of in vitro susceptibility testing of HSV have been published.[72] Resistance to acyclovir and other drugs has emerged as a clinical problem in immunocompromised patients receiving prolonged courses of continuous or intermittent suppressive therapy.[73,74] PCR, together with sequence analysis of the DNA polymerase and thymidine kinase genes, can be used to detect mutations conferring drug resistance and have significantly reduced the time for results.[75] However, this approach is limited by the fact that one can only interpret the presence of mutations that have been associated with HSV antiviral resistance. The significance of new or novel mutations requires confirmation.

Cytomegalovirus

CMV can be detected in a variety of clinical specimens by isolation, antigen detection, DNA probes, or NAATs.[18,76–79] It often is difficult to distinguish between asymptomatic shedding (from urine, cervical secretions, semen, saliva, and respiratory tract secretions) and active CMV disease. Isolation of CMV from tissues is good evidence of active infection. The preferred specimen(s) and test(s) for detection and diagnosis of CMV depends on the clinical syndrome and immune function of the patient (see Chapter 206, Cytomegalovirus).[18,80,81]

The shell vial method significantly shortens the detection time for CMV compared with conventional culture. To enhance detection of CMV in various clinical specimens, multiple shell vials should be inoculated (2 for urine, tissue, and BAL and 3 for blood specimens) with staining at 24 hours and 48 hours, and (for blood specimens) observed for CPE for 10 days.[82,83]

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 also is confounded by asymptomatic respiratory tract shedding. 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 of isolation from BAL fluid is 70% to 95% and the specificity 50% to 100%.[84,85] Demonstration of CMV antigen in cells from BAL specimens by direct fluorescent antibody (DFA) staining may be more specific for CMV infection, but sensitivity is reduced.[84] Histologic examination of cells obtained by BAL for the presence of characteristic CMV 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 and other immunocompromised patients.[86,87] However, the lack of sensitivity of culturing CMV from WBCs has led to the development of the CMV antigenemia assay (an immunocytochemical assay that detects the 65-kd lower-matrix phosphoprotein (pp65)

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	NP aspirate/wash, NP swab, throat swab, BAL, lung tissue	Culture[c]	6 days
			PCR	1–2 days
			Antigen detection/FA	2 hours
		Serum	IgG antibody[d]	1–5 days
Eye	Conjunctivitis	Conjunctival swab or scraping	Culture[c]	7 days
			Antigen detection	2 hours
		Serum	IgG antibody[c]	1–5 days
Intestinal (types 40/41)	Gastroenteritis	Stool	Antigen detection	2 hours
			EM	2 hours
Urinary bladder (immunocompromised host)	Hemorrhagic cystitis	Urine	Culture	6 days
			PCR[c]	1–2 days
			EM	2 hours
ARBOVIRUSES				
SLE, California, WEE, EEE, WNV	Fever, meningoencephalitis	Serum, CSF	IgG and IgM antibody[d]	1–5 days
Colorado tick fever	Fever, malaise, neutropenia	Serum	IgG antibody	7 days
Dengue	Febrile illness +/– rash, hemorrhagic fever	Serum	IgG and IgM antibody[c]	1–5 days
			PCR	1–2 days
CHLAMYDIA/CHLAMYDOPHILA				
Chlamydia trachomatis				
Genital	Urethritis, proctitis, cervicitis, salpingitis, pelvic inflammatory disease	Urethral, cervical swab, first-void urine, self-collected vulvovaginal swab, rectal mucosal swab	NAAT[c]	2–6 hours
			Antigen detection	4 hours
			DNA probe	4 hours
			Culture	48–72 hours
Neonatal	Conjunctivitis, pneumonitis	Eye swab, NP aspirate/wash	NAAT[c]	2–6 hours
			Antigen detection	4 hours
			Culture	48–72 hours
Sexual abuse, rape	Vaginitis, urethritis, proctitis	Cervical, urethral, rectal mucosal swab	Culture[e]	48–72 hours
Chlamydophila pneumoniae (TWAR)	Pneumonia, pharyngitis, bronchitis	NP aspirate/swab, throat swab/wash	Culture[c]	4 days
			Antigen detection	4 hours
		Serum	IgG and IgM antibody	1–5 days
Chlamydophila psittaci	Pneumonia	NP aspirate/wash, throat swab/wash	Antigen detection	4 hours
			Culture	2 days
		Serum	IgG antibody[d]	1–5 days
CYTOMEGALOVIRUS				
Congenital	Hepatosplenomegaly, thrombocytopenia, microcephaly, hearing loss, chorioretinitis	Urine, throat swab, EDTA blood, serum, amniotic fluid	Shell vial culture with antigen stain[c]	2 days
			Culture	3–4 weeks
			NAAT[f]	2–5 hours
			IgG and IgM antibody[c]	1–2 days
Postnatal infection	Heterophile-negative infectious mononucleosis	Throat swab, urine, EDTA blood	Shell vial culture with antigen stain[c]	2 days
			Culture	3–4 weeks
		Serum	IgG and IgM antibody[d]	1–2 days
Immunosuppressed patients	Pneumonitis, colitis, retinitis	EDTA blood	Antigenemia assay[c]	4–6 hours
			NAAT[c,f]	2–5 hours
		Bronchoalveolar lavage, rectal swab, vitreous fluid, tissue biopsy	Shell vial culture with antigen stain[c]	2 days
			Culture	3–4 weeks
			NAAT[c,f]	2–5 hours
Pretransplant screening/ immune status	Past infection (donor and recipient)	Serum	IgG antibody	1–2 days

Continued

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

Agent/Type or Site of Infection or Host	Major Diseases	Optimal Specimens	Available Tests[a]	Average Time to Positive Results[b]
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, rectal swab, EDTA blood, pericardial fluid, myocardium	Culture	4–7 days
			PCR[c,f]	6 hours
		Serum	Neutralizing[d,g] antibody panel (coxsackie B virus, echovirus and poliovirus)	5 days
EPSTEIN–BARR VIRUS				
Healthy individual	Mononucleosis syndrome	Serum	Slide agglutination test (monospot)[c]	1–3 days
			EBV-specific IgG and IgM antibody[d]	1–3 days
Immunocompromised	Posttransplant lymphoproliferative disease (PTLD)	Serum, plasma, whole blood, leukocytes	PCR (quantitative)[f]	2–5 hours
GASTROINTESTINAL VIRUSES				
Rotaviruses, caliciviridae (norovius and sapovirus), enteric adenoviruses, astroviruses	Gastroenteritis	Stool	EM[c] (rotavirus and enteric adenovirus)[c]	2 hours
			PCR[f]	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	Culture[c]	2 days
HEPATITIS VIRUSES				
Hepatitis A	Acute	Serum	IgM antibody	1–2 days
	Immunity	Serum	Total (IgG and IgM) antibody or IgG antibody	1–2 days
Hepatitis B	Acute	Serum	HBsAg, anti-HBc IgM	1–2 days
	Chronic	Serum	HBsAg, anti-HBc total antibody	1–2 days
		Serum/plasma	NAAT for HBV DNA (quantitative)[f]	1 week
	Immunity	Serum	HBsAb	1–2 days
Hepatitis C	Acute	Serum	Anti-HCV EIA screen	1–2 days
			Anti-HCV RIBA supplementary	5 days
	Chronic	Serum/plasma	NAAT for HCV RNA (quantitative/ qualitative)[f]	1 week
Hepatitis D (only occurs in patients with HBV coinfection/ superinfection)	Acute	Serum	HDV Ag, anti-HDV IgM	1–8 days
	Chronic	Serum	HDV Ag, anti-HDV total	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 in VTM	Shell vial culture with antigen stain[c,h]	16–24 hours
			Antigen detection (FA)	2 hours
			NAAT[f]	2–5 hours
Past infection	Recurrent genital symptoms but culture negative	Serum	IgG (group- or type-specific) antibody[d]	1–2 days
Neonatal infection	Disseminated disease; hepatitis; pneumonitis; encephalitis; skin, eye, mouth ulcers or vesicles	Swab of lesion(s), EDTA blood, CSF, conjunctiva/nose/mouth swab, rectal swab	Shell vial culture with antigen stain[c,h]	16–24 hours
			Antigen detection (FA)	2 hours
			PCR	2–5 hours
		Serum	IgG and IgM antibody[d]	1–2 days

Continued

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

Agent/Type or Site of Infection or Host	Major Diseases	Optimal Specimens	Available Tests[a]	Average Time to Positive Results[b]
Ocular herpes	Conjunctivitis, keratitis	Conjunctival or corneal swab or scraping in VTM	Shell vial culture with antigen stain[c,h]	16–24 hours
			Antigen detection (FA)	2 hours
			PCR	2–5 hours
Brain/Meninges	Encephalitis,[i] meningitis	CSF, brain biopsy[i]	PCR[c,f]	2–5 hours
			Antigen/antibody in CSF	2 hours
			Shell vial culture with antigen stain[h]	16–24 hours
		Serum	IgG and IgM antibody[d]	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 PBMC	PCR[f]	1–2 weeks
HUMAN IMMUNODEFICIENCY VIRUS				
Suspected HIV infection in adult or older child	Symptomatic or asymptomatic	Serum	Screening HIV EIA[c]	1–2 days
			Confirmatory Western blot or IFA	1–3 days
			HIV p24 antigen, NAAT[i]	2–4 days
Newborn	Suspected vertical or perinatal transmission	Serum	Screening HIV EIA	1–2 days
			Confirmatory Western blot or IFA	1–3 days
		EDTA blood	Virus culture	2–3 weeks
			NAAT[c,f,i]	1 week
OTHER VIRUSES				
Human metapneumovirus	Upper respiratory illness, bronchiolitis, pneumonia, croup	NP aspirate/wash, nasal/throat swab, BAL	PCR (including multiplex assays for respiratory viruses)[f]	1 day
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	PCR (including multiplex assays for respiratory viruses)[c,f]	1 day
			Antigen detection for influenza A and B	30 minutes–2 hours
			Culture[b]	7–9 days
Measles virus	Measles	NP aspirate/wash	Culture[c]	5 days
		Throat swab	Antigen detection[c]	2 hours
		Serum	IgG and IgM antibody[d]	1–2 days
Mumps virus	Parotitis, aseptic meningitis, meningoencephalitis	Urine, throat swab, saliva, CSF, blood	Culture	8 days
		Serum	IgG and IgM antibody[d]	1–2 days
Parainfluenza viruses	Croup, pneumonitis, bronchiolitis	NP aspirate/wash	Culture[c]	4–7 days
			Antigen detection using FA	2 hours
Parvovirus B19	Erythema infectiosum Aplastic crisis, congenital, hydrops fetalis	Blood, serum, bone marrow, amniotic fluid cells, placental tissue, cord	IgG and IgM antibody[d] PCR	2 days 2 days
Polyomavirus (JC and BK)	JC virus – progressive multifocal leukoencephalopathy (PML)	CSF	PCR	1 week
	BK virus – polyomavirus-associated nephropathy (PVAN)	Urine	PCR (quantitative)	1 week
Rabies virus	Encephalomyelitis Immune status post-vaccination	Postmortem CNS tissue, Antemorem nuchal biopsy Serum, CSF Saliva (antemortem) Serum, CSF	Direct antigen detection (DFA, IHC, DRIT)[c] IgG and IgM antibody[d] (IFA) Culture RT-PCR	24 to 72 hours 2 weeks 2 weeks 24 to 72 hours 2 weeks

Continued

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

Agent/Type or Site of Infection or Host	Major Diseases	Optimal Specimens	Available Tests[a]	Average Time to Positive Results[b]
Respiratory syncytial virus	Bronchiolitis, pneumonia, croup	NP aspirate/wash/swab, throat swab/wash, BAL	Antigen detection[c]	15 minutes–4 hours
			Shell vial with antigen staining	16–48 hours
			Culture	3–7 days
		Serum	PCR (including multiplex assays for respiratory viruses)	1 day
Rhinovirus	Common cold	NP aspirate/wash	Culture	7 days
Rubella	Acquired or congenital rubella	Serum	IgG and IgM antibody[d]	1–2 days
		Throat swab	Culture	5–7 days
VARICELLA-ZOSTER VIRUS				
Skin, disseminated	Chickenpox, herpes zoster, occasional CNS complications	Vesicle fluid, scraping of base of vesicle in VTM, CSF	Antigen detection[c]	2 hours
			Culture	3–7 days
			PCR[c,f]	1 day
		Serum	IgG and IgM antibody[d]	1–2 days
Immune status	Past infection or vaccination	Serum	IgG antibody	1–2 days
MYCOPLASMA PNEUMONIAE	Pneumonia, pharyngitis, Stevens–Johnson syndrome, meningoencephalitis	Throat swab	Culture	3 weeks
		CSF	PCR[f]	4–6 days
		Serum	IgG and IgM antibody[c]	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; EM, electron microscopy; FA, fluorescence antigen detection; 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; NAAT, 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.*

of CMV directly in neutrophils) and a variety of NAATs using WBCs, plasma, serum, or whole blood.[18,88–90] These assays are most widely used in immunocompromised 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.[91–94] These assays are used in pre-emptive treatment strategies, as well as for monitoring response to anti-CMV therapy. However, because of variability among commercial as well as in-house quantitative CMV assays, the exact level of CMV DNA or antigenemia that should be used to initiate pre-emptive therapy is not well established. Potential problems with these assays include inhibition of PCR when heparin is used as the anticoagulant,[12] false-negative findings with the CMV antigenemia assay when processing of blood samples is delayed beyond 4 to 6 hours,[95] cost, the need for technical expertise, and labor intensity (e.g., CMV antigenemia). Neither assay 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. However, IgM antibodies can be detected in both primary and reactivated CMV infections and can persist for months. False-positive CMV IgM results can occur in patients with acute EBV infection, as well as in patients with high levels of rheumatoid factor in the presence of CMV-specific IgG.[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 in up to 30% of cases and thus a negative result does not exclude congenital infection.[80]

The major use of CMV IgG serology is to determine susceptibility to infection in healthcare or childcare workers[97] and to identify the CMV status of blood and organ/tissue donors and recipients.[98] In pregnant women, CMV-specific IgG avidity assays may be of value.[81] The presence of low-avidity IgG anti-CMV may be a better predictor of recent infection than IgM alone, thus increasing the likelihood of CMV transmission to the fetus. However, substantial variability in performance of different CMV avidity assays precludes clear guidance on use and interpretation.[99] Additional testing such as PCR or virus isolation from amniotic fluid may be required to confirm infection of the fetus.

Standardization of in vitro CMV antiviral susceptibility testing has not been established despite utility in immunocompromised

patients in whom resistance correlates with clinical failures.[100] Phenotypic assays are limited by lengthy turnaround time and expertise required for performance. Genotypic assays can detect mutations in the CMV UL97 phosphotransferase gene and the UL 54 DNA polymerase gene conferring antiviral resistance but are not available widely.

Epstein–Barr Virus

In patients with suspected EBV mononucleosis (IM), heterophile antibody remains the serologic test of choice.[101] These IgM antibodies can be detected easily and rapidly using a simple spot agglutination assay (often referred to as a "monospot") or immunochromatographic assays.[102]

Heterophile antibodies develop in approximately 80% to 85% of adolescents and adults with EBV (IM)[101] within 2 to 3 weeks after the onset of illness. Responses can be delayed in some individuals; repeat testing may be required. The heterophile test can be negative in 70% to 80% of EBV infections in children <4 years of age.[102] Heterophile antibodies usually disappear within a few months but can persist for >1 year after acute illness in 20% to 70% of patients[48] and persistence should not be interpreted as recurrent or chronic IM. Cases of heterophile-negative IM in school-aged children are due to CMV in 70% and EBV (proven by EBV-specific serology) in 16%.[103]

EBV serology is indicated when the diagnosis of EBV infection is strongly suspected but the heterophile test is negative.[104] The immunofluorescence antibody (IFA) test is considered the "gold standard" although EIA and immunoblot assays can be used.[101] IFA tests have more uniform performance characteristics, whereas EIAs can vary because of the wide variety of antigen preparations used in different kits. The most useful diagnostic test is IgM anti-EBV viral capsid antigen (VCA), which appears within 1 to 2 weeks after the onset of symptoms, disappears within months, and is 91% to 98% sensitive and 99% specific.[48,101,104] 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. IgG anti-VC is elevated during symptoms of illness and can persist for life, and thus is less useful for the diagnosis of acute infection but remains the most reliable marker of EBV seropositivity. IgG anti-early antigen (EA) rises early, whereas IgG anti-EBNA (Epstein–Barr nuclear antigen) appears late (generally after 6 weeks) and persists for life. Several months after recovering from IM, an individual is expected to have IgG antibodies to VCA and EBNA, but low or absent VCA IgM antibodies as well as low or absent antibodies to EA[101] (see Figure 208-3).

Direct tests for EBV, such as cultivation in cord blood leukocytes, direct detection by immunofluorescence staining, or detection of DNA,[105,106] are performed in some laboratories. EBV can be isolated from oropharyngeal washings or circulating lymphocytes of 80% to 90% of patients with IM. PCR detection of EBV DNA in the CSF of patients with HIV infection is strongly associated with primary CNS lymphoma.[106] Following organ and marrow transplantation, the use of quantitative EBV PCR using blood specimens may help predict the development of posttransplant lymphoproliferative disease.[107] Relative merit of testing whole blood, leukocytes, plasma, or serum is unclear. Elevated levels of EBV DNA in peripheral blood may be an indication to decrease immunosuppressive therapy or to consider therapies such as $CD20^+$ monoclonal antibodies or EBV-specific cytotoxic T lymphocytes.[107]

Rarely, EBV infection is associated with an acute fulminant disease (e.g., X-linked lymphoproliferative syndrome and virus-associated hemophagocytic syndrome).[108] Persistent high-titer EBV antibodies, except against EBNA, are characteristic but may be absent. The diagnosis depends on detection of virus or its genome.

Varicella-Zoster Virus

The diagnosis of chickenpox or herpes zoster (shingles) usually can be made clinically. In selected circumstances, 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[109–114] may be useful. Skin lesions <4 days old are more likely to yield virus than older ones. Because VZV is extremely labile, transport of samples to the laboratory should occur within 12 hours of collection. Direct detection of VZV antigens by FA of smears from lesions is more sensitive than culture and is the preferred method for diagnosis of VZV skin lesions.[113] 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.

PCR for detection of VZV DNA compared with culture or FA has advantages of increased sensitivity, in scrapings of older lesions, and ability to distinguish vaccine- versus wild-type VZV. PCR analysis of CSF can confirm the etiology of CNS syndromes 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] Multiplex PCR assays capable of detecting VZV as well as other herpesviruses have been evaluated and may simplify the diagnosis in patients with overlapping clinical syndromes (e.g., vesicular rash).[33]

IgG anti-VZV is used primarily to assess susceptibility to infection, to determine the need for vaccination or risk of disease in exposed individuals, and to determine the duration of protection post-vaccination.[116,117] During acute VZV infection, VZV antibodies appear within a few days after the onset of rash and peak 2 to 3 weeks later. A >4-fold rise in IgG antibody between serum collected 10 to 14 days apart or the detection of VZV-specific IgM antibodies in a single sample supports a diagnosis of acute infection. However, serologic diagnosis can 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.[118] Fluorescent antibody against membrane antigen (FAMA) is considered the gold standard for detection of VZV antibodies.[117] Detection of neutralizing antibodies to VZV in healthy individuals by FAMA 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] EIA assays may have lower sensitivity when compared with FAMA and latex agglutination assays particularly in detecting antibodies post-vaccination.[117] Newer glycoprotein (gp) EIAs appear to have improved sensitivity over older ones.

Human Herpesvirus Types 6, 7, and 8

Primary infection with human herpesvirus 6 (HHV-6) occurs in most children before the age of 2 to 3 years and routine lab testing usually is not performed. Detectable antibodies in primary infection generally appear 3 to 8 days following onset of fever. The following serologic criteria are considered diagnostic of primary HHV-6 infection: (1) antibody seroconversion between acute- and convalescent-phase serum/plasma specimens collected 2 to 4 weeks apart; (2) detection of HHV-6-specific low-avidity IgG antibodies; (3) positive serum IgM in the absence of IgG antibodies; and (4) >4-fold rise in IgG antibody by IFA or anticomplementary immunofluorescence assays.[121] Current commercial assays for IgG anti-HHV-6 do not distinguish between variants A and B and can cross-react with HHV-7 and CMV.[122,123] Antibody avidity testing can be used to differentiate primary HHV-6 from HHV-7 infections. IgM anti-HHV-6 alone is not a reliable indicator of acute or recent infection because IgM also can be found during reactivation or reinfection and approximately 5% of adults have detectable IgM anti-HHV-6 at any time.[121] IgM may not be detectable in some culture-positive children.[122,124] During acute primary HHV-6 infection, virus can be recovered from cultures of peripheral blood mononuclear cells (PBMCs) in 100% of infants, but not after recovery,[125] whereas HHV-6 DNA can be detected by PCR during both acute illness and after recovery.[123,126] Monoclonal antibodies are available for direct detection of HHV-6 antigen and have been

used to confirm cell culture CPE and for immunohistochemical staining of tissues.

In immunosuppressed patients, HHV-6 infection can be associated with significant morbidity and mortality.[127,128] Proof of HHV-6 causation is difficult because specific antibodies can be absent and demonstration of viral DNA in PBMCs can represent latent infection. Although PCR detection of HHV-6 DNA in serum/plasma has low sensitivity, it may be a better marker for 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.[129,130]

Serologic tests for HHV-7 are not available widely. Some degree of cross-reaction between HHV-6 and HHV-7 antibodies occurs due to cross-reactive epitopes on the viruses.[122] 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[131] as well as from ill individuals, questioning the value of such testing. 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 diagnostic value.[132] Specific primers for PCR amplification of HHV-7 have been developed that do not amplify the DNA from any other human herpesviruses and have been included in a multiplex assay.[133,134]

Testing for HHV-8 is only available in research settings. PCR has been used for detection of HHV-8 DNA in PBMCs and tissues.[135] The use of plasma/serum for HHV-8 PCR has no value for identifying active infections.[121] Serologic assays can detect IgG- but not IgM-anti HHV-8. Useful for seroprevalence studies, the role of serologic tests in diagnosing and managing HHV-8 infections has not been established.[136]

Respiratory Syncytial Virus

NP wash or aspirate is superior to swab sampling for detection of RSV infection. Bronchoalveolar lavage (BAL) and endotracheal tube (ETT) aspirates also are acceptable. Specimens for culture or FA testing should be transported on wet ice or refrigerated as soon after collection as possible as there is substantial loss of cell culture infectivity at room temperature. Samples for antigen detection can be transported at room temperature. Culture for RSV requires a mean of 3 to 7 days. Shell vial culture appears to have a slightly greater sensitivity than standard culture.[137] Culture has the advantage of detecting other respiratory viruses that are recovered from 5% to 10% of specimens submitted for diagnosis of RSV infection. The use of mixed fresh cells has proven to be a rapid and sensitive method for detection of RSV. The sensitivity of antigen detection techniques such as EIA microtiter plate kits, membrane filter EIA, and DFA range from 84% to 96%, with specificity of 92% to 96%.[42,138–140] The membrane filter EIA offers the advantage of providing a result within 15 to 20 minutes.[138,139,141] Some assays can detect multiple respiratory viruses simultaneously.[142] In general, rapid antigen detection tests for RSV have a relatively lower sensitivity in adults than children, which likely reflects the decreased amount and duration of shedding of RSV in respiratory secretions of adults.

The use of serologic tests for the diagnosis of acute RSV infection has little clinical value. In primary RSV infection, detectable IgM antibodies appear approximately 5 to 9 days after onset of symptoms and persist for several weeks. The antibody response may be poor or absent in very young infants, older individuals with repeat infections, and immunocompromised patients.[143] RSV antibody detection may be useful for epidemiologic purposes and for evaluating responses to candidate RSV vaccines.[144] NAATs improve the detection of RSV in respiratory tract specimens and have been used to distinguish between RSV subgroups A and B during community and institutional outbreaks.[145] Multiplex PCR assays capable of detecting several respiratory viruses in the same test have been evaluated.[138,146]

Influenza Viruses

Clinical samples for the detection and isolation of influenza viruses should be collected within 3 days of symptom onset when virus shedding is maximal. Transport to the laboratory should be as prompt as possible and specimens can be stored at 4°C if processing will be delayed beyond 3 to 4 days. Standard tube culture for isolation of influenza viruses requires 3 to 5 days. Shell vial shortens the time for detection to 48 hours but may not be as sensitive as standard culture.[147] Serotyping of influenza A and B viruses isolated in culture can be achieved by inhibition of hemagglutination using serotype-specific antiserum.

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.[148–150] Evaluations of rapid tests for the detection of seasonal influenza virus as well as the pandemic 2009 H1N1 virus indicate relatively poor sensitivity but high specificity.[147,149,150] These tests have not been evaluated fully 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 a sensitivity of 80% to 90% and specificity of >90%.[151–153] 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 including multiplex respiratory virus assays have been evaluated in several studies and show a substantially increased sensitivity compared with other methods, including culture.[42,156–158] Multiplex assays capable of detecting influenza A (including pandemic 2009 H1N1) and B, antiviral resistance mutations (particularly the H275Y substitution conferring resistance to oseltamivir), and multiple other respiratory viruses are available.[156–160]

Other Respiratory Viruses

Numerous other viruses including human parainfluenza viruses types 1, 2, 3, and 4, adenoviruses (subtypes A to E), rhinoviruses, human coronaviruses 229E, OC43, severe acute respiratory syndrome (SARS) coronavirus, and human metapneumovirus (hMPV) can infect the respiratory tract and cause clinical signs and symptoms indistinguishable from influenza and RSV. 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. Most laboratories do not routinely attempt isolation of rhinoviruses. No routine culture methods are available for isolation of coronaviruses or hMPV. DFA staining is available for parainfluenza viruses and for adenoviruses.[161] 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 no value for the diagnosis of acute infection with these viruses. Several of the previously discussed multiplex molecular assays for the detection of respiratory viruses can detect many of these other respiratory viruses.[160]

Hepatitis Viruses

Routine diagnosis for all hepatitis viruses is based on serology. Serum or plasma can be used for most assays and should be separated from blood within 24 hours of collection. The diagnosis of acute HAV is made by demonstration of IgM anti-HAV.[162] Immunity to HAV following natural infection or immunization is determined by measuring hepatitis A IgG or total (IgG and IgM) anti-HAV.[162] Currently, there is no role for reverse transcription (RT)-PCR measurement of HAV RNA for routine diagnosis. In acute and chronic HBV infection both HBsAg and anti-hepatitis B core antibody (HBcAb) usually are present.[163] IgM anti-HBc generally is present in acute HBV infection and occasionally during a flare of inflammation in chronic carriers. Thus, IgM anti-HBc does not always distinguish acute from chronic infection. By definition, a person with persistently positive HBsAg for >6 months is

considered chronically infected. Isolated anti-HBc positivity can occur during: (1) acute infection between the loss of detectable HBsAg and emergence of detectable HBsAb ("core window"); (2) late chronic infection when HBsAg levels have fallen below detectable levels; (3) coinfection with HCV or HIV that suppress HBsAg production; (4) infection with a mutant HBV; or (5) a false-positive result. The role of quantitative HBsAg assays is being evaluated for monitoring patients with chronic hepatitis B infection. The presence of HBV e antigen (HBeAg) and the absence of anti-HBe are markers of greater infectivity and correlate with increased risk of progression to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[163] The presence of anti-HBe is an indicator of likely recovery. The presence of HBsAb at a level >10 IU/mL is considered to protective against acute infection. HBsAb levels can decline below 10 IU/mL after 10 to 12 years in a substantial number of vaccine responders. These individuals remain protected from acute infection likely as a result of immune memory.[164] The presence of HBsAb alone reflects prior immunization whereas the presence of HBsAb together with HBcAb reflects recovery from previous natural infection.[163]

For diagnosis of HCV infection, second- and third-generation EIA and supplementary recombinant immunoblot assay (RIBA) using recombinant structural proteins are available widely.[165,166] Seroconversion occurs by 8 to 12 weeks following acute infection, with sensitivity of 94% to 100% (except in immunosuppressed individuals) and specificity of >97% after the supplementary RIBA test.[32] HCV antibody (anti-HCV) frequently is negative at the onset of jaundice. The presence of HCV antibodies indicates current infection in most patients. No assay is available currently for the detection of IgM anti-HCV. The utility of measuring HCV antigen in serum or plasma has not been established.

Molecular assays for the detection and quantification of HBV and HCV viral nucleic acid in serum are useful for determining prognosis, selecting candidates for therapy, and monitoring response to therapy.[165,167,168] A lower baseline 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, whereas nonresponders do not. Molecular assays also are available for HBV and HCV genotyping and antiviral resistance testing.[169] HCV genotyping is useful for epidemiologic purposes and to identify patients most likely to respond to therapy. The role of HBV genotyping is less well established. NAATs that detect HCV RNA in serum 1 to 3 weeks after exposure are being used as part of blood and organ/tissue donor screening and in patients with indeterminate HCV antibody results when the RIBA is inconclusive.

In most clinical situations, testing for hepatitis A, B, and C can be grouped into one of three categories: (1) *acute hepatitis;* (2) *chronic hepatitis;* and (3) *immune status/previous exposure*. For suspected *acute hepatitis,* initial testing for IgM anti-HAV, HBsAg, and anti-HCV should be performed. If all 3 are negative, IgM anti-HBc should be tested. Repeat testing for anti-HCV is recommended in 3 to 4 weeks. In situations where *chronic hepatitis* is suspected, testing should include HBsAg and anti-HCV. Some also may test for anti-HBc. Patients being screened for *immunity* and/or *previous infection* should have the following tests: (1) total or IgG anti-HAV; (2) HBsAb or HBcAb or both (depending on whether previous infection is suspected); and (3) anti-HCV (which is a marker of previous infection and not immunity).

Serologic tests are available for both hepatitis D (delta agent) (HDV) and hepatitis E viruses (HEV) but none have been approved by the Food and Drug Administration (FDA).[170] Because infection with HDV occurs solely in conjunction with HBV infection, testing for anti-HDV only should be performed in patients acutely or chronically infected with HBV. During coinfection with HBV and HDV, anti-HDV disappears within months following recovery from acute infection. However, in HDV superinfection of a HBV chronically infected patient, anti-HDV generally persists indefinitely as infection becomes chronic in most cases. Measurement of HDV RNA by RT-PCR remains a research test. Both IgG and IgM anti-HEV can be measured using research or commercial assays.[171] Due to the use of different antigens, assays show significant variability in sensitivity and specificity.[172] IgG anti-HEV is positive in most patients 1 to 4 weeks after the onset of disease and becomes undetectable by 3 months. IgG anti-HEV typically declines after infection. In areas where HEV is not endemic, RT-PCR may prove useful as a confirmatory test.

Gastroenteritis Viruses

Stool samples placed in a clean sterile container without VTM or preservative for the detection of enteric viruses should be collected within the first 48 hours of illness. Rectal swabs may not contain sufficient virus for EM detection. Stool specimens are stable at 4 °C for up to 1 week. Although freezing at −70 °C can permit prolonged storage, EM detection is reduced by repeated freezing and thawing that destroys the morphology of viral structures. None of the enteric viruses can be cultivated readily in conventional cell culture systems, but all can be detected by EM. Commercial EIA, latex agglutination and membrane-based tests with >95% sensitivity and specificity are available for detection of rotaviruses, noroviruses, enteric adenoviruses, and astroviruses.[19,20,173] PCR-based assays for these viruses are becoming available in many state health departments.[41,174] PCR-based assays are now the method of choice for diagnosing enteric viruses, particularly rotaviruses and caliciviruses. There is no role for serologic testing for enteric viruses except during outbreak investigations.

Enteroviruses

Enteroviruses generally are stable and survive in the environment for weeks; rapid transport of clinical specimens to the laboratory is not critical. Enterovirus viability decreases slowly over days to weeks at room temperature and is preserved for decades at −70 °C. Appropriate specimens include CSF, serum or whole blood, pericardial fluid, tissue biopsies (e.g., myocardium), urine, stool, and rectal, nasal and throat swabs. Although many enteroviruses can be grown in cell culture, some serotypes (e.g., coxsackievirus A groups 1, 19, and 22) fail to grow in standard cell culture. Isolation of enterovirus requires 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%). Due to the lack of a common antigen among enteroviruses, immunoassays for direct detection are not available. EM is not useful for diagnosis because of the low numbers of viruses in most clinical samples.

RT-PCR has been used to test CSF, cardiac tissue, pericardial fluid as well as serum and has significantly improved the speed of detection of enteroviruses, with reported sensitivity of 81% to 100% and specificity of 92% to 100%.[176,177] In comparison, culture has a sensitivity of only 40% to 60%.[175] Detection in urine samples is poor, probably due to nonspecific inhibitors of PCR.[178] In respiratory specimens, cross-amplification of some rhinoviruses can occur.

Clinically, the detection of enteroviruses must be interpreted cautiously. Asymptomatic shedding of wild enterovirus from the gastrointestinal tract can occur for weeks or months. Additionally, oral polio vaccine virus can be shed in stool and, less commonly, in the throat of young vaccinated children. Detection of virus in CSF, the genitourinary tract, tissue, or blood is proof of a causative role.

Measuring antibody titers for enteroviruses is of limited diagnostic value. A separate neutralization assay must be performed for each enterovirus subtype.

Measles, Mumps, and Rubella (MMR)

The laboratory diagnosis of MMR viruses can be made by virus isolation, detection of antigen, the use of RT-PCR, or serologic testing. Suitable samples for isolation or detection of viral antigen include whole blood (particularly PBMCs for the isolation of measles), 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 4 °C prior to processing, but may be frozen at −70 °C if a delay >48 hours is anticipated. Isolation of virus from blood is greatest 3 to 5 days before rash onset and declines rapidly within 2 to 3 days thereafter. Conjunctival and NP samples for isolation of measles virus can be collected 2 to 4 days before and up to 4 days after the onset of rash. Throat swabs for rubella virus isolation usually are positive (~90%) if collected on the day of rash onset but rapidly become negative within 4 days. Mumps virus can be isolated from saliva 9 days before and up to 8 days after the onset of parotitis. 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] The shell vial method for measles virus has a sensitivity of 78% at 1 to 2 days and 100% at 5 days. Sensitivity of DFA staining of NP swab specimens for measles virus antigen is 100% compared with culture, but only 67% for throat swabs and 85% for urine specimens. Shell vial culture 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 and can be used for genotyping to help differentiate wild-type from vaccine-virus strains.[181,182]

Timing of serum specimen collection is critical as many patients do not have IgM antibody at the time of rash onset. For suspected measles virus infection, serum can be collected within 7 to 10 days of rash onset. For rubella virus infection, >90% of patients will have IgM positivity ≥5 days after rash onset. Although the traditional MMR serologic test is HAI for IgG antibody, a number of IFA and EIA IgG and IgM kits are available commercially.[183] With the declining prevalence of these viral diseases, the positive predictive value of IgM tests can be low. The presence of rheumatoid factor can lead to a false-positive IgM result and re-exposure in a previously vaccinated individual or individual with a history of natural infection can result in a secondary IgG or IgM response. Mumps IgM antibody can persist for months after acute illness.[184] Patients with IM,[185] parvovirus B19 infection,[186] measles virus, and CMV infection can have cross-reacting IgM anti-rubella. Similarly infection with parvovirus B19 and rubella virus can result in cross-reacting IgM anti-measles. In pregnant women, IgM anti-rubella should be confirmed with a second IgM assay or detection of a significant rise in IgG andtibodies.[187] Avidity assays for IgG antibodies to measles and rubella viruses are available. Measurement of virus-specific IgG antibodies can be used to determine immune status. For mumps virus, 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, IFA, and WB for HIV antibody, EIA for p24 antigen), culture, and NAATs for the detection of HIV-1 RNA in plasma or proviral DNA in whole blood or PBMCs. Culture for HIV is no longer used for routine diagnosis.[188] NAATs can be used for the diagnosis of HIV-1 infection in neonates with excellent sensitivity and specificity.[189] Screening tests for HIV-1 RNA have become part of routine blood and organ/tissue donor screening programs since 2002,[190] and also can be used for measuring HIV-1 in other specimen types including CSF, cervical secretions, seminal plasma/semen and serum. The use of NAATs in populations who are not known to be HIV-seropositive has yielded false-positive results.[191] The major use of quantitative HIV-1 viral load assays is for monitoring a patient's response to antiretroviral therapy.[28,192] Because of the intra-assay and biologic variability in HIV-1 RNA levels, a >3-fold change is considered clinically relevant. Different molecular assays also can produce significant differences in HIV-1 viral load and thus baseline values should be repeated when the laboratory testing is changed from one assay to another.[192] Some assays yield lower levels in the same patient if serum is used instead of plasma or if blood is collected in acid-citrate-dextrose anticoagulant rather than EDTA. Currently available commercial assays vary in specimen volume requirement (range from 50 µL to 2 mL), lower limit of detection, dynamic range, and time to result.[192] Regardless of assay format, plasma must be separated from the blood cells within 6 hours of collection. None of these assays is approved for use in individuals infected with HIV-2 or HIV-1 group O. Other molecular assays are available for HIV-1 genotyping for the detection of antiretroviral resistance mutations.[193] Resistance testing is recommended prior to initiating therapy and when treatment failure occurs. Phenotypic assays can also be performed for this purpose.

The mainstay of diagnosis for HIV remains HIV-specific serology using screening EIAs or, less commonly, particle agglutination assays followed by confirmatory testing using WB or other assay.[190] Both serum and plasma are acceptable specimens. Testing systems for dried blood spots, urine, and saliva also are available. Early EIA 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, 90% of positive results were false due, most commonly, to cross-reacting antibodies against human leukocyte antigens in the cell lysate used in antigen preparation.[190] False-negative results occurred due to antigenic heterogeneity among HIV strains, particularly group O.[194] Recent EIA kits use more purified viral antigens from cell lysates, recombinant viral proteins, and synthetic peptide antigens and can detect group O. These assays have increased sensitivity and specificity and fewer indeterminate results.[190] Most currently available assays detect IgG and IgM anti-HIV-1 and anti-HIV-2 in the same assay.[195] Fourth-generation screening tests can detect both HIV-1/HIV-2 antibodies and p24 antigen at the same time while reducing the seroconversion window period to approximately 16 days.[190] Detuned (also known as "sensitive/less sensitive") EIAs capable of measuring the affinity of HIV antibodies have been used to distinguish recent from past/distant HIV infection and to estimate incidence rates.[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. Separate WB tests must be used to confirm HIV-1 and HIV-2. WB measures the antibody response to 9 HIV-1 proteins (p) or glycoproteins (gp): gp160, gp120, p66, p55, p51, gp41, p31, p24, and p17 but is prone to give a high rate of indeterminate results due to detection of cross-reacting antibodies and nonspecific reactions.[190] The Centers for Disease Control and Prevention (CDC) criterion for confirmation of HIV-1 infection is presence of antibody to any two of the following: p24, gp41, or gp120/160.[197] No antibody response to HIV-1 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, and if WB results remain indeterminate persons are considered not to be infected.[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]

The IFA test can detect both IgG and IgM anti-HIV-1, is quite sensitive and specific, and can be used as an alternative to WB as a confirmatory test.[190] The line immunoassay (LIA) can be used for confirmation of HIV-1 (including group O) and HIV-2 in a single test. Rapid point-of-care tests for both screening and confirmation requiring minimal or no laboratory equipment have been developed that can yield a result in <30 minutes with comparable sensitivity and specificity to third-generation EIA-based tests and other confirmatory assays.[200]

Different laboratory diagnostic strategies are needed for the 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 an HIV-infected person*.

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 (in duplicate) if the test is positive; and (2) a

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.

confirmatory WB test if the repeat EIA is positive.[190] If the results of serologic testing are indeteminate, additional tests for p24 antigen, HIV DNA or RNA, or culture of PBMCs can be performed.[190] In the setting of high risk and clinical features of infection, p24 antigen test has specificity of 99%.[190] 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.[201]

Confirmation of vertical transmission of HIV using EIA or WB is confounded by the presence of maternal antibodies for up to 18 months of age.[189] In a symptomatic infant >4 to 6 months of age, detection of p24 antigen, or HIV genome and culture of the virus from PBMCs are reliable, definitive tests.[202–204] The sensitivities of culture, NAAT, p24 antigen, and IgA anti-HIV-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.[189,202–208] Although culture is considered the "gold standard" for pediatric HIV infection, NAAT for viral DNA or RNA is more sensitive.

In an individual with *known HIV exposure*, antibody to the virus usually can be detected within 2 to 8 weeks after infection. Based on third-generation 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.[190,209] Virtually all infected, immunocompetent 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]

Arboviruses

For the majority of arbovirus infections laboratory testing generally is not performed. For arboviruses causing CNS disease only a brief, low level of viremia occurs which clears before the patient seeks medical attention.[210] Thus blood specimen for virus isolation and NAAT rarely yield positive results unless collected prior to the neuroinvasive phase of illness. For some arboviruses, including dengue, yellow fever, sandfly fever, Venezuelan encephalitis, and Colorado tick fever, a relatively high level of viremia occurs that can persist for days or weeks making virus isolation or NAAT from blood specimens possible (in reference laboratories). Virus isolation of neurotropic viruses from brain tissue and CSF occasionally is successful during the acute phase of infection; NAAT is more sensitive in these cases.[211]

For most arbovirus infections, the diagnosis is established by IgG seroconversion or detection of specific IgM antibodies, or both.[210,212] Collection of paired acute (collected during the first week of illness) and convalescent (collected 2 to 3 weeks later) sera is recommended. A single sample may be sufficient for diagnosis if a specific IgM test is available (e.g., eastern equine encephalomyelitis, western equine encephalomyelitis, California (La Crosse) virus, St. Louis encephalitis (SLE), West Nile virus, dengue virus). However, in some cases (e.g., West Nile virus), virus-specific IgM can be detected in serum for ≥2 years following infection. For CNS disease, both serum and CSF specimens should be tested. The sensitivity of some of these tests approaches 100% by the 10th day of illness.[213] Traditional assays such as CF and HAI tests largely have been replaced by FA and EIAs.[210] Serologic cross-reactions can occur among antigenically related viruses (e.g., SLE, West Nile virus, Japanese encephalitis, dengue, Powassan, and other flaviviruses). The neutralization test remains the most specific test for serologic diagnosis of arbovirus infections. Neutralizing antibodies are also felt to be the best indicator of protective immunity.

Parvovirus B19

Parvovirus cannot be cultivated in routine cell culture and thus serology (rising IgG titers or presence of IgM antibody) is the mainstay of diagnosis.[214] IgM antibodies are detectable in serum approximately 10 to 12 days after infection, when the rash or joint symptoms begin, and can persist for several months. The sensitivity of IgM anti-parvovirus exceeds 90% in the first month after the onset of symptoms. IgG antibodies appear within several days after IgM and generally persist for years. Current IgG assays have a sensitivity of >90%; IgG indicates past infection. Re-exposure to parvovirus leads to a rise in IgG antibody levels. IgG avidity assays can help distinguish primary from secondary infections.[215] Immunocompromised individuals may not produce antibody; diagnosis can be made by NAAT detection of viral DNA in serum or other specimen types. Parvovirus-associated aplastic crisis, chronic infection, and congenital infection can be diagnosed by PCR analysis of serum.[214–216] PCR also can 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. However, parvovirus DNA may be detectable in serum for months after acute infection and for years in other tissues.[217] Thus the diagnosis of acute or chronic parvovirus infection may require both serology and quantitative PCR.

Other Viruses

The recommended specimens and lab tests for other viruses are listed in Table 287-2. For the majority of these viruses, testing is performed in highly specialized research or reference laboratories.

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 fetal infection.[218] Detection of virus (via culture, antigen detection, or NAAT) 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. Beyond 3 weeks of age, isolation of CMV from urine cannot distinguish congenital from perinatal or postnatal infection. IgM anti-CMV in a newborn is positive in only 50% to 70% of congenitally infected neonates and the test can yield false-positive results.[219]

Congenital VZV infection can be diagnosed by serology. Perinatal or postnatal infection with VZV, as well as with HSV and enteroviruses, usually can be diagnosed by conventional antigen detection or culture techniques, although NAAT testing is preferred for enteroviruses. Serologic diagnosis of neonatal HSV infections is inappropriate because response may not be detectable for 2 or 3 weeks after infection.[220] Demonstration of rubella IgM in a neonate with features consistent with congenital rubella confirms the diagnosis; virus isolation can require 3 to 4 weeks.[218,220,221]

Parvovirus infection during pregnancy can be diagnosed in the mother by serology; detection of IgM or 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 NAAT for viral DNA in fetal blood, amniotic fluid cells, or both.[215,221]

CHLAMYDIA AND *CHLAMYDOPHILA*

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

Chlamydia trachomatis

Specimen Collection and Transport

Because *C. trachomatis* is an intracellular pathogen, optimal specimens for diagnosis are mucosal epithelial cells rather than purulent material. Preferred specimen types vary for testing methods and age groups tested. The following specimens are acceptable for culture: in postpubertal women, a swab or Cytobrush specimen collected from the cervical os; for prepubertal girls, a vaginal swab; for adult males, a urethral swab inserted 3 to 4 cm and rotated; and for boys, a swab of the urethral meatus (if discharge is present).[222] Infants with suspected chlamydial conjunctivitis should have the purulent discharge removed, followed by swabbing or scraping of the palpebral conjunctiva. The yield of culture is related directly to the quality of the specimen and the transport and storage conditions before testing.[223–225] Urine specimens should not be used for culture because of poor sensitivity.

For culture, Dacron-, cotton- or rayon-tip swabs on an aluminum or plastic shaft are recommended. Swabs with wooden shafts and those with a calcium alginate tip may inhibit growth.[226] In females, pooling of urethral and cervical swab specimens increases culture sensitivity by approximately 20%.[223] Swabs should be placed immediately into chlamydial transport media (containing sucrose phosphate or sucrose phosphate glutamate supplemented with bovine serum and antimicrobial agents) at 2°C to 8°C and transported to the laboratory within 24 hours. Some culture transport media also are acceptable for use with NAAT. Freezing at −70°C can result in a 20% loss of viability. Freezing at −20°C should be avoided.

Collection of endocervical and urethral swab specimens for EIA, DFA, hybridization tests or NAAT generally is similar to that for culture and should follow the instructions of the manufacturer. For EIA and DFA testing, swab specimens do not require refrigeration. Swab specimens for NAAT are stable at room temperature for up to 10 days. Urine, vaginal, rectal, NP, or female urethral specimens should not be tested using EIA, DFA, and hybridization tests due to poor sensitivity.[227]

Acceptable specimens for NAATs include endocervical, vaginal, and urethral swabs as well as urine from adolescents and adults.[223] NAATs are not approved for use with specimens collected from extragenital sites. NAAT for any specimen type from children has not been approved. First-void urine (10 to 50 mL) collected into a clean sterile container from men and women and self-collected vulvovaginal swab specimens are acceptable for use with NAATs.[228–233] Urine specimens for NAAT are stable for up to 24 hours at room temperature, after which they may be refrigerated for up to 4 days or stored at −20°C or lower for up to 2 months before processing.

Laboratory Test Methods

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

Serologic tests for *C. trachomatis* genital tract infections are not useful for diagnosis in individual patients.[226] Antibodies to *C. trachomatis* persist for life. In infants, detection of IgM anti-*C. trachomatis* using the microimmunofluorescence (MIF) test is the diagnostic test of choice for chlamydial pneumonia.[223] Maternal IgG antibodies can persist in infants for 6 to 9 months.[226] The MIF test is the most sensitive serologic test and is the only one that detects species- and serovar-specific responses.[223] EIAs for the detection of IgM antibodies in infants have variable performance compared with the MIF test.[226] EIAs detect antibodies to the genus-specific antigen, or lipopolysaccharide (LPS) of chlamydial elementary or reticulate bodies and are not specific for *C. trachomatis.* Interpretation of a single IgG antibody test result is difficult because 50% to 70% of people can have antibodies to *C. pneumoniae.*[234,235] CF tests have been used widely for the diagnosis of psittacosis and lymphogranuloma venereum, but have no value in diagnosing genital tract or neonatal chlamydial infections.

Cell culture has specificity approaching 100%; however it is relatively insensitive compared with NAATs, requires cell culture facilities, and has slow turnaround time (3 to 7 days).[223,226] Barring evaluation using other testing methods, the CDC continues to recommend culture for urethral specimens from women and asymptomatic men, NP specimens from infants, rectal specimens from all patients, and vaginal specimens from prepubertal girls.[222] The shell vial culture method has improved the sensitivity and shortened the detection time (48 to 72 hours) of *C. trachomatis* inclusions.[226]

Diagnosis most often is accomplished by *direct detection* of antigens (EIA or DFA assays) or nucleic acid (hybridization assays), or by cytologic examination for the presence of intracellular inclusions. EIAs use monoclonal or polyclonal antibodies to detect chlamydial LPS and are suited for processing large numbers of specimens; sensitivity generally is less than culture and NAATs. A positive EIA usually requires validation by a second nonculture method, especially in low-prevalence populations.[227] Point-of-care EIAs can provide a result in <30 minutes but their performance is poor and evidence regarding their impact on clinical outcomes is lacking.[236–238] DFA assays using monoclonal antibodies directed against the major outer-membrane protein (MOMP) permit direct visualization of the cellular material obtained (as an assessment of the quality of the specimen) and both elementary bodies and intracellular inclusions can be detected within 30 minutes. However, DFA testing requires a skilled laboratory microscopist, and large numbers of specimens cannot be processed expediently.[239–241] DFA has been used for conjunctival and respiratory specimens from infants. Nucleic acid probes are similar in sensitivity to other antigen detection methods and are relatively specific. However, DNA probe tests (without previous amplification) require special transport media, thus precluding the use of another test on the same specimen to confirm a positive result. DNA probe tests have a sensitivity for male genital secretions inferior to that of other methods. Cytologic examination of direct smears for the presence of intracellular inclusions is useful for detection of chlamydial conjunctivitis in neonates, but not for diagnosing conjunctivitis or genital infection in adolescents.[223]

Three FDA-approved *molecular diagnostic tests* are available for the simultaneous detection of *C. trachomatis* and *Neisseria gonorrhoeae* based on PCR, TMA, and SDA.[226] All have excellent sensitivity and specificity and can be performed in 2 to 5 hours. However, they do not all perform equally well with all specimen types.[242] In 2006, a genetic variant of *C. trachomatis* was identified in Sweden that was undetectable by PCR.[243] Although remaining localized, dissemination to other areas could render the current PCR test useless, highlighting the potential vulnerability of these tests to mutations within the target regions of the organism.

Comparison of Methods

Culture previously was considered the gold standard because of its 100% specificity and excellent sensitivity when optimal techniques are used.[223] However, for genital specimens, its sensitivity is approximately 70% to 80% compared with NAATs, which have become the preferred tests for diagnosis of genital tract infections.[226] For cervical swab specimens, EIA and DFA are less sensitive than hybridization tests and NAATs, whereas NAATs provide the best specificity and positive predictive value. Rapid EIA tests have relatively poor sensitivity using urethral swabs from males and cervical swabs from females and their accuracy for other specimen types has not been well evaluated.[237,238] Testing of first-void urine in men and women by any of the 3 NAATs has a median specificity of >97% and excellent sensitivity resulting in a high positive predictive value.[231]

In infants with conjunctivitis or pneumonitis, testing of conjunctival and NP specimens by culture, DFA, or EIA produces acceptable results. In a study of children ≤13 years of age, SDA and TMA had a sensitivity of 100% for urine specimens and 85% for vaginal swabs.[244] In the same study, the sensitivity of vaginal swab cultures for *C. trachomatis* was only 39%. In cases of suspected rape or sexual abuse, recent studies and guidelines support the use of FDA-approved NAATs when culture is not available; a positive result is confirmed using a different NAAT.[222,245]

Chlamydophila pneumoniae

Accurate laboratory confirmation of acute infection with *C. pneumoniae* is difficult and is most often based on serology.[246,247] The MIF test appears to be the most reliable serologic test, and the following criteria for a positive test have been used: (1) >4-fold 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.[235] However, the limitations of the MIF test are lack of standardization and availability of high-quality reagents, and inability to distinguish past from persistent infection.[248] Comparison of EIAs (using species-specific assays) to MIF have shown good sensitivity and specificity in children with respiratory tract diseases and control children.[249] Because some EIAs detect antibodies to LPS, these tests detect antibodies to all *Chlamydia* species. Due to the poor sensitivity, CF tests should not be used for diagnosis.[235,250]

Isolation of *C. 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.[226,251] Throat swabs, sputum, NP, BAL, and other respiratory tract specimens placed in transport media have been used with variable success. Detection of the organism in respiratory secretions does not prove causality because asymptomatic infections occur in children and persistent shedding can occur for months after acute disease in adults.[252,253] Additional problems of culture include: small numbers of organisms present in respiratory secretions, poor recovery unless special transport media and optimal transport and storage conditions are used, and limited availability.

Molecular diagnosis with noncommercial conventional and real-time PCR tests has been evaluated.[254,255] Sensitivities appear to be as good as culture, but specificity is difficult to determine given the lack of a gold standard for comparison.

MYCOPLASMA

Mycoplasma pneumoniae

Rapid and accurate diagnosis of *M. pneumoniae* infection is problematic due to the lack of well-standardized tests.[256] Culture is the most widely accepted method for testing respiratory tract secretions, but availability is limited, specialized broth and agar media are required, and yield is relatively low.[257] For optimal isolation, specimens (BAL, tracheobronchial secretions, sputum, NP aspirates/swabs, tissues, blood, CSF, joint fluid) should be inoculated into appropriate media (e.g., SP4) at the bedside. Most media are acceptable for both isolation and PCR assay. 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. Shedding of *M. pneumoniae* can persist for several weeks after the onset of illness (particularly in children), confounding the interpretation of a positive culture result.

Direct antigen testing (EIA, DFA, immunoblotting) for respiratory tract secretions such as sputum and NP aspirates perform well in research settings (sensitivity of 90%), but are not available widely.[258,259] Cross-reactivity with other commensal mycoplasmas can occur. Persistent shedding and detection of antigen in asymptomatic individuals confound interpretation of positive results. At present, these tests are not used routinely in the clinical setting.

Conventional and real-time PCR tests for detection of *M. pneumoniae* in respiratory secretions have been widely evaluated.[257–263] PCR tests consistently are more sensitive than culture and antigen detection. Despite relatively high sensitivity, most studies suggest that PCR cannot be used alone to make a diagnosis of acute/recent infection; results must be used in conjunction with other results such as serology.[257,263,264] When performed on CSF, PCR can be useful for the diagnosis of *M. pneumoniae*-associated meningoencephalitis.[265]

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. Measurement of IgG, IgM, and IgA anti-*M. pneumoniae* can be performed with commercially available EIA, FA, and latex agglutination kits, which are more sensitive and specific than CF and have replaced CF in many diagnostic laboratories.[256,266,267] Usefulness is limited by the fact that many children can be IgM-negative at the time of presentation, and time to seroconversion can be 2 to 4 weeks.[268] In children, adolescents, and young adults, a single positive IgM result can be considered diagnostic, although false-positive test results occur. The combination of serologic results together with culture and/or PCR may provide the most reliable approach to the diagnosis.[257,262,263]

Cold agglutinin antibody titers are simple to perform and widely available. Because only 50% to 75% of those infected with *M. pneumoniae* develop cold agglutinin antibodies and the test lacks sensitivity/specificity, it should not be used for the serologic diagnosis of *M. pneumoniae* infections.[269,270]

Genital Mycoplasmas

The major means for laboratory diagnosis of *U. urealyticum* and *M. hominis* infections is culture of the organism using specialized broth and agar media. Organisms grow rapidly and cultures are positive within 2 to 5 days. PCR (including multiplex assays) has been used to detect *U. urealyticum* and *M. hominis* in clinical specimens.[271–273] *M. genitalium* grows slowly; cultures may not be positive for ≥6 days. PCR-based assays are the mainstay for diagnosis,[271,274] but none is available commercially. Serologic tests for genital mycoplasmas have not been standardized, and none is available commercially. Serologic testing (using EIA, WB, IFA) has little utility except as an epidemiologic tool.[275] *M. genitalium* cross-reacts strongly with *M. pneumoniae*. Patients with invasive *M. hominis* infection almost always have seroconversion or a significant rise in antibody titer.

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