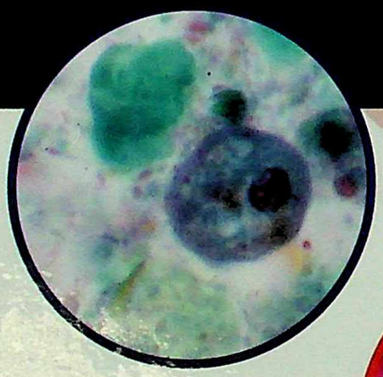
TEXTBOOK OF DIAGNOSTIC MICROBIOLOGY



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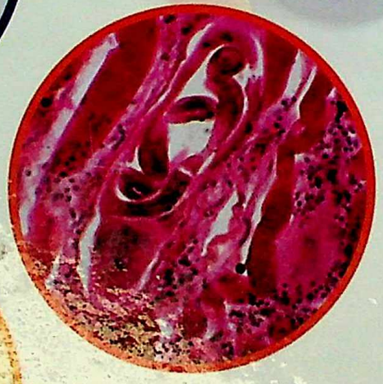

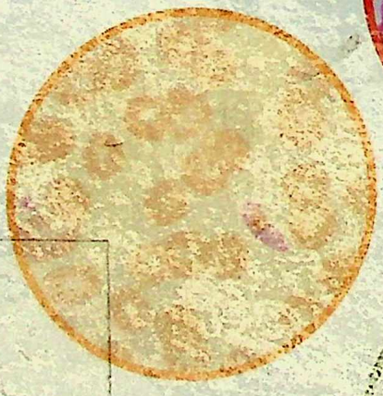

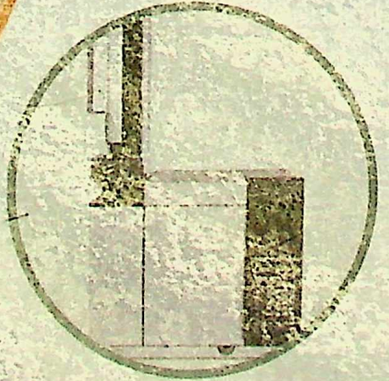

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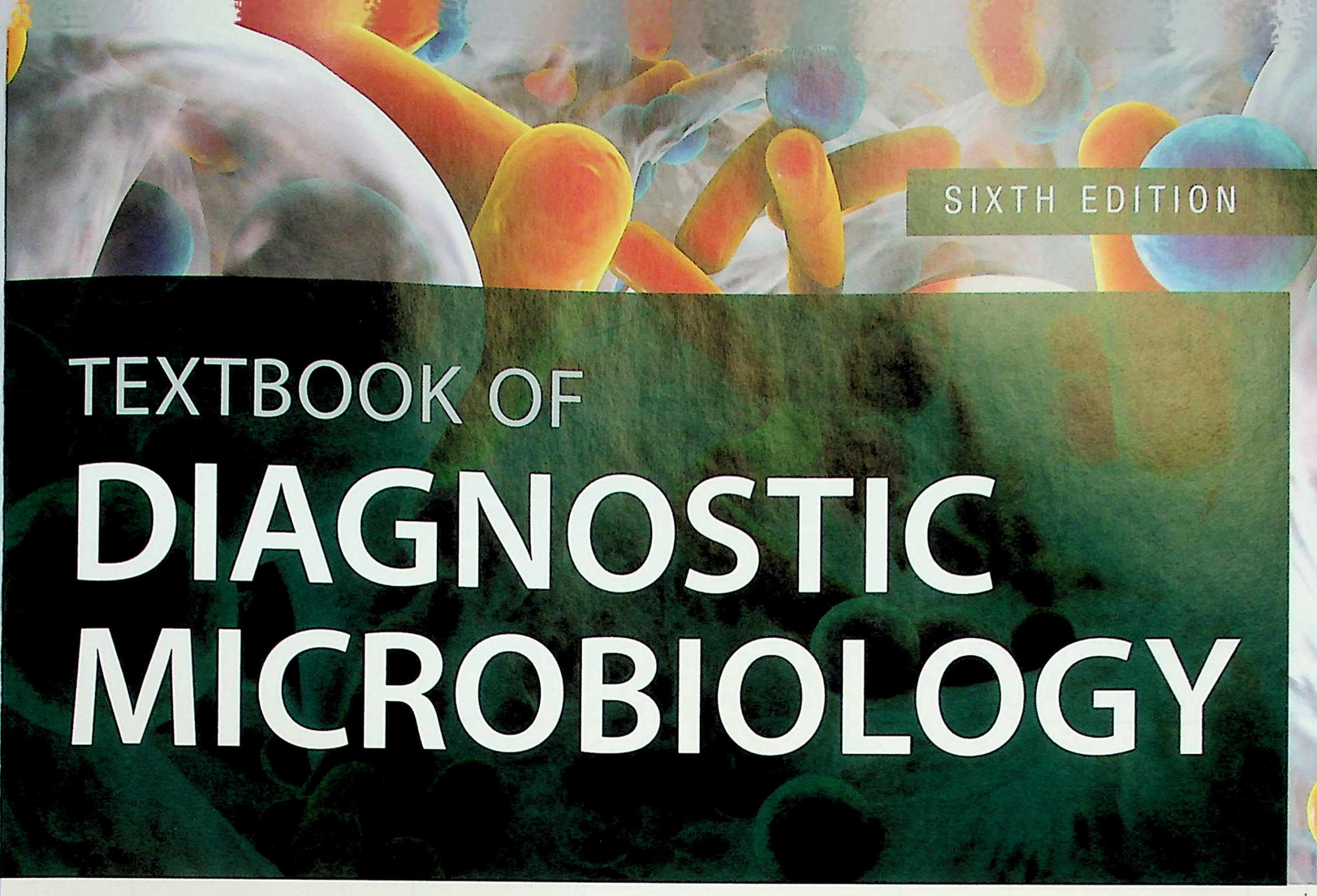

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TEXTBOOK OF DIAGNOSTIC MICROBIOLOGY

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Contents

CHAPTER

29 Clinical Virology

Kevin M. McNabb

CHAPTER OUTLINE

OBJECTIVES

After reading and studying this chapter, you should be able to:

1. Describe the characteristics of viruses and how they differ from bacteria.
2. Describe how viruses replicate.
3. Describe the proper procedures for collection and transport of viral specimens.
4. Name the appropriate specimen for maximum recovery of the suspected viral agent.
5. Compare the different methods used in the diagnosis of viral infections.
6. Explain the advantages and limitations of conventional cell cultures for diagnosing viral infections.
7. Explain the advantages and limitations of rapid viral antigen detection methods.
8. Discuss the indications and limitations of serologic assays in the diagnosis of viral infections.
9. Define *cytopathic effect* and describe how it is used to presumptively identify viral agents.
10. Evaluate the vaccine program for influenza.
11. List common opportunistic infections and other indicators of acquired immunodeficiency syndrome.
12. Create an algorithm for the serologic diagnosis of human immunodeficiency virus infection.
13. Compare the genomes and modes of transmission of the human hepatitis viruses.
14. Develop an algorithm for the serologic diagnosis of viral hepatitis.
15. Interpret the results of a hepatitis serologic profile.
16. For each of the viral agents presented in this chapter, discuss how the virus is transmitted or acquired, infection produced by the virus, and most effective method of laboratory diagnosis.

Case in Point

A 36-year-old man was admitted to the hospital after presenting at the emergency department with a self-reported, 7-month history of numbness and weakness in his right leg. He had lost 25 lb in body weight, was experiencing fecal incontinence, and had been unable to urinate for 3 days. Two years previously, the patient had been diagnosed with human immunodeficiency virus (HIV) infection. A physical examination demonstrated bilateral lower extremity weakness, and his reflexes were slowed throughout

his body. Kaposi sarcoma (KS) lesions were noted, especially on the lower extremities, along with thrush and herpes lesions in the perianal region. The patient had no fever, and magnetic resonance imaging (MRI) ruled out spinal cord compression. The patient had a history of intravenous (IV) drug abuse, chronic diarrhea for 1.5 years, KS for 2 years, and pancytopenia for several weeks. The patient had large right arachnoid cysts of congenital origin. No previous laboratory reports indicated infectious agents in cerebrospinal fluid (CSF). Meningitis was suspected, and the patient was admitted with a diagnosis of polyradiculopathy (neuropathy of the spinal nerve roots) secondary to acquired immunodeficiency syndrome (AIDS). Blood and CSF specimens were collected. Although numerous white blood cells (WBCs) were found, CSF produced no growth on routine bacteriologic culture. The blood cultures were also negative. Acyclovir was administered after culture results were received.

Issues to Consider

After reading the patient's case history, consider:

- How the patient's history relates to his current symptoms
- What information is obtained from the laboratory and MRI results
- What information provided helps determine the most likely cause of the patient's symptoms

Key Terms

Antigenic drift	Heteroploid
Antigenic shift	Koplik spots
Arboviruses	Nucleocapsid
Capsid	Obligate intracellular parasites
Cell cultures	Primary cell cultures
Continuous cell cultures	Prions
Cytopathic effect	Syncytia
Diploid	Tissue culture
Envelope	Vaccinia virus
Hemagglutinin	Virion

Clinical virology is a challenging and exciting area of clinical microbiology. It has changed over the years from viral diagnostic testing performed in only a very few, highly specialized laboratories to the modern, high-complexity laboratory of today. Many of the older, traditional diagnostic methods were slow and cumbersome and required significant expertise because they were primarily based on cell culture, serology, and microscopy (both bright-field and electron). Results were often too slow to come to be clinically useful and were perhaps even irrelevant. Over the last decade, diagnostic advances have transformed the field of virology by developing newer methods that are many times faster so that results are useful clinically. However, faster laboratory-directed diagnostics must be followed by appropriate medical interventions, or patients will receive poor care. With the emergence of molecular diagnostic testing for viral infections over the last few years, detection is much faster, much more sensitive, and much more specific, resulting in earlier intervention, early treatment, and better outcomes. This technology is becoming much more cost-effective and much more common in the clinical microbiology laboratory and allows for much better patient care.

Virology is very relevant today and perhaps even more so as shown by the viral threats that have literally burst into our lives, including the following:

- In Brazil, outbreak of dengue fever (DF), with over 1.5 million reported cases
- In West Africa, Ebola outbreak that started in 2014 and lasted until well into 2015
- In Brazil, the spread of Zika virus in November 2015, with a link to microcephaly and subsequent spread to the southern United States
- In the United States, the spread of chikungunya virus from the Caribbean countries and territories, first seen in late 2013 and reported in Florida, Puerto Rico, and the U.S. Virgin Islands
- Introduction of West Nile virus (WNV) into North America, with resurgence in 2012 that resulted in double the yearly cases seen prior to that time
- Explosion, spread, mortality, and then withdrawal of severe acute respiratory syndrome (SARS)
- Unexpected transfer of monkeypox from Africa to Midwestern United States
- New variant of influenza A (H3N2) affecting humans and swine throughout the United States and implicated in infections in visitors to county fairs

Viral illness continues to be a significant problem for large segments of people throughout the world. For example, HIV continues to devastate entire continents, effectively reducing large portions of each generation. Mosquitoes continue to spread dengue virus throughout the world and Zika virus in Brazil, with significant impact. Over the years, there has been a rise in enterovirus 71 (EV71), which has killed hundreds of children throughout parts of the Asian continent. Despite influenza surveillance programs, reliable vaccines, and dependable antiviral medications, more than 30,000 U.S. citizens die each year of influenza. This chapter discusses basic virology, including the advances and challenges in clinical virology in the modern clinical laboratory and how the laboratory helps diagnose viral illnesses.

Characteristics of Viruses

Structure

At a minimum, viruses contain a viral genome of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) and a protein coat—the **capsid**. The genome can be double stranded (ds) or single stranded(ss). The genome and its protein coat together are referred to as the **nucleocapsid**. The entire virus particle is called the **virion**. Some viruses also have a phospholipid labile **envelope** surrounding the virion. Enveloped viruses are typically more susceptible to inactivation by high temperature, extreme pH, and chemicals compared with non-enveloped (naked) viruses. The envelopes are of host origin but contain virus-encoded proteins. The viruses acquire the envelope from the host membrane as they bud from host cells.

The morphology of virions is helical, *icosahedral* (a geometric shape with 20 triangular sides), or complex. The envelope masks the shape of the virion, so most enveloped viruses are variably shaped or pleomorphic. The poxviruses are the largest viruses

(260 × 450 nm), and the smallest human virus is the poliovirus, which is 25 nm in diameter.

Taxonomy

Originally, viruses were classified by the diseases they caused and their host range. Now, viruses are classified in orders, families, genera, and species based on genome type (RNA or DNA), number of strands in the genome (ds or ss), morphology, and presence or absence of an envelope. Our growing knowledge of the nucleotide sequences also becomes a valuable tool for the taxonomic placement of viruses. A summary of the clinically significant viruses is shown in Table 29.1.

Viral Replication

Viruses are **obligate intracellular parasites**; that is, they must be inside a living cell and use the host cell machinery to replicate. In the first step for infection of a cell to occur, virions must absorb or attach to the cell surface. Absorption is specific for certain cell receptors, and receptor distribution will determine the entry point into the host. Most host cell receptors are glycoproteins, some of which include the immunoglobulin superfamily molecules (for poliovirus), acetylcholine (for rabies virus), sialic acid (for influenza virus), CD4 (for HIV), and complement receptor C3d (for Epstein-Barr virus [EBV]). The virus attaches to specific receptors on the surface of a susceptible cell by means of specialized structures on its surface called *adhesion molecules*.

The next step in viral replication is penetration. Viruses can penetrate the cell by several different mechanisms and penetration is virus dependent. Naked virions can penetrate the cell membrane directly. Enveloped viruses may enter the cell by fusion with the cell membrane, and a third method of penetration is endocytosis, whereby the enveloped virus enters the cell in a cytoplasmic vacuole. Once inside the cell, the virus loses its protein coat, releasing the genome. This process is called *uncoating*. RNA viruses usually release the genome into the cytoplasm, whereas most DNA viruses release their genome into the host nucleus. The viral genome then directs the host cell to make viral proteins and replicate the viral genome. Depending on the virus, the metabolism of the host cell may be completely stopped (as with polioviruses) or may continue on a restricted scale (as with influenza viruses).

The next step is the assembly or maturation of the virus particles. The capsid protein subunits aggregate to form capsomers, and the capsomers combine to form the capsid. The capsid and genome associate to form the nucleocapsid. The new virions are then released by lysis if they are naked viruses or by budding if they are enveloped viruses. During budding, part of the host cell plasma membrane surrounds the viral capsid and becomes the viral envelope.

Laboratory Diagnosis of Viral Infections

Laboratories can provide different levels of services, depending on the mission, financial resources, and need. All these must be balanced to provide the most cost-effective and complete diagnostics that will meet the needs of the clinical staff. Full-service virology laboratories provide viral culture and identification by using different mammalian **cell cultures** to support the growth of viruses in clinical specimens. Although not all medical treatment facilities provide full virology services, these laboratories can still obtain information about viral infections through performance of rapid tests that detect specific viruses in clinical specimens. These tests can involve the detection of viral antigens by using

TABLE 29.1 List of Viruses Causing Human Disease, Based on Nucleic Acid Characteristics and Taxonomy

Genome Strand	Family (Subfamily)	Genus	Species
dsDNA	Adenoviridae	*Mastadenovirus*	Human mastadenoviruses A to G
	Herpesviridae	*Simplexvirus*	Human herpesviruses 1 and 2, macacine herpesvirus 1
	(Alphaherpesvirinae)	*Varicellovirus*	Human herpesvirus 3
	(Betaherpesvirinae)	*Cytomegalovirus*	Human herpesvirus 5
		Roseolovirus	Human herpesvirus 6
			Human herpesvirus 7
	(Gammaherpesvirinae)	*Lymphocryptovirus*	Human herpesvirus 4
		Rhadinovirus	Human herpesvirus 8
	Papillomaviridae	*Papillomavirus*	Human papillomavirus
	Poxviridae	*Molluscipoxvirus*	Molluscum contagiosum virus
	(Chordopoxvirinae)	*Orthopoxvirus*	Cowpox virus, monkeypox virus, vaccinia virus, variola virus
		Parapoxvirus	Orf virus
		Yatapoxvirus	Yaba monkey tumor virus
dsDNA, ssDNA	Hepadnaviridae	*Orthohepadnavirus*	Hepatitis B virus
ssDNA	Parvoviridae	*Bocaparvovirus*	Human bocavirus
	(Parvovirinae)	*Dependoparvovirus*	Adeno-associated dependoparvoviruses A and B
		Erythroparvovirus	Human parvovirus B19
dsRNA	Picobirnaviridae	*Picobirnavirus*	Human picobirnavirus
	Reoviridae	*Rotavirus*	Rotaviruses A, B, and C
	(Sedoreovirinae)	*Orbivirus*	Changuinola virus, Corriparta virus, Great Island virus, Lebombo virus, Orungo virus
		Seadornavirus	Banna virus
	(Spinareovirinae)	*Coltivirus*	Colorado tick fever virus
		Orthoreovirus	Mammalian orthoreovirus

Continued

TABLE 29.1 List of Viruses Causing Human Disease, Based on Nucleic Acid Characteristics and Taxonomy—cont'd

Genome Strand	Family (Subfamily)	Genus	Species
ssRNA	Arenaviridae	*Arenavirus*	Lymphocytic choriomeningitis virus, Lassa virus, Chapare virus, Guanarito virus, Junín virus, Lujo virus, Machupo virus, Sabiá virus
	Astroviridae	*Mamastrovirus*	Human astroviruses 1, 6, 8, and 9
	Bunyaviridae	*Orthobunyavirus*	California encephalitis virus, Bunyamwera virus, Bwamba virus, Guama virus, Madrid virus, Nyando virus, Oropouche virus, Tacaiuma virus
		Hantavirus	Hantaan virus, Sin Nombre virus, Puumala virus, Thottapalayam virus
		Nairovirus	Crimean-Congo hemorrhagic fever virus, Dugbe virus
		Phlebovirus	Rift Valley fever virus, Punta Toro virus, Sandfly fever Naples virus
	Caliciviridae	*Norovirus*	Norwalk virus
		Sapovirus	Sapporo virus
	Coronaviridae	*Alphacoronovirus*	Human coronavirus 229E, human coronavirus NL63
	(Coronavirinae)	*Betacoronavirus*	Betacoronavirus 1, human coronavirus HKU1, severe acute respiratory syndrome (SARS)–related coronavirus
	(Torovirinae)	*Torovirus*	Human torovirus
	Filoviridae	*Marburgvirus*	Marburg virus
		Ebolavirus	Zaire ebolavirus, Tai Forest ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus
	Flaviviridae	*Flavivirus*	Yellow fever virus, West Nile virus, dengue virus, Zika virus, Japanese encephalitis virus, Kyasanur Forest disease virus, Langat virus, louping ill virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, St. Louis encephalitis virus, tickborne encephalitis virus, Wesselsbron virus, Yellow fever virus
		Hepacivirus	Hepatitis C virus
	Hepeviridae	*Hepevirus*	Hepatitis E virus
	Orthomyxoviridae	*Influenzavirus A*	Influenza A virus
		Influenzavirus B	Influenza B virus
		Influenzavirus C	Influenza C virus
	Paramyxoviridae	*Respirovirus*	Human parainfluenza viruses 1 and 3
	(Paramyxovirinae)	*Morbillivirus*	Measles virus
		Rubulavirus	Human parainfluenza viruses 2 and 4, mumps virus
		Henipavirus	Hendra virus, Nipah virus
	(Pneumovirinae)	*Pneumovirus*	Human respiratory syncytial virus
		Metapneumovirus	Human metapneumovirus
	Picornaviridae	*Enterovirus*	*Human enterovirus A* (human coxsackievirus A2, human enterovirus 71), *Human enterovirus B* (human coxsackievirus B1, human echovirus), *Human enterovirus C* (human polioviruses 1 to 3, human coxsackievirus A1), *Human enterovirus D* (human enterovirus 68, 70 and 94), *Human rhinovirus A, Human rhinovirus B, Human rhinovirus C*
		Parechovirus	Human parechovirus
		Hepatovirus	Hepatitis A virus
	Rhabdoviridae	*Lyssavirus*	Rabies virus
	Retroviridae		
	(Orthoretrovirinae)	*Lentivirus*	Human immunodeficiency viruses 1 and 2
	Togaviridae	*Alphavirus*	Barmah Forest virus, chikungunya virus, eastern equine encephalitis virus, Mayaro virus, O'nyong-nyong virus, Ross River virus, Semliki Forest virus, Sindbis virus, Venezuelan equine encephalitis virus, western equine encephalitis virus
		Rubivirus	Rubella virus

dsDNA, Double-stranded deoxyribonucleic acid; *ssDNA,* single-stranded deoxyribonucleic acid; *ssRNA,* single-stranded ribonucleic acid.

a number of methods, such as immunofluorescence (IF) or enzyme immunoassay (EIA). Some tests have waivers from the Clinical Laboratory Improvement Act (CLIA), and this helps bring viral identification services into physicians' offices and clinics. Other laboratories limit their virology services to *viral serology*—determining the patient's immune response to viruses—rather than detecting the viruses directly. Although this is sometimes useful, it is usually takes 3 to 4 weeks after infection before these antibodies are produced, which may mean that treatment would be too late or not needed. Many new molecular methods based on nucleic acid detection and amplification, once used in only highly complex laboratories, are being used more by many clinical laboratories. This technology can detect viral infections very early in infection, and many tests are completed in less than an hour.

Specimen Selection, Collection, and Transport

A number of different clinical specimens are suitable for the diagnosis of viral diseases. The clinical signs and symptoms of diseases often point to the target organ(s) involved, which can help determine the most appropriate specimen(s) to collect. This, combined with a basic understanding of the viral pathogenesis,

can help in specimen selection for each specific virus. It is important to ensure, however, that the specimen collected can be used to isolate a wide range of viral pathogens because similar syndromes may overlap.

Because viral shedding is usually greatest during the early stages of infection, the best specimens are those collected as early as possible, which, in many infections, is even before symptoms occur. The sensitivity of viral culture can decrease rapidly 3 days after the acute onset of symptoms, so care needs to be taken to collect specimens appropriately to maximize detection and identification. Specimens should be collected aseptically. Depending on the anatomic site and the method of collection, specimens may be nonsterile (i.e., contaminated with bacteria and/or fungi) or sterile. This will impact how much specimen processing will be required prior to viral culture. Non–culture-based test methods are typically not impacted by contamination, but that varies with the system. Often, sterile specimens are obtained from sites that are free of microorganisms, such as blood, CSF, or tissue. Identification of a virus in sterile sites usually means that the isolated virus is the cause of the disease. Nonsterile specimens are obtained from sites that contain normal flora, such as the respiratory tract, genital tract, skin, or stool. These specimens may require processing to reduce contaminants and promote viral growth. Aspirated secretions are often preferable, but swabs are easier to use for collection. Swabs must be made of Dacron or rayon. Calcium alginate swabs inhibit the replication of some viruses and can interfere with nucleic acid amplification tests. Tissue samples must be kept moist and must not be placed on media unless it is specifically designed for viral preservation.

Viral transport medium, saline, or trypticase soy broth can be added to sterile containers to keep tissues from drying. Several viral transport systems are commercially available. Most transport media consist of a buffered isotonic solution with a protein, such as albumin, gelatin, or serum, to protect less stable viruses. Often, antibacterial and antifungal agents are added to transport systems to inhibit contamination of microorganisms. Samples that can be collected with viral transport media are respiratory, swab, and tissue samples. Samples that should be collected without viral transport media include blood, bone marrow, CSF, amniotic fluid, urine, pericardial fluid, and pleural fluid. The transport container should be unbreakable and able to withstand freezing and thawing.

It is optimal to process viral specimens for culture immediately. Some viruses, such as respiratory syncytial virus (RSV), become much more difficult to recover even a few hours after collection. If specimens cannot be processed immediately after collection, they should be stored at 4° C. Specimens should not be frozen unless a significant delay (>4 days) in processing is anticipated. In that case, specimens should be frozen and held at –70° C. Specimens should never be stored at –20° C because this temperature facilitates the formation of ice crystals that will disrupt the host cells and result in loss of viral viability. Repeated freeze–thawing cycles are to be avoided because they can also result in loss of viral viability.

Appropriate Specimens for Maximum Recovery

For optimal recovery, specimens for viral isolation should be collected from the affected site. For example, secretions from the respiratory mucosa are most appropriate for viral diagnosis of respiratory infections. Aspirates, or surface swabs, are usually appropriate for lesions. If the intestinal mucosa is involved, a stool specimen is most appropriate. However, if systemic, congenital, or generalized disease is involved, specimens from multiple sites, including blood (buffy coat), CSF, and the portals of entry (oral or respiratory tract) or exit (urine or stool), are appropriate. Enteroviruses can cause respiratory infections and may be recovered from the stool after the respiratory shedding has ceased. In addition, enteroviruses are a major cause of aseptic meningitis and can also be isolated from urine specimens. Table 29.2 lists recommended specimens to be collected for viral diagnosis according to the body site affected. Incorrect or poor specimen collection can result in a false-negative diagnostic result.

Methods in Diagnostic Virology

The clinical laboratory uses four major methods to diagnose viral infections:

- Direct detection of the virus in clinical specimens
- Nucleic acid–based detection
- Isolation of viruses in cell cultures
- Serologic assays to detect antibodies to virus

Each laboratory must decide on the method to offer on the basis of the spectrum of infections encountered, population of patients served, and financial resources. In most laboratories, a combination of several methods is used to optimize detection and reduce cost.

Direct Detection

In general, direct detection methods are not as sensitive as culture methods but can offer quick results to allow rapid therapy. Many of these tests can be performed in a few minutes. Viral detection allows clinicians to make relevant decisions about therapy, infection control measures, and hospitalization. In many cases, virology results may be available before routine bacteriology culture results are.

Microscopy. Bright-field light microscopy is best for detecting poxviruses because all other virus particles are too small to be seen. Electron microscopy has a greater magnification and can be used to detect virions and is useful to detect nonculturable viruses, such as Norwalk virus, in stool filtrates. However, electron microscopy is expensive, labor-intensive, and not a very sensitive method of detecting viruses. Therefore electron microscopy is rarely used in clinical laboratories and is more suited for large teaching or research institutions.

Many viruses produce distinctive and characteristic visual changes in infected cells referred to as a **cytopathic effect** (CPE). Although virus particles cannot be visualized, the CPE can be detected in cell scrapings from infected sites with bright-field microscopy. For instance, a Tzanck smear can detect Cowdry type A bodies from herpes simplex virus (HSV) and varicella-zoster virus (VZV) lesions, and Papanicolaou (Pap) smears can reveal human papillomavirus (HPV)–associated koilocytes, which are squamous cells with an enlarged nucleus surrounded by a nonstaining halo. Rabies is sometimes diagnosed by detecting Negri bodies, which are eosinophilic cytoplasmic inclusions in neurons.

IF can be a valuable tool to detect various viral agents directly in clinical specimens. IF-labeled antibodies allow direct visualization

TABLE 29.2 Tests Available for Common Viral Pathogens and Specimens for Culture

Body System Affected	Antigen Detection	Virus Isolation	Serology	Culture Specimens	Molecular Testing
Respiratory tract	Adenovirus, herpes simplex virus (HSV), cytomegalovirus (CMV), influenza virus types A and B, parainfluenza virus, respiratory syncytial virus (RSV)	Adenovirus, coxsackie group A virus, coxsackie group B virus, echovirus, HSV, CMV, influenza virus types A and B, parainfluenza virus, RSV, reovirus, rhinovirus	Adenovirus, coxsackie group A virus, coxsackie group B virus, echovirus, HSV, CMV, influenza virus types A and B, parainfluenza virus, RSV	Nasal aspirate, nasopharynx (NP) or throat swabs, bronchoalveolar lavage, lung biopsy	Single marker molecular testing available or panels for typical respiratory pathogens available
Gastrointestinal tract	Adenoviruses 40 and 41, rotavirus	Adenoviruses 40 and 41, coxsackie group A virus, reovirus	Adenoviruses 40 and 41, coxsackie group A virus	Stool, rectal swab	Panels for multiple markers available
Liver			Hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), Epstein-Barr virus (EBV)		
Cutaneous	HSV, adenovirus, varicella-zoster virus (VZV)	HSV, adenovirus, coxsackie group A virus, coxsackie group B virus, echovirus, enterovirus, measles virus, VZV, reovirus, rubella virus, vaccinia virus	HSV, adenovirus, coxsackie group B virus, dengue virus, echovirus, human herpesvirus 6 (HHV-6), measles virus, VZV, parvovirus B19, rubella virus, vaccinia virus	Vesicle aspirate, NP aspirate and stool, lesion swab	Molecular testing is available for HSV-1 and HSV-2
Central nervous system	HSV-1 and HSV-2, mumps virus, CMV, enterovirus, HHV-6, human parechovirus, VZV	Coxsackie group A virus, coxsackie group B virus, echovirus, enterovirus, poliovirus, HSV-1 and HSV-2, mumps virus	Coxsackie group A virus, coxsackie group B virus, echovirus, poliovirus, HSV, HHV-6, mumps virus	Cerebrospinal fluid (CSF), brain biopsy, NP swabs, stool	Molecular panel available for CSF that includes CMV, enterovirus, HSV-1 and HSV-2, HHV-6, human parechovirus, and VZV
Ocular	Adenovirus, HSV	Adenovirus, HSV, coxsackie group A virus, enterovirus	HSV, coxsackie group A virus	Corneal swabs, conjunctival scrapings	
Genital	HSV	HSV	HSV	Vesicle aspirate, vesicle swab	Molecular testing available for HSV-1 and HSV-2

of virus infection, and some tests can amplify signals, which enhance sensitivity. In direct fluorescent antibody (DFA) tests, cells from a patient are fixed to a microscope slide and fluorescence-labeled antibodies are added. If viral antigens are present in the sample, the labeled antibody will bind and fluorescence will be seen microscopically (see Chapter 10 for a more detailed description). DFA assays are available for numerous viruses, including adenovirus, influenza viruses A and B, measles virus, parainfluenza viruses (PIVs) 1 through 4, and RSV from respiratory specimens, HSV-1, HSV-2, and VZV from cutaneous lesion material, and cytomegalovirus (CMV) from blood.

Enzyme Immunoassays. Many EIA tests for viral detection are commercially available, with most using multiwell microtiter plate assays. These tests can detect RSV and influenza A virus from respiratory specimens, hepatitis B virus (HBV) and HIV-1 from serum or plasma, enteric adenoviruses from the stool, and HSV from cutaneous lesions and conjunctival swabs. Other tests are packaged in single-test platforms, with positive specimens detected by colorimetric or optical density changes on membrane or silicon surfaces (Fig. 29.1). These tests can be used to detect RSV, influenza viruses A and B from respiratory specimens, rotavirus and enteric adenovirus from rectal swabs, and WNV from serum. EIA is often less sensitive than cell cultures or IF, so negative results are confirmed with cell culture or IF or nucleic acid–based tests. These assays are, by far, the most popular viral testing methods in hospital-based laboratories, but as nucleic acid–based detection becomes cheaper and easier, they may be supplanted by this newer technology.

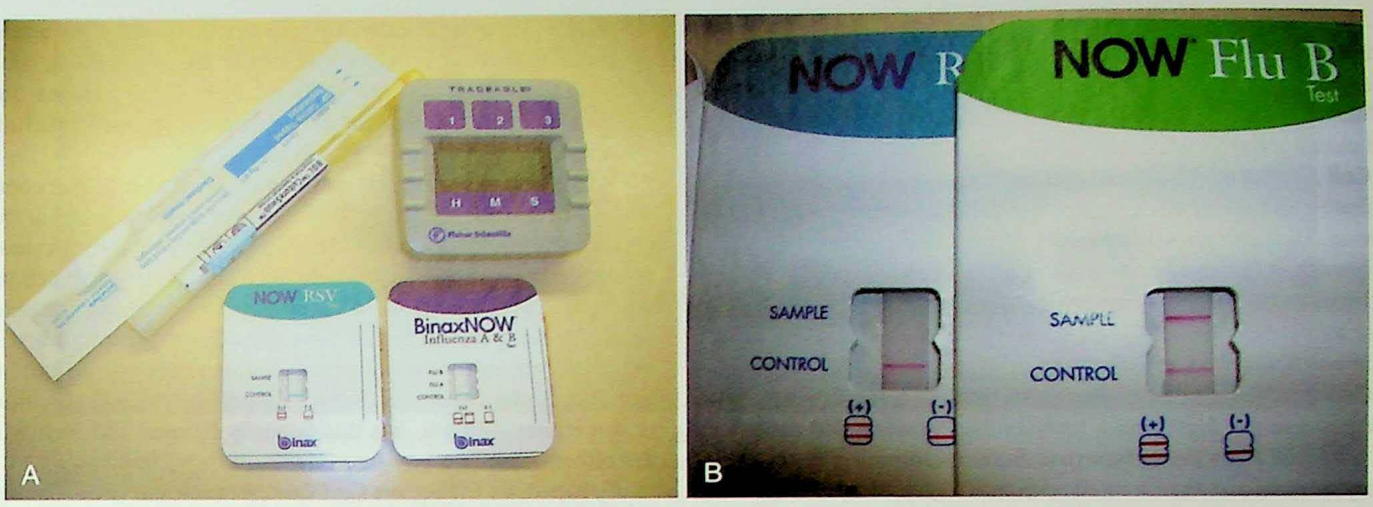

FIG. 29.1 A, Card format rapid immunochromatographic membrane assay, BinaxNOW (Scarborough, ME), for three common respiratory viruses—influenza A and B and respiratory syncytial virus. B, Examples of positive and negative results.

Nucleic Acid–Based Detection

An increasing interest in nucleic acid–based detection assays compared with traditional cell culture methods has shifted the focus of clinical virology. Not only can the presence or absence of a virus be determined with nucleic acid–based analysis but, depending on the assay used, a quantitative result can also be obtained. The use of these assays has led to a better understanding of viruses and helped develop better therapies.

Advantages of nucleic acid–based detection assays include a much faster turnaround time (TAT), better sensitivity compared with cell culture and DFA, assays that can be quantitative, detection of viruses nonculturable by cell culture (e.g., norovirus [NoV], hepatitis viruses), ability to detect multiple viruses simultaneously (multiplex), and potentially characterization of the virus genetically (genotype). Disadvantages include detection of active and inactivated virus, higher cost, need for specialized training and more complex facilities, and lack of assays approved by the U.S. Food and Drug Administration (FDA). Smaller clinical laboratories often rely on sending many of these tests to larger reference laboratories at a higher cost and longer TAT. However, as the technology for virology develops, it will get easier and cheaper to perform, leading to much higher specificity and sensitivity of results.

Examples of nucleic acid–based assays include the hybridization assay, traditional polymerase chain reaction (PCR) and real-time PCR (rtPCR) assays, branched DNA assay, nucleic acid sequence–based amplification, and a combination of PCR and flow cytometry, such as the Luminex system (Luminex, Austin, TX) for multiplex detection. Nucleic acid hybridization tests can detect viruses from various clinical specimens. Assays are available to detect a number of viruses, including HPV from endocervical specimens, and classify them into types that have a high risk or a low risk for cancer. Other hybridization tests can detect CMV from blood and HBV from plasma and serum.

Numerous gene amplification techniques are available for amplification and detection of viral genomes, primarily bloodborne pathogens, such as HIV-1, HBV, hepatitis C virus (HCV), and WNV. With a dramatic rise in the incidence of West Nile fever in the United States, there has been an increased demand for WNV testing with PCR assay. The recent influx of Zika virus has resulted in an "emergency use only (EUO)" approval from the FDA for this rtPCR assay, which is geared toward patients who suspect they have been exposed. Detection of influenza A virus by PCR assay was shown to be not only more sensitive than the traditional cell culture and shell vial methods, but it also allowed earlier administration of antiviral therapy to patients, resulting in better overall treatment. A microarray assay for rapid subtyping of influenza A virus isolates has been developed and would be valuable in the event of an outbreak or pandemic. A Luminex assay to detect and type or subtype 20 different viral pathogens within 5 hours has also been described. These types of systems will help epidemiologists, infectious disease physicians, and others in the public health community by rapidly identifying viral pathogens during an outbreak. Newer isothermal nucleic acid amplification technology is now becoming more prevalent (Alere, Waltham, MA); it does not require temperature cycling and can deliver results in as quickly as 20 minutes with performance that is a vast improvement on slower PCR-based assays. All of these molecular assays will lead to faster treatment and better patient outcomes.

Viral Isolation

In clinical virology, isolating viruses is still the gold standard against which all other methods are compared. Three methods are used for the isolation of viruses in diagnostic virology—cell culture, animal inoculation, and embryonated eggs. Of these three methods, the most commonly used by clinical virology laboratories is cell culture. Animal inoculation is extremely costly, used only as a special resource and in reference or research laboratories. For example, certain coxsackie A viruses require suckling mice for isolation of the virus. Embryonated eggs are rarely used; isolation of influenza viruses is enhanced in embryonated eggs, but this is generally accomplished more easily in cell culture.

Establishing at least a limited clinical virus isolation capability in routine laboratories can be justified, provided qualified personnel and space are available. Most of the clinical workload focuses

on the detection of HSV in genital specimens and respiratory viruses. A significant number of common clinical viruses can often be identified within 48 hours of inoculation, including HSV, influenza A and B viruses, PIVs 1 through 4, RSV, adenovirus, and many enteroviruses.

Cell Culture. The term *cell culture* is technically used to indicate culture of cells in vitro; the cells are not organized into a tissue. The term **tissue culture** or *organ culture* is used to denote the growth of tissues or an organ so that the architecture or function of the tissue or organ is preserved. Many clinical virologists use these terms interchangeably; however, *cell culture* is the technically more correct term.

Cell cultures can be divided into three categories—primary, low passage (or finite), and continuous. **Primary cell cultures** are obtained from tissue removed from an animal. The tissue is finely minced and then treated with an enzyme, such as trypsin, to disperse individual cells further. The cells are then seeded onto a surface to form a monolayer, such as in a flask or a test tube. With primary cell lines, only minimal cell division occurs. Cell viability is maintained by periodically removing cells from the surface, diluting them, and placing them into a new container. This process is referred to as *splitting* or *passaging*. Primary cell lines can only be passaged a few times before new cells must be obtained. An example of commonly used primary cell culture is one with primary monkey kidney (PMK) cells.

Finite cell cultures can divide, but passage is limited to about 50 generations. Finite cell lines, like primary cell lines, are **diploid**; that is, they contain two copies of each chromosome. Diploid is the normal genetic makeup for eukaryotic cells. As the number of passages increases, these cells become more insensitive to viral infection. Human neonatal lung is an example of a standard finite cell culture used in diagnostic virology.

Continuous cell cultures are capable of infinite passage and are **heteroploid**; that is, they have an abnormal and variable number of chromosomes that is not a multiple of the normal haploid number. HEp2 (derived from a human laryngeal epithelial carcinoma), A549 (derived from a human lung carcinoma), and Vero (derived from monkey kidney) are examples of continuous cell lines used in diagnostic virology. Both HEp2 and A549 were developed from cancer tissue obtained from patients during treatment. Each laboratory must decide which cell lines to use on the basis of the spectrum of viral sensitivity, availability, and cost. Optimally, several different cell lines will be used for a single specimen to recover different viruses that may be present, similar to the strategy used with media for the recovery of bacteria. Table 29.3 lists some cell culture lines commonly used in clinical virology.

Mixed or engineered cell cultures are lines of cells that contain a mixture of two different cell types or are made up of cells genetically modified to make identification of viral infection easier. Mixed cell lines have been developed by combining two cell lines susceptible to certain types of viruses, such as respiratory or enteric viruses. The mixed line can have greater sensitivity to a wider range of viruses and therefore reduce the number of culture vials that need to be incubated. Interpreting these mixed cell cultures is sometimes difficult, but this is easily learned and well worth the effort.

Cytopathic Effect on Cell Cultures. Some viruses produce a very characteristic CPE that can provide a presumptive identification of a virus isolated from a clinical specimen. For example, HSV grows rapidly on many different cell lines and frequently produces a CPE within 24 hours. A predominantly cell-associated virus, HSV produces a focal CPE (in which adjacent cells become infected) and plaques, or clusters of infected cells. The combination of rapid growth, plaque formation, and growth on many different cell types, such as MRC-5 (Medical Research Council cell strain 5), human fibroblasts, Vero, HEp2, mink lung, and PMK cells, is presumptive evidence for the identification of HSV. HSV is one of the few viruses that can grow on rabbit kidney cells (Fig. 29.2); therefore it is a useful cell line for HSV detection.

CMV produces an HSV-like CPE (Fig. 29.3) but grows much more slowly and only on diploid fibroblasts. VZV grows on several types of cells, including diploid fibroblasts, A549 cells, and Vero cells. Enteroviruses characteristically produce rather small, round infected cells that spread diffusely on PMK cells, diploid fibroblasts, human embryonal rhabdomyosarcoma (RD) cells, and A549 cells. Adenoviruses also produce cell rounding (Fig. 29.4) on a number of cell types, including diploid fibroblasts, HEp2 cells, A549 cells, and PMK cells, but this is usually larger than that caused by enteroviruses. The rounding may be diffuse or focal, appearing like a cluster of grapes.

The respiratory viruses may not produce a characteristic CPE. RSV can produce classic syncytial formation in HEp2 or MKC cells. **Syncytia** are giant multinucleated cells resulting from cell

TABLE 29.3 Cell Cultures Commonly Used in the Clinical Virology Laboratory

Virus	PMK	HDF	HEp2	RK	A549	CPE
Herpes simplex virus	–	+ + +	+ + +	+ + +	+ + +	Large, rounded cells
Cytomegalovirus	–	+ + +	–	–	–	Large, rounded cells
Varicella-zoster virus	–	+ + +	–	–	±	Foci or rounded cells; possible syncytia
Enterovirus	+	+	+ +	–	+	Refractile, round cells in clusters
Adenovirus	+	+ +	+ + +	–	+ +	Large, rounded cells in clusters
Respiratory syncytial virus	±	±	+ + +	–	+ +	Syncytia
Influenza virus, parainfluenza virus	+ + +	±	–	–	–	Variable—none to granular appearance

A549, Human lung carcinoma cell line; *CPE,* cytopathic effect; *HDF,* human diploid fibroblasts; *HEp2,* human laryngeal carcinoma cell line; *PMK,* primary monkey kidney; *RK,* rabbit kidney; –, negative; +, acceptable; ++, good viral recovery; +++, recommended; ±, positive or negative.
Modified from Costello MJ et al: Guidelines for specimen collection, transportation, and test selection, *Lab Med* 24:19, 1993.

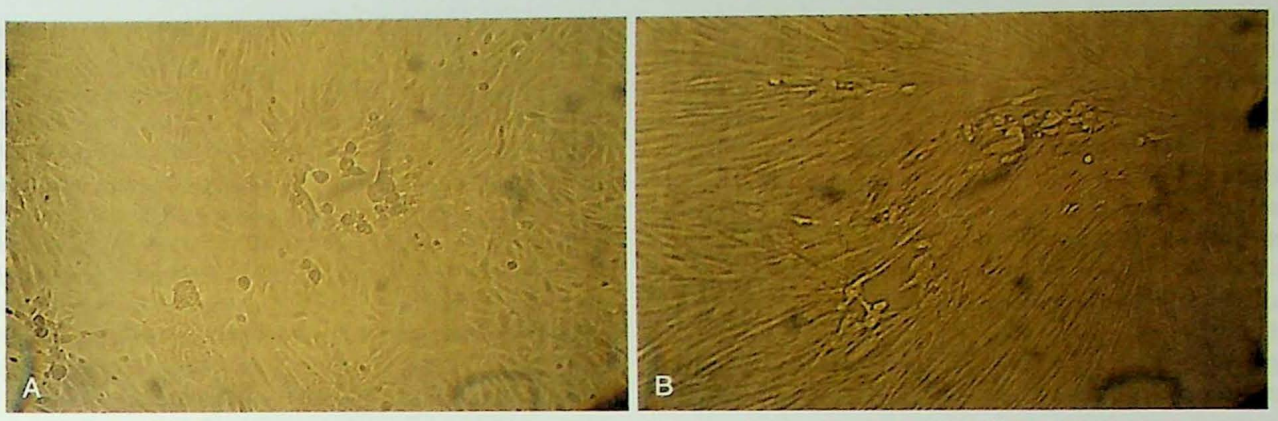

FIG. 29.2 A, Herpes simplex virus (HSV) from the skin, showing the cytopathic effect (CPE) in less than 1 day on rabbit kidney cells. B, HSV showing the CPE in less than 1 day on HeLa cells. (Unstained, ×400.)

FIG. 29.3 Cytomegalovirus from cerebrospinal fluid forming a cytopathic effect on diploid fibroblast cells (unstained, ×400).

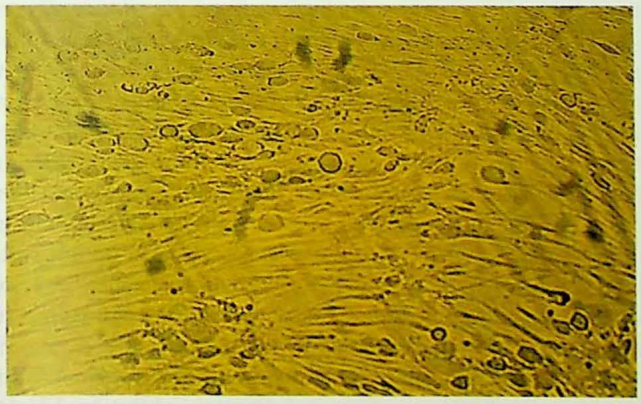

FIG. 29.4 Cytopathic effect of adenovirus on HeLa cells (unstained, ×400).

fusion as a consequence of virus infection. PIV type 2, and to a lesser extent PIV type 3, can also produce syncytia. Influenza virus commonly does not exhibit a well-defined CPE. Specimens submitted for influenza virus cultures are usually inoculated onto PMK cells, LLC-MK2 (a continuous line derived from rhesus monkey kidney), or MDCK (Madin-Darby canine kidney epithelial cells) cells. Because influenza viruses typically do not produce a CPE, a hemagglutination or hemadsorption test is done to detect these viruses. Cells infected with influenza virus express a viral **hemagglutinin** (H) protein on their surface that binds red blood cells (RBCs). In the hemadsorption test, a suspension of RBCs is added to the infected cell monolayer. If influenza virus is present, the RBCs will adsorb or stick to the infected cells. In the hemagglutination assay, supernatant from the infected monolayer containing influenza virus is mixed with a suspension of RBCs. Influenza viruses also have the H protein on their surface; therefore the RBCs will visibly agglutinate. Fluorescent antibody stains that detect viral antigen, such as those used directly on clinical specimens, can also be used to screen cell cultures before a final negative result is reported. IF, EIA, and nucleic acid amplification assays can also be used to detect and identify viruses in cell cultures to ensure true positives are not missed.

Centrifugation-Enhanced Shell Vial Culture. The shell vial culture technique can more rapidly identify viruses than the traditional cell culture method. Cells are grown on a round coverslip in a shell vial. A shell vial is a small, round, flat-bottomed tube, generally with a screw cap. The shell vial is inoculated with the clinical sample and then centrifuged to promote viral absorption. The shell vial is incubated for 24 to 48 hours, after which the coverslip is removed and the IF technique performed. Based on the type of clinical specimen and suspected viruses, a variety of fluorescent-labeled antibodies can be used. A modification of this procedure is to use flat-bottomed microtiter plates. Although this is better than looking for a CPE, in many cases it can be labor-intensive, and often cultures are done in duplicate, which results in reading at 24 hours then again at 48 hours, thus increasing the TAT.

Serologic Assays

Viral serology detects circulating antibodies to viruses after exposure. This method provides limited information and has certain inherent problems. First, serologic assays measure the host response rather than directly detecting the virus. Second, the antibody-producing capabilities of human hosts differ widely. For example, despite being actively infected, immunocompromised individuals may not produce enough antibodies to be detected. This is typically seen in HIV-positive individuals. Third, the antibody level does not necessarily correlate with the acuteness or activity level of the infection because this is also host dependent.

With few exceptions, paired sera (acute and convalescent) demonstrating seroconversion or a fourfold rise in titer are required to establish a diagnosis of recent infection. Therefore serologic studies are usually retrospective. Some assays are able to distinguish between immunoglobulin M (IgM) and immunoglobulin G (IgG); the presence of IgM indicates an acute (recent) infection. Cross-reactions with nonspecific antibodies can occur, which makes interpretation of results difficult. Interpretation is also difficult because of passive transfer of antibodies, such as in transplacental or transfusion transmission. The following are indications for serologic testing:

- Diagnosis of infections with nonculturable agents, such as hepatitis viruses
- Diagnosis of a past (IgG) or acute (IgM) infection from various viral pathogens
- Determination of immune status in regard to rubella virus, measles virus, VZV, hepatitis A virus (HAV), and HBV
- Monitoring of patients who are immunosuppressed or have had transplantations
- Epidemiologic or prevalence studies

Double-Stranded DNA Viruses

Viruses are discussed in this chapter in groups based on nucleic acid types—double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), and single-stranded RNA (ssRNA) viruses. Hepatitis viruses are the only exception, and they will be discussed as one group because they do not all have the same type of nucleic acid.

Adenoviridae

Adenovirus was first isolated from adenoid tissue and was thus named for the initial isolation location. Human adenoviruses belong to the family Adenoviridae and the genus *Mastadenovirus*. Adenoviruses are naked icosahedral viruses with dsDNA (Fig. 29.5). Adenovirus has 51 distinct serotypes (seven subgenera, A through G), and the different serotypes are associated with numerous common clinical manifestations. The clinical manifestations seen are dependent on the age and immune status of the infected person. The most common serotypes are 1 to 8, 11, 21, 35, 37, and 40.

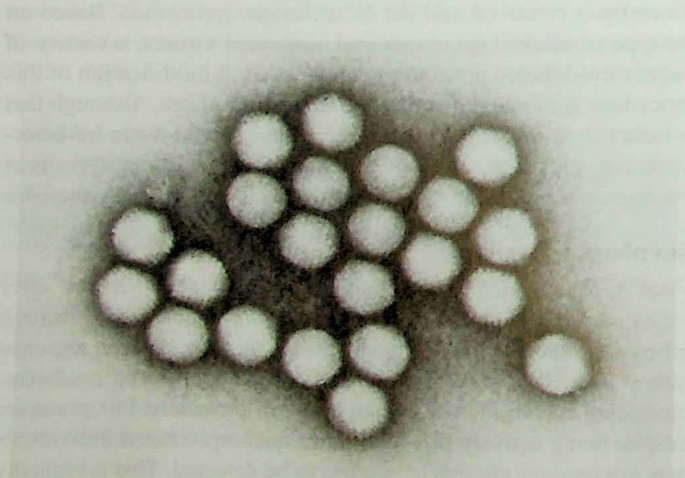

FIG. 29.5 Transmission electron micrograph of adenovirus (×60,000). (Courtesy Dr. G. William Gary, Jr., Centers for Disease Control and Prevention, Atlanta, GA.)

Although half of all adenovirus infections are asymptomatic, the virus causes about 10% of all cases of pneumonia and 5% to 15% of all cases of gastroenteritis in children. Adenovirus infections affect the respiratory tract, eye, and gastrointestinal (GI) tract, with lesser involvement of the urinary tract, heart, central nervous system (CNS), liver, pancreas, and genital tract. The viruses can also cause epidemic keratoconjunctivitis, acute hemorrhagic cystitis, and pharyngoconjunctival fever. Adenovirus infections occur throughout the year and affect every age group. Adenovirus serotype 14 is rarely reported but causes severe and sometimes fatal acute respiratory disease (ARD) in patients of all ages. In the United States, an outbreak of adenovirus 14 was reported in four states from 2006 to 2007. The outbreak included one infant in New York and 140 additional cases from the states of Oregon, Texas, and Washington. Although no link could be found between the New York case and the other cases, all isolates were identical by hexon and fiber gene sequencing. Since 2007, adenovirus has been associated with outbreaks of ARD in U.S. military recruits and the general public. Adenovirus types 3, 4, and 7 are most commonly associated with ARD and can be fatal.

Adenovirus is shed in secretions from the eyes and respiratory tract. Viral shedding in feces and urine can occur for days after the symptoms have disappeared. The viruses are spread by aerosols, fomites, the oral-fecal route, and personal contact. Most infections are mild and require no specific treatment. Until the sole manufacturer ceased production, oral vaccination was available from 1971 to 1999 for types 4 and 7 and was used only for preventing ARD in military recruits. The development of a new vaccine was directed by the military after outbreaks occurred among its personnel, and this vaccine became available in October 2011. Good infection control measures, including adequate chlorination of swimming pools, prevent adenovirus infections, such as adenovirus-associated conjunctivitis.

Adenovirus types 40 and 41 are called *enteric adenoviruses* because they cause epidemics of gastroenteritis in young children, with diarrhea being a prominent feature of the illness. There is far less vomiting and fever than with rotavirus infections. Enteric adenoviruses have a worldwide, endemic distribution, and the number of cases increases during the warmer months. These adenoviruses can be identified but not serotyped by EIA. Commercial antigen detection kits are available, and although inexpensive, they lack sensitivity. There is a new molecular panel (FilmArray, BioFire, Salt Lake City, UT) that is specific for adenovirus types 40 and 41. Adenoviruses are quite stable and can be isolated in human embryonic kidney and many continuous epithelial cell lines. They produce a characteristic CPE, with swollen cells in grapelike clusters. Isolates can be identified by fluorescent antibody and EIA methods, along with nucleic acid tests. Serotyping is accomplished by serum neutralization or hemagglutination inhibition. Electron microscopy has been used in several epidemiologic studies but is not routinely used as a clinical tool.

Herpesviridae

The herpesviruses belong to the family Herpesviridae. The herpesviruses have a genome of linear dsDNA, an icosahedral capsid, an amorphous integument surrounding the capsid, and an outer envelope. All herpesviruses share the property of producing latency and lifelong persistence in their hosts. The virus is latent between active infections. It can be activated from latency by various

stimuli, including stress, caffeine, and sunlight. Activation can cause lesions to reappear.

Eight species of human herpesviruses (HHV) are currently known:

- HSV-1, also known as HHV-1
- HSV-2, also known as HHV-2
- VZV, also known as HHV-3
- EBV, also known as HHV-4
- CMV, also known as HHV-5
- HHV-6
- HHV-7
- HHV-8, also known as KS herpesvirus

There are other herpesviruses that infect only primates, except for herpes B virus, which has produced fatal infections in animal handlers and researchers working with primates.

Herpes Simplex Viruses

HSV-1 and HSV-2 belong to the genus *Simplexvirus*. HSV infections are very common. By adulthood, about 80% of Americans have been infected with HSV-1. Approximately 20% of Americans have had HSV-2 infections. These figures indicate that about one in six persons in the United States has had HSV infection, and most infections are asymptomatic. Disease caused by HSV infection is generally divided into two categories—primary (first or initial infection) and recurrent (reactivation of the latent virus).

Infections are generally spread by contact with contaminated secretions. Lesions usually occur on mucous membranes after an incubation period of 2 to 11 days. Infected individuals are most infectious during the early days of a primary infection. Virus-infected cells are usually found at the edge and in the base of lesions; however, the virus can be transmitted from older lesions as well as from asymptomatic patients.

Types of Infections

HSV infections can cause a wide spectrum of clinical manifestations, including those discussed below.

Oral Herpes. Oral herpes infections were thought to have been caused by HSV-1, but it is now known that a number of cases are caused by HSV-2. The incubation period ranges from 2 days to 2 weeks. Primary infections are usually asymptomatic, but when apparent, they commonly manifest themselves as rarely seen mucosal vesicles inside the mouth or as ulcerations that may be widespread and involve the buccal mucosa, posterior pharynx, and gingival and palatal mucosae. In young adults, a primary HSV infection can involve the posterior pharynx and look like acute pharyngitis. Recurrent, or reactivation, HSV infection usually occurs on the border of the lips at the junction of the oral mucosa and skin. An early symptom of burning or pain followed by vesicles, ulcers, and crusted lesions is the typical pattern.

Genital Herpes. Genital herpes infections are usually caused by HSV-2, although HSV-1 can cause as many as one third of the infections. Many individuals with antibodies to HSV-2 have not been diagnosed with genital herpes. The infection manifests itself in females as vesicles on the mucosa of the labia, vagina, or both. Involvement of the cervix and vulva is not uncommon. In males, the shaft, glans, and prepuce of the penis are the most commonly affected sites. The urethra is commonly involved in both men and women. Recurrent herpes infections involve the same sites as primary infections, but the urethra is less commonly involved. The symptoms are usually less severe in recurrent disease. Genital herpes infections can as much as double the risk of sexual transmission of HIV.

Neonatal Herpes. Transmission of HSV from infected mothers to neonates is less common than might be expected, but the risk of mother-to-infant transmission is 10 times higher when mothers have an unrecognized primary infection during labor and delivery. However, mortality associated with disseminated neonatal disease is about 60% in treated neonates but exceeds 70% in untreated neonates. Infection can be acquired in utero, intranatally (during birth), or postnatally (after birth). The infection is usually transmitted during a vaginal delivery and is more severe when HSV-2 is involved. The rate of transmission is about 50% when the mother has a primary infection. Most newborns are infected by mothers who are asymptomatically shedding the virus during a primary infection. The risk of transmission is very low when the mother has recurrent herpes. Cesarean delivery or suppressive antiviral therapy at delivery significantly reduces the risk of transmission.

Herpes Simplex Virus Encephalitis. HSV encephalitis is a very rare but devastating disease with a mortality rate of about 70%. In the United States, HSV encephalitis may account for up to 20% of all encephalitis cases. HSV is the leading cause of fatal sporadic encephalitis in the United States. Encephalitis is usually caused by HSV-2 in neonates and HSV-1 in older children and adults. HSV encephalitis is also associated with an immunocompromised status. Survival rates and clinical outcomes are greatly improved with IV antiviral treatment. A new diagnostic panel for the detection of HSV-1 and HSV-2, as well as several other viruses and bacteria that cause meningitis, is available (FilmArray, BioFire, Salt Lake City, UT), and this test is frequently being performed to reduce the need for antiviral therapy especially in infants.

Ocular Herpes. A herpes simplex infection of the conjunctiva can manifest itself as swelling of the eyelids associated with vesicles. Corneal involvement can result in destructive ulceration and perforation of the cornea, leading to blindness. HSV is the most common cause of corneal infection in the United States. Fortunately, most infections involve only the superficial epithelial layer and heal completely with treatment.

Diagnosis

Diagnosis of HSV infections is best made by antigen detection or viral isolation. The best specimens for culture are aspirates from vesicles, open lesions, or host cells collected from infected sites. Culture of CSF is usually not productive. To obtain a culture-confirmed diagnosis of encephalitis, brain biopsy material is required. Alternatively, CSF can be assayed by PCR for HSV. In many studies, gene amplification for HSV in CSF approaches 100% sensitivity. Some of the newer nuclear assays are becoming easier to perform and less costly, so it is expected that they will be used more frequently in clinical laboratories. The newest meningitis panel will detect HSV-1 and HSV-2 as well as other viruses, bacteria, and yeasts.

In culture, HSV replicates rapidly, and the CPE can be seen within 24 hours (Fig. 29.6; also see Fig. 29.2). Therefore diagnosis and appropriate therapy can be initiated quickly, resulting in better patient outcomes. HSV can be isolated in numerous cell lines, including human embryonic lung, rabbit kidney, HEp2, and A549 cells. HSV is one of the most frequently isolated viruses in the

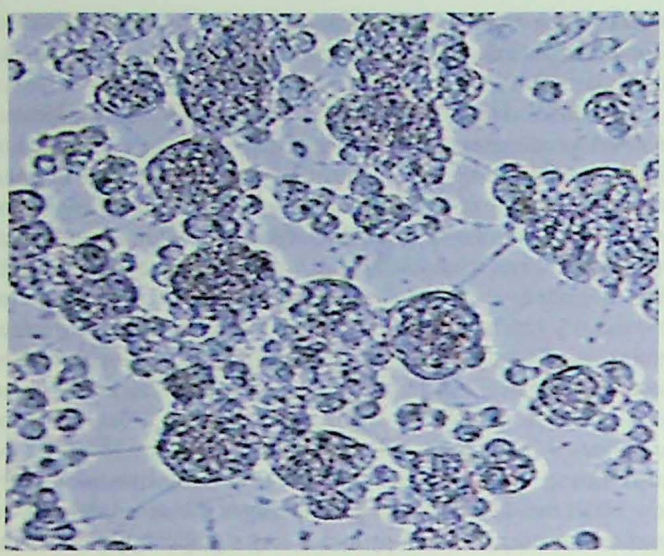

FIG. 29.6 Advanced cytopathic effect in an A549 cell line caused by herpes simplex virus infection (unstained, ×400). (Courtesy Sarah Pierson.)

clinical virology laboratory. Once isolated, monoclonal antibodies can be used to type the virus. Typing genital lesion isolates can be prognostic in that HSV-2 reactivation occurs more readily than HSV-1 reactivation. In addition, typing genital lesions from children has been used to provide legal evidence supporting potential sexual abuse.

Commercially available engineered cell lines improve the detection of HSV. In the ELVIS (enzyme-linked, virus-inducible system) test, a gene for the enzyme β-galactosidase linked to a virus-induced promoter has been inserted into baby hamster kidney cells. If HSV-1 is present in the cell line, a viral protein will activate the promoter, resulting in β-galactosidase expression. Detection is accomplished by addition of a reagent, which is cleaved by the enzyme produced in virus-infected cells and results in the formation of a blue color, which is easily seen by light microscopy.

Formerly, serology provided only limited information to aid in the diagnosis of HSV infections. Reagents that could distinguish between antibodies to HSV-1 and to HSV-2 were not previously available. This was problematic because most adult patients have antibodies to HSV-1. Now, however, several FDA-approved, type-specific assays that differentiate antibody response to HSV are available. The tests come in a variety of formats, including EIA, strip immunoblot, and even simple membrane-based, point-of-care assays. The difference between the newer tests and those of the previous generation is the antigens used. The newer tests use recombinant or affinity-purified, type-specific glycoprotein G1 or G2, giving the tests the ability to distinguish between HSV-1 and HSV-2. Older-generation tests used crude antigen preparations from lysed cell culture of the virus and have been shown to have cross-reactivity rates of as much as 82% in positive specimens.

Cytomegalovirus

CMV is in the genus *Cytomegalovirus,* and the name originates from the enlargement of infected cells (from Latin *cyto,* meaning cell, and *mega,* meaning large). It is a typical herpesvirus, but it replicates only in human cells much more slowly compared with HSV or VZV. CMV is typically spread by close contact with an infected person. Most adults demonstrate antibody against the virus, with a prevalence rate in the United States of 55% among adult women and 32% among adult men. The seroprevalence of CMV increases with age in all populations; it is highest among lower socioeconomic groups living in crowded conditions. Persons who live in overcrowded conditions can acquire CMV at an early age. The virus is shed in saliva, tears, urine, stool, and breast milk. CMV infection can also be transmitted sexually via semen and cervical and vaginal secretions and through blood and blood products. CMV infection is the most common congenital infection in the United States.

Most CMV infections are asymptomatic in the immune-competent host but can manifest themselves as a self-limiting, infectious mononucleosis-like illness, with fever and hepatitis. In immunocompromised hosts, such as transplant recipients and patients with HIV infection, CMV infection can become a significant, life-threatening, systemic disease involving almost any organ, including the lungs, liver, intestinal tract, and retina, as well as the CNS.

Congenital infections and infections in immunocompromised patients are often symptomatic and can be serious. Serious clinical manifestations can develop if the mother acquires the primary infection during pregnancy; congenital infection, however, is unlikely to occur if the mother was seropositive at the time of conception. Symptomatic congenital infection is characterized by petechiae, hepatosplenomegaly, microcephaly, and chorioretinitis. Other manifestations are reduced birth weight, CNS involvement, mental impairment, deafness, and even death. CMV infection is one of the leading causes of mental retardation, deafness, and intellectual impairment.

The diagnosis of CMV infection is best confirmed by isolation of the virus from normally sterile body fluids, such as the buffy coat of blood or other internal fluids or tissues. The virus can also be cultured from urine or respiratory secretions, but because shedding of CMV from these sites is common in normal hosts, isolation from these sources must be interpreted with extreme caution. Over the last several years, a viral antigenemia test has gained wider use by clinical virology laboratories. The antigenemia assay is specific, sensitive, rapid, and relatively easy to perform. The test is based on the immunocytochemical detection of the 65-kilodalton (kDa), lower-matrix phosphoprotein (pp65) in the nuclei of infected peripheral WBCs. The antigenemia test may prove helpful in assessing the efficacy of antiviral therapy. However, there are now several newer nucleic acid assays using PCR that may replace this test in smaller clinical laboratories as they become more cost-effective. Nucleic acid assay is offered at major U.S. reference laboratories and is the preferred method for determining viral loads. CMV produces a characteristic CPE, which can sometimes be seen in clinical specimens (Fig. 29.7).

Molecular-based testing is also widely used to detect virus particles in clinical samples. PCR, branched DNA, and hybridization assays are all used for blood donor screening and diagnostic applications. A new meningitis panel, including CMV isolation, is available and is perfect for use in pediatric populations. A congenital infection is best confirmed by isolation of CMV from the infant within the first 2 weeks of life. Isolation after the first 2 weeks does not confirm congenital infection. Urine is the most common specimen submitted for viral detection in these patients.

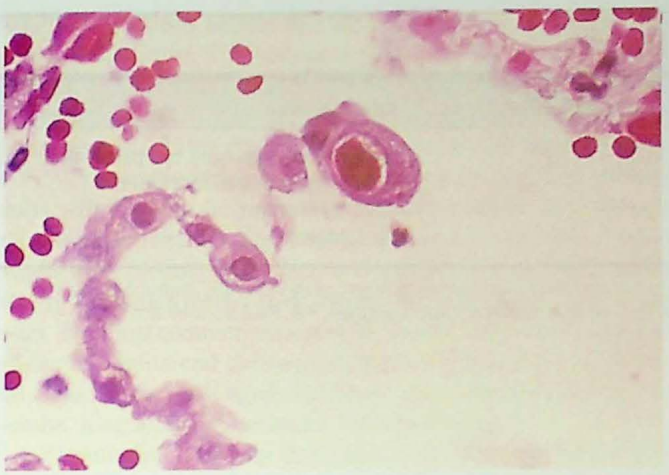

FIG. 29.7 Active cytomegalovirus lung infection in a patient with acquired immunodeficiency syndrome. Lung histopathology shows cytomegalic pneumocyte containing characteristic intranuclear inclusions, hematoxylin and eosin (×1000). (Courtesy Edwin P. Ewing, Jr., Centers for Disease Control and Prevention, Atlanta, GA.)

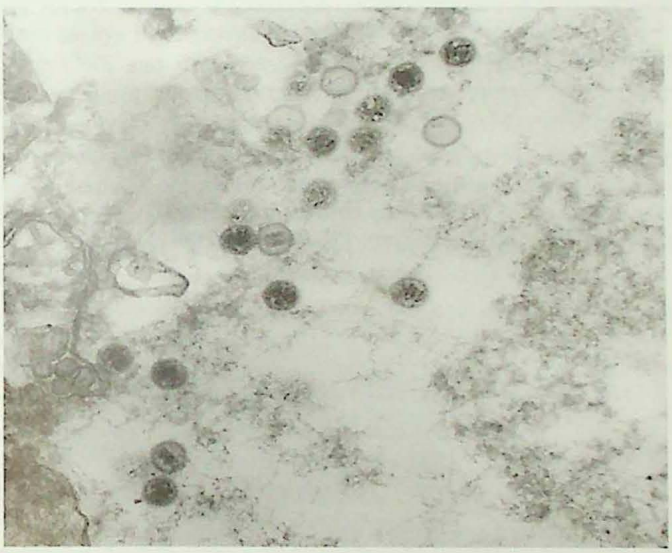

FIG. 29.8 Negatively stained transmission electron micrograph revealing the presence of numerous Epstein-Barr virus' virions (×40,000). (Courtesy Fred Murphy, Centers for Disease Control and Prevention, Atlanta, GA.)

As with HSV, serology is not as helpful as a culture in diagnosing the infection. CMV can be isolated in cell culture only by using human diploid fibroblast cell lines, such as human embryonic lung or human foreskin fibroblasts (see Fig. 29.3). The virus replicates slowly, so it may take up to 3 weeks for the CPE to appear in culture. However, the use of shell vials can reduce the time for detection to as little as 1 day.

Epstein-Barr Virus

Epstein-Barr virus (EBV), in the subfamily Gammaherpesvirinae and the genus *Lymphocryptovirus*, causes infectious mononucleosis (Fig. 29.8). Up to 95% of adults aged between 35 and 40 years have been infected. Many children become infected with EBV and show few signs of infection. When infection with EBV occurs in adolescence, it presents as infectious mononucleosis 35% to 50% of the time. The signs and symptoms of EBV infection include sore throat, fever, lymphadenopathy, hepatomegaly, splenomegaly, and general malaise. These usually resolve within a few weeks, although malaise can be prolonged in some cases. Complications of EBV infections include splenic hemorrhage and rupture, hepatitis, thrombocytopenia purpura with hemolytic anemia, Reye syndrome, encephalitis, and other neurologic syndromes. EBV can be recovered from the oropharynx of symptomatic as well as healthy persons, who can transmit the virus to susceptible persons via infected saliva.

The incubation period for EBV infection ranges from 2 weeks to 2 months. As with the other herpes group viruses, infection is very common and results in latency, and most adults demonstrate antibody against the virus. Young children with the infection are almost always asymptomatic. As the age at the time of infection increases to young adulthood, a corresponding increase occurs in the ratio of symptomatic to asymptomatic infections. Some cancers have been associated with EBV, including Burkitt lymphoma, Hodgkin disease, and nasopharyngeal carcinoma (NPC). Burkitt lymphoma is a malignant disease of the lymphoid tissue seen most commonly in African children. The virus has also been increasingly recognized as an important infectious agent in transplant recipients. The most significant clinical effect of EBV infection in these patients is the development of a B-cell lymphoproliferative disorder or lymphoma.

Viral culture for EBV requires human B lymphocytes, and is beyond the capabilities of most clinical virology laboratories. Therefore laboratory diagnosis of EBV infection is often accomplished with serologic tests. EBV infects circulating B lymphocytes and stimulates them to produce multiple heterophile antibodies, including antibodies to sheep and horse RBCs. The Paul-Bunnell heterophile antibody test is an excellent rapid screening test for these antibodies, although some false-positive reactions do occur. A large number of rapid test kits, generally based on EIA or latex agglutination, are commercially available for detecting heterophile antibodies. These tests are 80% to 85% effective. Some false-positive test results represent patients who have had infectious mononucleosis and still have low levels of antibody. Young children can have false-negative results with the heterophile test; performing an EBV-specific antibody test on these individuals is appropriate. EBV-specific serologic tests (Table 29.4, Fig. 29.9) measure the presence or absence of the following:

- *Anti-VCA (antibodies against the viral capsid antigen):* IgM to the VCA occurs early in the infection and disappears in about 4 weeks, so its presence indicates current infection. IgG often appears in the acute stage and will persist for life at lower titers.
- *Anti-EA IgG (IgG antibody to early antigen):* IgG to EA can appear in the acute phase, and its presence indicates current or recent infection. The antibody usually cannot be detected after 6 months.
- *Anti-EA/D (antibody to early antigen, diffuse):* Antibodies to EA/D appear in the acute phase, and their presence indicates current or recent infection. The antibodies usually cannot be detected after 6 months. Patients with NPC often have elevated levels of IgG and IgA anti-EA/D antibodies.

TABLE 29.4 Interpretation of Epstein-Barr Virus Serologic Markers

PB	Anti-VCA IgM	Anti-VCA IgG	Anti-EA IgG	Anti-EBNA	Interpretation
–	–	–	–	–	No previous exposure to Epstein-Barr virus
+	+	+	±	–	Acute infectious mononucleosis
±	±	+	±	+	Recent infection
—	–	+	–	+	Past infection

Anti-EA IgG, Immunoglobulin G antibodies to early antigen; *anti-EBNA,* antibodies to Epstein-Barr vius nuclear antigen; *anti-VCA IgG,* immunoglobulin G antibodies against the viral capsid antigen; *anti-VCA IgM,* immunoglobulin M antibodies against the viral capsid antigen; *PB,* Paul-Bunnell antibody; –, negative; +, positive; ± positive or negative.

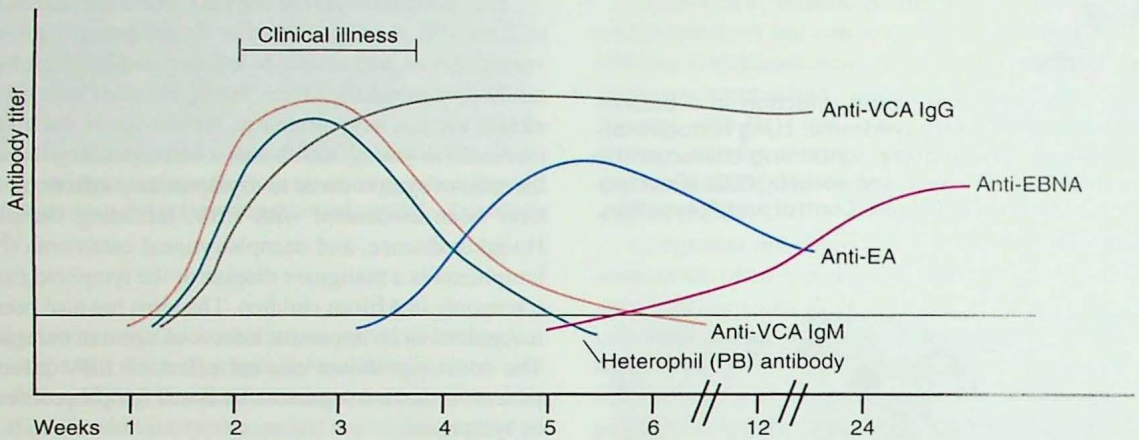

FIG. 29.9 Serologic evaluation of Epstein-Barr virus infection (infectious mononucleosis) showing the rise and fall of detectable antibodies. *Anti-EA,* Antibody to early antigen; *anti-EBNA,* antibody to Epstein-Barr virus nclera antigen; *anti-VCA IgG,* immunoglobulin G antibody to the viral capsid antigen; *anti-VCA IgM,* immunoglobulin M antibody to the viral capsid antigen; *PB,* Paul-Bunnell.

- *Anti-EA/R (antibody to early antigen, restricted):* Antibodies to EA/R appear in the acute phase and disappear soon after anti-EA/D, but can persist for up to 2 years and may be lifelong in some patients. Anti-EA/R IgG antibody level is elevated in patients with Burkitt lymphoma.
- *Anti-EBNA (antibody to the EBV nuclear antigen):* Antibodies appear about 1 month after infection, with titers peaking in 6 to 12 months.

There are several molecular assays coming to market that will use rtPCR to both detect and quantitate viral load that will be key in patient treatment and also to measure the effectiveness of treatment for EBV-positive patients. This will especially critical in persons who also have other medical conditions that lower immune status, such as HIV infection or diabetes.

Varicella-Zoster Virus

VZV is in the subfamily Alphaherpesvirinae and the genus *Varicellovirus.* VZV spreads by droplet inhalation or direct contact with infectious lesions. Cell-free virus is produced at very high levels in the skin vesicles, and thus the fluid from these vesicles is highly infectious. The virus causes two different clinical manifestations—varicella (chickenpox) and zoster (shingles). In the United States, more than 90% of adults have antibody to VZV. Varicella is the primary infection and is highly contagious (Fig. 29.10). In contrast to infections with the other herpesviruses that do not usually manifest symptoms, varicella is generally clinically apparent. It commonly appears in childhood and includes symptoms such as a mild febrile illness, rash, and vesicular lesions.

FIG. 29.10 Electron micrograph of a varicella virus (×100,000). (Courtesy Erskine Palmer and B.G. Partin, Centers for Disease Control and Prevention, Atlanta, GA.)

Usually, the lesions appear first on the head and trunk and then spread to the limbs. The lesions dry, crust over, and heal in 1 to 2 weeks. Painful oral mucosal lesions may develop, particularly in adults.

Herpes zoster is the clinical manifestation caused by reactivation of VZV; it usually occurs in adults. Approximately one in three adults will develop herpes zoster in their lifetime. It is thought that the virus remains latent in the dorsal root or cranial nerve ganglia after primary infection. In a small proportion of patients, the virus becomes reactivated, travels down the nerve, and causes zoster. The most common presentation is rash, followed by vesicular lesions in a unilateral dermatome pattern. These lesions may be associated with prolonged disabling pain that can remain for months, long after the vesicular lesions disappear.

VZV infection is usually diagnosed on the basis of characteristic clinical findings. In atypical cases, such as in immunosuppressed patients, the diagnosis may be more difficult or questionable. In such patients, culture of fresh lesions (vesicles) or the use of fluorescent-labeled monoclonal antibodies against VZV confirms the diagnosis. VZV can be cultured on human embryonic lung or Vero cells. Cytopathic changes may not be evident for 3 to 7 days.

Over the last few years amplified nucleic assays, such as PCR assays, have become the standard for the diagnosis of VZV disease. These assays have revolutionized the diagnosis of VZV disease of the CNS and of disseminated VZV infection, especially in immunocompromised patients (HIV infection, diabetes), and the identification of herpes zoster in patients who do not develop the rash associated with VZV. The advantages of these molecular assays are that they require small specimen volumes and are highly sensitive, rapid, and specific.

An attenuated vaccine to prevent chickenpox was approved for use in the United States in 1995. Before routine use of the vaccine in children, an estimated 4 million to 5 million cases occurred annually. The vaccine is expected to give lifelong immunity. In 2006 a single dose-attenuated vaccine for shingles, Zostavax (Merck, Whitehouse Station, NJ), which uses an attenuated VZV, was approved. The vaccine is recommended for individuals 50 years of age or older. Antiviral treatment of VZV infection and reactivation is possible and is quite effective in reducing the infection time for patients especially if coupled with more rapid molecular diagnostics.

Human Herpesvirus 6

HHV-6 is in the genus *Roseolovirus*. The two variant of the virus, A and B, are indistinguishable serologically, but variant B appears to be the cause of disease. HHV-6 is a common pathogen. About 95% of young adults are seropositive. Studies have shown that the virus persists in the salivary glands and has been isolated from stool specimens, but most evidence indicates that saliva is the most likely route of transmission. Inhalation of respiratory droplets from and close contact with infected individuals is the primary portal of entry.

HHV-6 has been associated with the childhood disease *roseola*, which is also called *roseola infantum, exanthem subitum*, and *sixth disease*, reflecting its role as the sixth childhood rash. Children are protected by maternal antibodies until approximately 6 months of age. Seroconversion occurs in 90% of children between the ages of 6 months and 2 years. In immunocompetent individuals, most infections are mild or asymptomatic. When symptoms occur, the disease is acute and febrile; a maculopapular rash appears as the fever resolves. About 30% to 40% of infected children with symptoms experience seizures. As with all members of the family Herpesviridae, reactivation of latent infections can become clinically significant in immunocompromised individuals. HHV-6 has also been proposed as having some involvement in the development of progressive multifocal leukoencephalopathy and multiple sclerosis.

The diagnosis of HHV-6 infection is usually made clinically. Isolation of the virus is most sensitive with lymphocyte cell culture, which is not practical for routine diagnosis. Serology may not be helpful unless paired sera are available. Patients do not usually have a positive IgM result until about 5 days after infection; IgG appears several days later. PCR and viral load testing offer the most sensitive and specific means of diagnosing primary HHV-6 infection.

Human Herpesvirus 7

HHV-7 is in the genus *Roseolovirus* with HHV-6. The CD4 molecule serves as a receptor for HHV-7 to infect T lymphocytes. It also uses other receptors and has a broad range of host cells. Like HHV-6, HHV-7 is extremely common and is shed in the saliva of 75% of adults. The virus causes roseola, which is clinically identical to that caused by HHV-6. HHV-7 causes latent infections in T lymphocytes. Despite the similarities between HHV-6 and HHV-7, their antigenic diversity is such that antibodies to one virus do not protect against infection from the other. In addition, exposure to HHV-7 seems to occur later in life than exposure to HHV-6. Most 2-year-olds are seronegative for HHV-7, but most children are seropositive by the age of 6 years.

HHV-7 can be isolated in culture in peripheral blood lymphocytes or in cord blood lymphocytes. Although the virus can be isolated from the saliva of healthy individuals, it is rarely isolated from peripheral blood mononuclear cells. PCR assay can detect the virus, but the ubiquitous nature of the virus can lead to difficulties in interpreting the results. Serologic results can be confusing because of cross-reactions, but patients with rising levels of antibody to HHV-7 but not to HHV-6 may have an active HHV-7 infection.

Human Herpesvirus 8

HHV-8, in the genus *Rhadinovirus*, can be detected in all forms of KS, including AIDS-related, Mediterranean, and HIV-1–negative KS, which is endemic to Africa, as well as posttransplantation KS. This association has earned it as the more common name *Kaposi sarcoma–associated herpesvirus*. It has also been shown to play a role in the development of primary effusion lymphomas and multicentric Castleman disease.

In North America and much of Europe, HHV-8 appears to be transmitted primarily through sexual contact, but studies in Africa and some Mediterranean populations suggest transmission by more casual means. The pattern of infection is similar to that of HSV-2, although men who have sex with men (MSM) seem to be more susceptible than heterosexuals. Prevalence ranges from close to zero in a study of Japanese blood donors to more than 50% in some parts of Africa. In HIV-positive persons, the seroprevalence can be as much as 20% to 50% higher than that of the surrounding healthy population. In the United States, as many as 20% of normal adults have antibodies to HHV-8, as do 27% of patients with HIV-1 who do not have KS and 60% of patients with HIV and KS.

Currently, the virus cannot be recovered in cell culture. Nucleic acid testing using PCR assay, although considered very sensitive, has been shown to be less sensitive than some immunologic assays. However, PCR assay has been used to detect the virus in various specimens, including tissue, blood, bone marrow, saliva, and semen. HHV-8 DNA or antigens are rarely detected in immunocompetent individuals, even if they are seropositive. In situ hybridization can detect HHV-8–affected tissue. The availability of commercially prepared monoclonal antibodies has made the identification of HHV-8–infected cells in various types of lesions by immunohistochemistry more common. Serologic tests are being evaluated and may soon be available.

Papillomaviridae

Papillomas, or warts, caused by HPVs, are clustered in five genera: *Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus,* and *Nupapillomavirus.* The majority of clinically significant HPVs are found in the genus *Alphapapillomavirus,* which includes types infecting the genital and nongenital mucosa and genital cutaneous surfaces as well as types most often seen in human cancers. Although associated with the common wart, some HPV types are linked to cancers, including cervical cancer. There are more than 100 types of these small dsDNA viruses; more than 40 types are sexually transmitted and are known as the genital types. HPV 1, 2, 3, and 4 are thought to infect all children and young adults universally, with no significant consequences. Different HPV types exhibit different tissue tropism based on the type of epithelial cells that the viruses preferentially infect, cutaneous or mucosal. The genital HPVs are further categorized as low, intermediate, or high risk based on their association with genital tract cancers. Table 29.5 lists some HPV types and their clinical significance.

Cervical HPV lesions typically consist of flat areas of dysplasia and are often difficult to see. Rinsing the area with 5% acetic acid, which turns the lesion white, makes the lesions more visible; however, this method is not used for diagnosis. Some types of HPV will result in genital wart formation (condylomata acuminata) that can easily be identified. Lesions can be removed by several methods, including surgery, cryotherapy, and laser.

The HPVs cannot be grown in cell cultures; therefore laboratory diagnosis of HPV infection often involves cytology sections. Cytotechnologists and cytopathologists read Pap smears and look for koilocytes, cells with perinuclear clearing accompanied by an increased density of the surrounding rim of cytoplasm, which are indicative of HPV infection. Additional testing, such as nucleic acid probe tests, can help detect HPV DNA in endocervical cells and identify the HPV type. PCR techniques are more sensitive and have shown that HPV is present in 95% or more of invasive cervical cancers, but the presence of the virus alone is not the sole factor in cancer development. As many as one third of all college-age women are infected with HPV, and most develop only subclinical infections. Because finding HPV in cervical tissue is not the sole predictor of invasive disease, there is some debate about whether it is useful to look routinely for the virus in cervical specimens. A quadrivalent vaccine, Gardasil (Merck, Kenilworth, NJ), against HPV types 6, 11, 16, and 18 to prevent cervical cancer was approved by the FDA in 2006 for females aged 9 to 26 years; the vaccine was approved for use in males in 2009. A second vaccine that protects against HPV types 16 and 18, Ceravix (GlaxoSmithKline, Brentford, UK), was approved for use in women in 2009. HPV types 16 and 18 are linked to the majority of cervical cancers and other HPV-associated cancers. There has been controversy about the use of these vaccines because it is recommended that they be administered at a young age, preferably before sexual activity.

Poxviridae

Poxviruses belong to the family Poxviridae and subfamily Chordopoxvirinae, and they are among the largest of all viruses. They are about 225 to 450 nm long and about 140 to 260 nm wide. These viruses have a characteristic brick shape and contain a dsDNA genome. Variola virus belongs to the genus *Orthopoxvirus.* Other

TABLE 29.5 Human Papillomaviruses and Their Clinical Significance

Human Papillomavirus Type	Clinical Manifestation	Association with Malignancy
Cutaneous		
1	Plantar warts	None
2–4	Common warts	None
5, 8, 9, 12, 14, 15, 17, 19-25, 36–38	Flat and macular warts	>30% of patients with epidermodysplasia verruciformis (a rare autosomal disease) with types 5, 8, 14, 17, and 20 develop malignancy
26–29, 34	Common and flat warts	Frequent, especially in immunosuppressed patients
Mucosal		
6, 11	Papillomatosis, primarily laryngeal, also upper respiratory tract and condylomata acuminata (genital warts)	Low risk
42, 43, 44	Condylomata acuminata	Low risk
31, 33, 35, 51, 52	Condylomata acuminata	Intermediate risk
16, 18, 45, 56, 58, 59, 68	Condylomata acuminata	High risk

Adapted from Gravitt PE, Ginocchio CC: Human papillomaviruses, In Versalovic J, et al, editors: *Manual of Clinical Microbiology,* ed 10, Washington, DC, 2011, American Society for Microbiology, p. 1612.

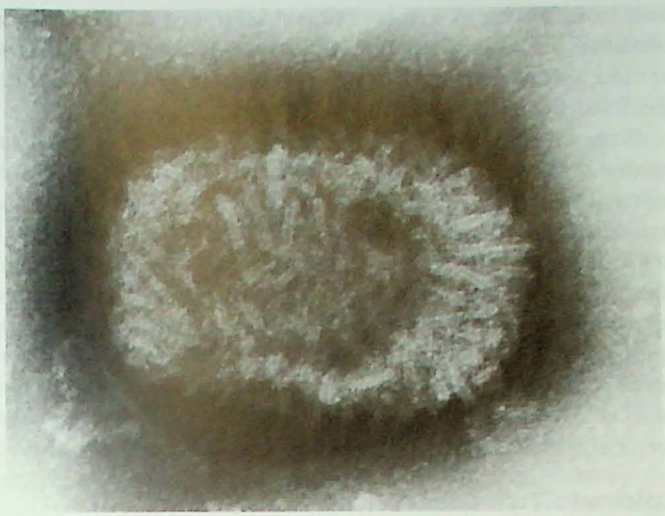

FIG. 29.11 Negatively stained transmission electron micrograph of the smallpox (variola) virus (×100,000). (Courtesy J. Nakano, Centers for Disease Control and Prevention, Atlanta, GA.)

members of the genus include **vaccinia virus** (the smallpox vaccine strain), monkeypox virus, cowpox virus, and other poxviruses.

Variola Virus

Variola virus causes smallpox, a disease that was common throughout early history (Fig. 29.11). Edward Jenner demonstrated the efficacy of vaccination against smallpox in 1796, which ultimately led to control and eradication of the disease. The last reported case of smallpox in the United States was in 1949, and the last case of smallpox worldwide was in Somalia in 1977. The World Health Organization (WHO), after decades of aggressive efforts toward vaccination, education, and eradication, officially declared the world free of smallpox in May 1980. Although extinct in nature, at least two cultures are known to be maintained. One culture is kept in the United States at the Centers for Disease Control and Prevention (CDC) and one is kept in Russia. These cultures are kept under strict security measures, and their very existence remains a point of contention between scientists who advocate their destruction and those who wish to continue studying the virus. The two countries continue to defend maintaining these stocks on the grounds that further study is needed for the production of better vaccines and as countermeasures to bioterror.

Typically, smallpox is characterized as a synchronous progressive rash accompanied by fever. The incubation period is approximately 10 to 17 days. The patient becomes febrile, and oral lesions can appear. At this point, the patient is infectious. Within 24 to 48 hours, a faint macular rash develops on the body. The rash appears on all parts of the body, but lesions are present in greater concentration on the head and limbs (centrifugal distribution), including the palms and soles. The macular rash progresses into papules, then vesicles, and finally into pustules that resemble chickenpox lesions. Pustules are deeply embedded into tissues. All lesions change at the same time—hence the term *synchronous*. Infected persons with dark skin tones typically scar as a result of this infection because of the depth of the pustules. The mortality rate for smallpox was, on average, 30%. Other forms of smallpox, including flat and hemorrhagic smallpox, occurred rarely and were almost always fatal.

Routine vaccination against smallpox in the United States ended in 1972, and other countries have also stopped their vaccination programs. The modern vaccine, ACAM2000, is manufactured by Sanofi Pasteur Biologics (Canton, MA). Although smallpox was eradicated with the use of a relatively safe vaccine, the vast majority of today's population is now susceptible to infection because vaccination is thought to offer protection for only up to 10 years. Because most humans are now susceptible to variola virus, it remains a threat as a bioterrorist weapon. Most military personnel moving into areas of conflict receive the vaccination, with little to no ill effects. If an outbreak of smallpox were to occur, there is sufficient stock of smallpox vaccine to mount an effective vaccination program in the United States. The antiviral compounds cidofovir and brincidofovir have been shown to be effective in combating disease in humans. More information on smallpox as an agent of bioterrorism can be found in Chapter 30.

Monkeypox Virus

Monkeypox was first described in primates in 1958, with the first case of human monkeypox in 1970. In 2003, a multistate outbreak of monkeypox occurred in the United States. It is thought that the virus was introduced into the country by rodents imported from Africa (Gambian rats). Human monkeypox infection occurs primarily in central and western Africa. Human infections are rare but result in a vesicular, pustular febrile illness that is very similar to smallpox. Monkeypox infections are less severe in humans compared with smallpox, and mortality rates are significantly lower.

Single-Stranded DNA Viruses

Parvoviridae

The smallest of the DNA viruses are the Parvoviridae, which are naked ssDNA viruses that measure about 22 to 26 nm in diameter. Parvovirus B19 is the principal pathogen in the family. It is classified in the genus *Erythrovirus*. Parvovirus B19 was named after the serum sample (number 19 of panel B) in which the initial viral isolate was observed by electron microscopy.

Infections range from symptomless to potentially fatal. The most commonly recognized syndrome is erythema infectiosum, more commonly referred to as *fifth disease*. Patients with erythema infectiosum experience a prodrome of fever, headache, malaise, and myalgia, with respiratory and GI symptoms (nausea and vomiting). The prodromal phase lasts a few days, after which a rash often appears. The rash gives a slapped cheek appearance and then spreads to the trunk and limbs. The rash occurs more commonly in children than in adults, lasts as long as 2 weeks, and can recur after exposure to heat and sunlight. Adults may also experience arthralgia, arthritis, or both. In some cases, this connective tissue manifestation occurs without the prodrome or rash stage. Most infections occur in children and adolescents, and 80% of adults are seropositive by the age of 65 years.

Parvovirus B19 viremia can cause transient aplastic crisis, a self-limiting erythropoietic arrest. Erythroid precursor cells contain a receptor for the virus, allowing viral infection and replication. The disease is characterized by a decrease in RBC production in bone marrow. In otherwise normal individuals, this decrease results in a short-lived anemia. The disease can be severe; complications include viremia, thrombocytopenia,

granulocytopenia, pancytopenia, flulike symptoms, and congestive heart failure. Within about 1 week, reticulocytosis occurs, and the patient recovers. Persons with weakened immune systems caused by HIV infection, organ transplantation, cancer, or leukemia are at risk for serious complications from fifth disease.

The viremia caused by parvovirus B19 creates a risk for blood donors and fetuses. In utero infection can cause hydrops fetalis resulting from anemia. Although the most vulnerable period for the fetus is the third trimester, most women exposed to the virus do not develop an acute disease, and few infections result in loss of the fetus. The infection usually does not require therapy other than relief of symptoms, such as anti-inflammatory agents for painful joints and generalized aches.

A novel parvovirus was described in 2005. It was named the *human bocavirus* (HBoV) and causes a variety of upper and lower respiratory tract illnesses. HBoV is closely related to the bovine parvovirus and canine minute virus, both members of the genus *Bocavirus* of the family Parvoviridae. Clinical symptoms of HBoV infection include cough, rhinorrhea, fever, difficulty breathing, diarrhea, conjunctivitis, and rash. HBoV has been increasingly present as a co-infection with RSV and human metapneumovirus (hMPV). Some studies have indicated a potential link to HBoV respiratory illness and gastroenteritis. However, researchers concluded that HBoV is shed in high quantities in stool, but its link to gastroenteritis has not been demonstrated.

HBoV infection is highest during the winter months and has been detected worldwide in 5% to 10% of children aged 7 to 18 months with upper and lower respiratory tract infections. Since its first description, HBoV has been described in at least 19 countries on five continents. HBoV was detected less frequently than common respiratory agents (e.g., influenza virus, parainfluenza virus, adenovirus, RSV) in South African children. Detection of HBoV has improved with the development of sensitive and specific rtPCR assays.

Double-Stranded RNA Viruses

Reoviridae

Rotaviruses

Rotaviruses are naked viruses about 75 nm in diameter, with two protein layers surrounding the capsid. They belong to the genus *Rotavirus*. Rotaviruses are the most common cause of viral gastroenteritis in infants and children. Gastroenteritis is a major cause of infant death and failure to thrive. Rotaviruses have a worldwide distribution and cause an estimated 611,000 deaths annually. Most outbreaks occur in the winter months in the temperate zones and year-round in subtropical and tropical regions.

Rotaviruses are spread by the fecal-oral route and have an incubation period of 1 to 4 days. Symptoms generally occur suddenly and include vomiting, diarrhea, fever, and in many cases, abdominal pain and respiratory symptoms. Vomiting and diarrhea can cause rapid loss of fluids and fatal dehydration. The rotavirus replicates in the epithelial cells in the tips of the microvilli of the small intestine. The microvilli are stunted and adsorption is reduced. The virus is shed in large quantities in the stool and can cause nosocomial outbreaks in the absence of good hygiene. Although the rotavirus is present in large numbers in stools, it can be isolated only with special procedures. Enzyme-linked immunosorbent assay (ELISA) and latex agglutination tests detect the viral antigens in fecal material. Rapid membrane-bound colorimetric tests are also available. Electron microscopy examination of stool samples can be used; however, this method is not very sensitive and is usually restricted to large reference or research laboratories.

With the introduction in 2006 of a human-bovine rotavirus vaccine (RV5; RotaTeq, Merck), a delay in the onset of the rotavirus season from mid-November to late February was seen. RotaTeq is a series of three oral vaccines administered beginning at age 6 to 12 weeks. A second vaccine, Rotarix (RV1; GlaxoSmithKline, Brentford, UK), was approved in June 2008. In clinical trials, both vaccines were shown to be safe and effective. During approximately the first year of an infant's life, rotavirus vaccine prevented 85% to 98% of severe rotavirus illness episodes and prevented 74% to 87% of all rotavirus illness episodes, which was a significant reduction in both populations.

Colorado Tick Fever Virus

The genus *Coltivirus* contains the Colorado tick fever virus that causes a dengue-like infection in the western United States and Canada. It is an 80-nm spherical particle with two outer shells containing 12 RNA segments. Because Colorado tick fever is not reportable, actual numbers of cases are unknown. It is thought to be one of the most common diseases transmitted by ticks in the United States. Viruses transmitted by arthropods, such as ticks and mosquitoes, are referred to as arboviruses. The vector for the infection is *Dermacentor andersoni*, which has many hosts in nature, including deer, squirrels, and rabbits. Infected individuals develop fever, photophobia, myalgia, arthralgia, and chills. As with dengue, patients can also have a biphasic fever with a rash, and children can experience hemorrhagic fever. No commercially produced laboratory tests are available, but recombinant immunoassays to detect Colorado tick fever IgG have been developed. Some nucleic acid–based assays using rtPCR in research laboratories are becoming available and may be helpful in the future. Many clinicians rely on ruling out other tickborne diseases to diagnose Colorado tick fever.

Single-Stranded RNA Viruses

Arenaviridae

The arenaviruses get their name from the Latin *arena*, meaning "sand." Under an electron microscope, arenaviruses appear sandy and granular. There are 43 named arenaviruses; the family includes many species that cause hemorrhagic fever. Arenaviruses are commonly divided into two groups—Old World and New World viruses. The New World complex includes Tacaribe, Junín, Machupo, Amapari, Cupixi, Parana, Latino, Pichinde, Tamiami, Flexal, Guanarito, Sabiá, Oliveros, whitewater Arroyo, Pirital, and Bear Canyon, Ocozocoautla de Espinosa, Allpahuayo, Tonto Creek, Big Brushy Tank, Real de Catorce, Catarina, Pampa, Skinner Tank, and Chapare viruses. The Old World complex contains lymphocytic choriomeningitis (LCM) virus and the Lassa viruses, including the Lassa-like viruses Mopeia, Mobala, and Ippy viruses. More recently, new arenaviruses have been isolated or identified through molecular detection and sequencing from rodents, and these include Merino Walk virus, Menekre and Gbagroube viruses, Kodoko virus, Morogoro virus, Lemniscomys and Mus minutoides viruses, and Luna virus; or they have been identified from humans, in the case of Lujo virus from an outbreak of human fatal

hemorrhagic fever, in which person-to-person transmission was documented. Some of the viruses have not yet been isolated and are known only from molecular sequencing data.

The first arenavirus to be described was LCM virus in 1933. Subsequently, several others were identified. The first arenavirus found to cause hemorrhagic fever was Junín virus, which causes Argentine hemorrhagic fever. In 1969, Lassa virus was isolated in Africa, that became the basis of the novel *Fever* by John Fuller. The book details the emergence of Lassa fever, that in retrospect is eerily similar to the emergence of other hemorrhagic fevers, including Ebola virus disease, which would be identified later in Africa.

The arenaviruses infect rodents, and humans are exposed to the disease by zoonotic transmission. All have been isolated from rodents of the family Muridae. The rodents are infected for long periods and typically do not become ill when infected by the viruses, which they shed in urine, feces, and saliva. In some parts of the United States, as many as 20% of *Mus musculus* mice carry LCM virus. Pet hamsters are also reservoirs. Humans become infected when they inhale the aerosolized virus or come into contact with fomites. LCM virus causes flulike illness; about 25% of infected individuals develop meningitis.

Lassa virus is the most well known of the arenaviruses. Most exposed individuals develop an asymptomatic infection, but some patients experience fever, headache, pharyngitis, myalgia, diarrhea, and vomiting. Some patients develop pleural effusions, hypotension, and hemorrhaging. CNS involvement includes seizures and encephalopathy. The mortality rate is about 15% for patients who become so ill that they need hospitalization. West African nations are most affected, with more than 200,000 cases and approximately 3000 deaths occurring annually.

Spread of the virus through airline travel by people from endemic areas highlights the vulnerability of large populations; this combined with the resurgence of the infection in Nigeria has prompted vaccine development efforts, with some using a genetically engineered virus offering promising early results. Most cases of Lassa fever are community acquired, primarily through contact with excretions from the multimammate rat *Mastomys natalensis*, which, once infected, sheds the virus throughout its life. Humans inhale the aerosolized virus or contract the virus directly through breaks in skin. Lassa virus is present in throat secretions, can be transmitted from person to person, and can also be transmitted through sexual contact and nosocomially. If therapy begins within the first 6 days of exposure, Lassa virus infection can be effectively treated with the antiviral drug ribavirin. Diagnosis of Lassa virus infection is typically made with ELISA to detect IgM and IgG antibodies.

Bunyaviridae

The family Bunyaviridae includes the genera *Orthobunyavirus*, *Phlebovirus*, and *Nairovirus*, which are classified as arboviruses. These viruses replicate initially in the gut of the arthropod vector and eventually appear in saliva. The arthropod transmits the virus when feeding on the blood of vertebrate hosts, including humans. After a few days, the infected host usually develops an asymptomatic viremia; however, some hosts become febrile, which is far less common. Most members of the family Bunyaviridae cause a febrile illness, hemorrhagic fever, or encephalitis. Rift Valley fever virus targets the brain and liver to cause encephalitis and hepatitis. LaCrosse virus (LACV) and California encephalitis virus cause encephalitis, and Crimean-Congo hemorrhagic fever (CCHF) virus infects the vascular endothelium and liver. The hantaviruses, which also belong to this family, do not infect arthropod hosts. They are rodent-borne viruses. Hantaviruses typically affect the peritoneal cavity, kidneys, thoracic cavity, or lungs.

CCHF virus causes a high-mortality infection in humans. Infection begins with fever, myalgia, arthralgia, and photophobia. Patients exhibit mental status changes, ranging from confusion and agitation to depression and drowsiness. Petechiae and ecchymoses can form on mucosal surfaces and on skin. The patient may bleed from the bowel, nose, and gums. About 30% of patients die. Others begin recovering after about 10 days of illness. Nosocomial transmission of CCHF virus has been reported. In the United States, LACV infects as many as an estimated 300,000 persons annually; about 80 to 100 have severe CNS disease. The incidence is underestimated because the disease manifests itself as a nonspecific fever, headache, nausea, vomiting, and lethargy. The disease is commonly found in children and usually develops in the summer, frequently referred to as the *summer flu* or *summer cold*. Because serologic tests for LACV are not offered in most laboratories and because this disease has very low mortality (about 1%), a definitive diagnosis is not often seen.

The genus *Hantavirus* includes Hantaan virus, Seoul virus, Puumala virus, and Dobrava virus, which cause a disease called *hemorrhagic fever with renal syndrome* (HFRS). These viruses are present in Asia and Europe, with the exception of Seoul virus, which is found worldwide. Hantaviruses endemic to Europe and Asia are called *Old World hantaviruses*. Puumala virus is the most common member of this genus in Europe and causes a mild form of HFRS, called *nephropathia epidemica*. Viruses causing HFRS target the kidneys. Patients develop a febrile prodrome and enter a phase of fever and shock, accompanied by oliguria. The kidneys gradually regain function as the patient recovers. The mortality rate for HFRS is 1% to 15%.

In 1993, two adults from the same household in New Mexico died of an unusual respiratory illness. Serologic testing indicated that these patients had been exposed to an unknown agent that was antigenically related to one of the Asian hantaviruses in spite of the different clinical presentation. Serosurveys also determined that 30% of the deer mice tested in the New Mexico area were seropositive for the same unknown virus. The virus was ultimately characterized as a new hantavirus and was named *Sin Nombre* ("no name") *virus* (SNV). The disease caused by this virus became known as *hantavirus pulmonary syndrome* (HPS). Molecular techniques were subsequently developed to detect many new hantaviruses in the Americas (Table 29.6), sometimes called the *New World hantaviruses*.

SNV is transmitted through inhalation of contaminated aerosolized mouse urine, saliva, and feces. Generally, person-to-person transmission does not occur with hantavirus. Patients with HPS have a 3- to 5-day febrile prodrome, with fever, chills, and myalgia. Patients then enter a phase of hypotensive shock and pulmonary edema. The patient develops tachycardia, hypoxia, and hypotension. In severe cases, the patient can develop disseminated intravascular coagulation. The mortality rate for HPS is about 50%. Treatment for HPS is primarily supportive. No FDA-approved laboratory tests for the identification of a hantavirus infection are available; however, some European tests are being evaluated. Some state health laboratories and the CDC perform EIAs to detect anti-SNV

TABLE 29.6 Hantaviruses That Cause Hantavirus Pulmonary Syndrome

Hantavirus	Host	Location
Sin Nombre	*Peromyscus maniculatis* (deer mouse)	United States, western Canada
Black Creek Canal	*Sigmodon hispidus* (cotton rat)	United States, South America
Bayou	*Oryzomys palustris* (rice rat)	Southeastern United States
Monongahela	*Peromyscus maniculatis* (deer mouse)	Eastern United States
New York	*Peromyscus leucopus* (white-footed deer mouse)	New York
Oran	*Oligoryzomys longicaudatus*	Argentina
Andes	*Oligoryzomys longicaudatus*	Argentina, Chile
Lechiguanas	*Oligoryzomys flavescens*	Argentina
Laguna Negra	*Calomys laucha* (vesper mouse)	Paraguay, Bolivia

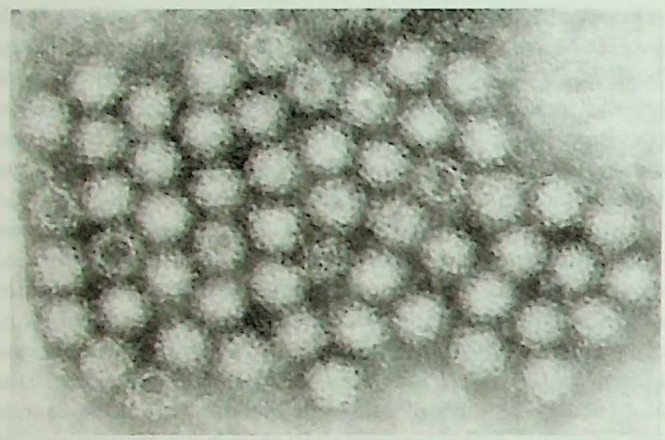

FIG. 29.12 Transmission electron micrograph revealing the ultrastructure morphology of norovirus virions (×100,000). (Courtesy Charles D. Humphrey, Centers for Disease Control and Prevention, Atlanta, GA.)

IgM and IgG antibodies, which may provide some useful information on the infection. Immunohistochemistry is a sensitive method used to detect hantavirus antigens in the capillary endothelium; a high concentration of antigens is found in capillary tissue specimens from the lung.

Caliciviridae

The family Caliciviridae contains five genera (*Norovirus, Sapovirus, Nebovirus, Lagovirus, Vesivirus*) and four newly proposed genera (*Becovirus, Nacovirus, Valovirus, Recovirus*). The five genera include Sapporo virus, Norwalk virus, rabbit hemorrhagic disease virus, and feline calicivirus. Sapoviruses (SaVs) and noroviruses (NoVs) are causative agents of human gastroenteritis.

NoVs (Fig. 29.12) are the most common cause of infectious gastroenteritis in the United States, accounting for as many as 23 million cases annually. These small, ssRNA, round viruses, 27 to 30 nm in diameter, were, until recently, called *Norwalk-like viruses, caliciviruses,* and *small round structured viruses.* They are currently placed in the genus *Norovirus.* They cause outbreaks of acute gastroenteritis in schools, colleges, nursing homes, and families, as well as on cruise ships and in resort areas. NoVs have been found in drinking water, swimming areas, and contaminated food. Transmission is most commonly foodborne, although waterborne and person-to-person transmission can be significant.

The incubation period is 24 to 48 hours; the onset of severe nausea, vomiting, diarrhea, and low-grade fever is abrupt. The infection rate can be as high as 50%. The illness usually subsides within 72 hours. Immunity may be short-lived, leading to the potential for multiple infections throughout life.

The viruses cannot be grown in culture, so diagnosis relies on electron microscopy, immune electron microscopy, and real-time PCR. A newer assay using real-time, reverse transcriptase–polymerase chain reaction (RT-PCR) is now the most commonly used diagnostic assay for detecting NoV. This assay detects viral RNA and can be used to test stool, vomitus, and environmental samples. Stool is the best sample to use to detect NoV. It should be collected when a person has acute illness (within 48 to 72 hours after onset of symptoms). In some cases, NoV can be detected in stool samples collected 2 weeks after recovery. Several EIAs for detecting NoV in stool samples are available. The FDA has approved an EIA for detecting NoV during outbreaks. However, at this time, EIAs are not sensitive enough for diagnosing individuals suspected of being infected.

SaVs are small (30–35 nm in diameter) diarrheagenic viruses distinguished by a cup-shaped morphology. They usually cause diarrhea and vomiting in infants, young children, and older patients. Originally discovered in Sapporo, Japan, in 1977, these viruses are detected by electron microscopy, molecular (e.g., RT-PCR), and/or immunologic methods (e.g., ELISA).

Coronaviridae

Coronaviridae is one of four families within the order Nidovirales and is divided into two subfamilies—Coronavirinae and Torovirinae. Within the Coronavirinae, there are four genera, *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus.* Many new coronaviruses (CoVs) have recently been discovered and categorized genetically, and several hundred CoV genome sequences are now available. This has led to a reorganization of the taxonomic structure and the current phylogenetic relationship of the 20 recognized species within the Coronavirinae. CoVs have very large, linear, positive-stranded RNA genomes, ranging from approximately 25 to 32 kilobases (kb) in size, and they are enveloped helical. They were first identified by electron microscopy and were named for the distinctive club-shaped projections on their surfaces (Fig. 29.13). CoVs infect a number of different animals; however, most individual strains of virus typically infect only a single animal species. Some are able to infect more than one related species.

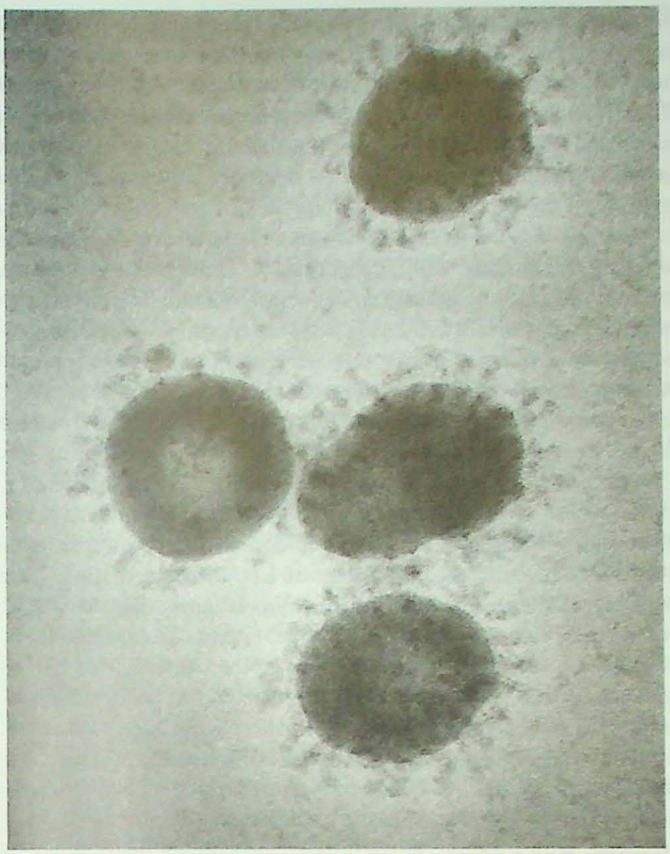

FIG. 29.13 Electron micrograph of a coronavirus (CoV). This virus derives its name from the fact that under electron microscopy, the virion is surrounded by a corona, or halo (×100,000). (Courtesy Fred Murphy and Sylvia Whitfield, Centers for Disease Control and Prevention, Atlanta, GA.)

CoVs may be responsible for 15% of coldlike infections in adults, but higher seroconversion rates have been seen in children. A few CoVs are responsible for a small percentage of pediatric diarrhea cases. In general, the illness lasts about 1 week, and blood may appear in the stool. CoVs are extremely fragile and difficult to culture, but it is possible to test specimens directly by IF and EIA methods. Today, the most common diagnostic approach for the identification of CoVs is amplification and detection of virus-specific RNA. Assays using a one- or two-step RT-PCR procedure for the amplification stage are the most popular. RT-PCR assays have demonstrated greater sensitivity and specificity and much shorter TATs. Other nucleic acid–based tests include isothermal amplification methods and loop-mediated amplification assays. These are typically used in research or larger reference laboratories.

A novel CoV was the causative agent of a pandemic respiratory disease that emerged from Hong Kong in late 2002. During a 6-month period, the infection spread rapidly to 26 countries in Asia, Europe, South America, and North America. The virus infected at least 8000 people and resulted in a mortality rate of approximately 10%. The disease was characterized by high-grade fever, pneumonia, and in some patients, acute respiratory distress syndrome. The disease was ultimately termed *severe acute respiratory syndrome* (SARS), and the causative agent was designated as the SARS-associated CoV (SARS-CoV). No vaccine or antiviral agent was available to fight the pandemic, which was ultimately ended through intense public health intervention, including massive screening programs, voluntary quarantine, and travel restrictions.

This human infection apparently started as a CoV that jumped from its normal animal host, possibly a civet cat, to humans. SARS-CoV highlights the public health risk that can occur when animal viruses suddenly appear in a susceptible human population. In 2012, there were reports of another possible SARS outbreak in China in Baoding City in the province of Hebei. It was reported that 300 soldiers received treatment. Although it was not confirmed to be SARS, the signs and symptoms indicated a high likelihood that this was the cause of this outbreak. In 2012, a SARS-like virus was linked to severe respiratory tract infections in the Middle East. This virus was named the Middle East respiratory syndrome CoV coronavirus (MERS-CoV). As of August 2013, MERS-CoV had been associated with 94 infections and 46 deaths, primarily in Saudi Arabia. MERS-CoV is unlike any other CoV that infects humans. In 2014, there was another outbreak of MERS-CoV in Saudi Arabia, with 14 people infected and four reported deaths. To provide a global perspective, to date, there have been 852 confirmed cases of MERS-CoV infection and 301 deaths.

The 2002 SARS outbreak created much interest in understanding the epidemiology, reservoir-host relationship, and vaccination possibilities associated with this human CoV. In 2007, SARS-CoV antibodies were detected in 47 of 705 South African and Democratic Republic of the Congo (DRC) bat sera samples collected from 1986 to 1999. Researchers in the United States tested bats from the Rocky Mountain region in 2006 and detected CoV RNA in two species of bats—*Eptesicus fuscus* and *Myotis occultus*. Animals, such as civets, may acquire infections with SARS-like CoV from contact with infected bats. Current data indicate that bats, primarily horseshoe bats, are the most likely reservoir for SARS-CoV, although the bat CoVs are species specific. More than 10 mammalian species have been identified as being susceptible to SARS-CoV by natural or experimental infection. Infections of these secondary hosts may give rise to strains that could potentially infect humans.

SARS-CoV targets the epithelial cells of the respiratory tract and is transmitted from person to person by the direct contact, droplet, or airborne routes. Other organ systems affected by SARS infection include the spleen, lymph nodes, digestive tract, urogenital tract, CNS, bone marrow, and heart. Virus can also be isolated from urine and feces, suggesting other potential routes of transmission. Certain individuals may be genetically more susceptible to SARS than others.

Detection methods include electron microscopy, ELISA (EUROIMMUN, Lübeck, Germany), and RT-PCR. Antibody can be detected through Western blot analysis. Treatment is mostly supportive, and precautions are taken to isolate and/or quarantine infected individuals.

Filoviridae

The family Filoviridae includes two genera—*Marburg virus* and *Ebolavirus*. Lake Victoria Marburg virus (formerly Marburg virus) is in the former genus, and Ebola virus Zaire strain (EBO-Z), Ebola virus Sudan strain (EBO-S), Ebola virus Reston strain (EBO-R), and Ebola virus Tai Forest strain all belong to the latter. Both Marburg virus and Ebola virus share a common morphology and similar genome and structural proteins. These viruses have many

similarities—they rarely cause human infections, they cause infections with high mortality rates, and they have unknown reservoirs in nature, although human infection can result from contact with infected monkeys.

Lake Victoria Marburg virus hemorrhagic fever was named after the location of one of the first outbreaks, Marburg, Germany. In 1967, 32 people from Marburg and Frankfurt, in Germany, and from Belgrade, Serbia, and Yugoslavia contracted an unknown infection, and seven died. Epidemiologists noted that the deceased individuals all worked in vaccine-producing facilities and had contact with African green monkeys that had arrived recently from Uganda. The virus had been transmitted to 24 other people through nosocomial transmission, casual contact, and sexual contact. Patients with secondary infection had a milder illness, and all survived the infection.

Outbreaks of Lake Victoria Marburg virus are noticeably rare, but some have been fierce. In 1998, in the DRC, formerly Zaire, a large outbreak associated with gold miners resulted in 149 cases, with a fatality rate of almost 83%. Another outbreak began in northern Angola in late 2004; by mid-2005, when reports of new cases began to decline, more than 350 people had died. Outbreaks have occurred in Angola (2005), Uganda (2007), and the Netherlands as a result of Ugandan importation (2008).

Lake Victoria Marburg virus hemorrhagic fever begins with a febrile prodrome. The fever can last 12 to 22 days. At the end of the first week of infection, a maculopapular rash appears on the trunk and extremities, usually followed by the development of worsening nausea, vomiting, and diarrhea. Patients begin bleeding from the nose, gums, and GI tract during the latter part of the first week. Liver hemorrhaging, myocarditis, kidney damage, and mental status changes occur, often followed by death. The infection can be diagnosed by using PCR, immunohistochemistry, and IgM-capture ELISA. Treatment of infected patients is primarily supportive and includes replacement of blood and clotting factors.

The Ebola viruses are named after the Ebola River in the DRC, where the infection first emerged in 1976 (Fig. 29.14). The virus emerged almost simultaneously in Sudan. In Zaire, a patient treated at a village hospital for a bloody nose probably introduced the virus into the hospital, where it was then transmitted nosocomially and via contact with infected individuals at home. Nuns in the hospital routinely reused syringes without sterilizing them. Therefore the hospital amplified the number of cases. Infections were also passed to the victims' families, often during a process in which the intestines of infected deceased males were cleaned to prepare the bodies for funerals.

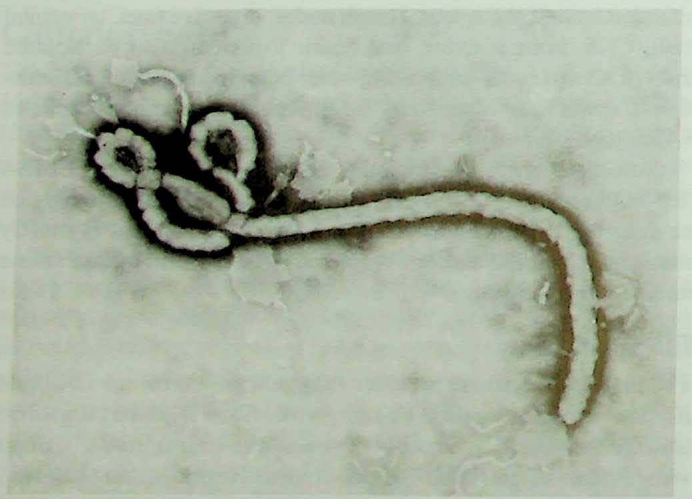

FIG. 29.14 Transmission electron micrograph of the Ebolavirus (×100,000). (Courtesy Fred A. Murphy, Centers for Disease Control and Prevention, Atlanta, GA.)

The two simultaneous outbreaks of Ebola fever were caused by two different species, EBO-Z and EBO-S; of the two, EBO-Z is the more virulent. In the initial outbreak, 318 individuals were infected in Zaire; the mortality rate was 88%. In the Sudan outbreak, 284 people were infected, and a mortality rate of 53% was reported. After the initial two large outbreaks, smaller outbreaks occurred. In Sudan, 34 cases occurred in 1979, and 65% of the 34 patients died. The virus seemed to retreat into the jungles for the next 15 years. However, an unusual sequence of events occurred in 1989, when monkeys imported to Reston, Virginia, from the Philippines were afflicted by an epidemic of infection by what eventually became the third type of Ebola virus, EBO-R. Four workers in the animal facility developed antibodies to EBO-R but did not develop the disease. EBO-R was isolated from the bloodstream of one of the workers. Another outbreak of EBO-R occurred in monkeys at a Texas quarantine facility in 1996. EBO-Z continued to emerge periodically in the DRC (2003 and 2007), southern Sudan (2004), and Uganda (2007). In 2012, several more outbreaks were reported. Beginning in March 2014, West Africa had the largest outbreak of Ebola in history, with multiple countries involved, with Liberia, Sierra Leone, and Guinea being impacted the most through early 2016. According to the CDC, there were 28,652 probable cases, with 15,261 laboratory confirmed cases and 11,325 deaths occurring during this outbreak. This outbreak resulted in precautions in both Europe and the United States to handle suspected cases of infected people flying in from West Africa. Many clinical laboratories have now established protocols to handle these suspected cases, ensuring safely for both staff and other patients. Newer molecular testing is being developed to detect Ebola from serum, and this will likely get rapid FDA review and approval.

Symptoms of Ebola hemorrhagic fever include fever, chills, myalgia, and anorexia 4 to 16 days after infection. Patients develop a sore throat, abdominal pain, diarrhea, and vomiting and also start to bleed from the injection sites and the GI tract. Hemorrhaging in skin and the internal organs may occur as well. Diagnosis of the infection can be made by using PCR, IF, or viral culture methods. Sometimes electron microscopy of clinical samples will yield the characteristic virions.

Flaviviridae

The family Flaviviridae contains a number of important human pathogens, many of which are zoonotic arboviruses, including Japanese encephalitis virus, dengue virus, yellow fever virus, St. Louis encephalitis (SLE) virus, Zika virus, Kyasanur Forest disease virus, Langat virus, Löuping ill virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, tickborne encephalitis virus, Wesselsbron virus, and WNV. Japanese encephalitis virus is a major cause of encephalitis in Asia and is the most common cause of arboviral encephalitis in the world. Because it is being reported in regions previously free of Japanese encephalitis, including Australia, Japanese encephalitis virus is

considered an emerging pathogen. Currently, 30,000 to 50,000 cases of Japanese encephalitis are reported annually. Most patients are asymptomatic. Disease ranges from a flulike illness to acute encephalitis. Children are mostly affected by this infection, with mortality as high as 30%; mortality in adults is much lower.

Another important member of the family Flaviviridae is dengue virus, which causes two distinct diseases—classic dengue fever (DF) and dengue hemorrhagic fever (DHF). Worldwide, tens of millions of cases of DF and approximately 500,000 cases of the more serious DHF occur annually. The average mortality associated with DHF is 5%, accounting for 24,000 deaths each year. Most deaths occur in children younger than 15 years. The virus is transmitted by *Aedes* mosquitoes, including *Aedes aegypti* and *Aedes albopictus*. These mosquitoes infest more than 100 countries and bring the risk of DF to 2.5 billion people.

Dengue virus has four serotypes (1 through 4). DF, which is a relatively mild infection, occurs when patients are bitten by mosquitoes carrying the virus. Patients with DF develop fever, headache, myalgia, and bone pain (resulting in the nickname "breakbone fever"). Some patients also develop a rash. The disease is self-limiting and often resolves in 1 to 2 weeks. Although classic DF is a mild disease, DHF is not. Patients develop DHF after they have already been exposed to one serotype of dengue virus and are then exposed to one of the other three serotypes. Exposure to two different serotypes of dengue virus appears to be necessary for development of DHF. Patients with DHF develop the symptoms of classic DF, along with thrombocytopenia, hemorrhage, shock, and sometimes death.

Yellow fever, caused by yellow fever virus, is also considered an emerging infection. Although a safe vaccine has been available for decades, about 200,000 cases of yellow fever and 30,000 resulting deaths are reported annually worldwide. The actual incidence may greatly exceed these numbers. The emergence results from increased spread of the mosquito vectors, deforestation of Africa and South America, and increased travel to endemic regions. The vaccine has greatly reduced or eliminated the transmission of yellow fever in some countries. However, yellow fever is still epidemic in parts of Africa and South America, where about 80% of the population must be vaccinated to reduce the impact of the disease.

Patients bitten by mosquitoes carrying yellow fever virus can develop an asymptomatic or acute infection involving fever, myalgia, backache, headache, anorexia, nausea, and vomiting. Most patients experiencing acute disease recover after about 4 days. However, about 15% enter a systemic toxic phase in which fever reappears. The patient develops jaundice (hence the name "yellow" fever) and bleeding from the mouth, eyes, nose, stomach, or other areas. The kidneys may fail, and about 50% of patients in the toxic phase die. The other 50% recover without serious sequelae.

The three different transmission cycles for yellow fever virus are the sylvatic, urban, and intermediate cycles. In the sylvatic cycle, yellow fever virus is maintained in monkey populations and transmitted by mosquitoes. Monkeys become sufficiently viremic to pass the virus to mosquitoes as they feed on the monkeys, thus keeping the transmission cycle active. Humans are not the usual hosts when they enter jungle areas in which the sylvatic cycle exists.

The urban cycle occurs in larger towns and cities when infected *A. aegypti* mosquitoes transmit the infection to humans. Because infected humans can continue the transmission when bitten by uninfected mosquitoes, large outbreaks can occur from a single case of yellow fever. The intermediate transmission cycle of yellow fever occurs in smaller villages in Africa. In the intermediate cycle, humans and monkeys are reservoirs, whereas mosquitoes are the reservoirs and vectors in the high-morbidity, low-mortality outbreaks. In the intermediate cycle, mosquitoes can transmit yellow fever virus from monkeys to humans, and vice versa. If patients who develop yellow fever from the intermediate cycle travel to larger cities and are bitten by mosquitoes, they can trigger an outbreak of urban yellow fever.

In the United States, the most common flavivirus infection is SLE. During the past 35 years, an average of 193 cases have been reported annually in the United States. Epidemics are more likely to occur in the Midwest or the Southeast, but cases have been reported in all of the lower 48 states. Patients with SLE are most likely to be asymptomatic. Symptomatic patients may develop a fever only, whereas some patients develop meningoencephalitis. The mortality rate of symptomatic patients is 3% to 20%. Unlike many of the arboviral infections, SLE is milder in children than in adults; older patients have the greatest risk of serious illness and death. SLE is transmitted to humans by the bird-biting *Culex* mosquitoes. Most infections occur in the summer months.

First isolated and identified in 1937 from a febrile patient in the West Nile district of Uganda, WNV is an ssRNA virus and member of the Japanese encephalitis antigenic complex, similar to SLE. The virus is transmitted by a mosquito vector between birds and humans. The virus replicates actively inside the avian host; however, the virus does not replicate well in humans, making humans the dead-end host of infection. The incidence of WNV infection in the United States increased during the mid-1990s, prompting the development of a national surveillance system in 1999. In 2002, the CDC documented 4156 human cases of WNV infection in 44 states. By 2008, the incidence of human WNV infections had dropped to 1356, with 44 deaths. In 2012, there was a resurgence of WNV infections. A total of 5387 cases and 243 deaths were reported; 51% of all cases were described as neuroinvasive. About one third of the total WNV infection cases were seen in Texas.

Approximately 80% of individuals infected with WNV are asymptomatic. The remaining 20% display symptoms of what is termed *West Nile fever*, which includes fever, headache, fatigue, occasional rash on the trunk, swollen lymph glands, and/or eye pain. The primary risk factor for serious neuroinvasive disease is age greater than 50 years. Neuroinvasive disease typically manifests itself as meningitis or encephalitis.

Laboratory tests approved for the detection of WNV include IgG and IgM ELISA, including a rapid WNV ELISA assay and an indirect IF assay to screen for antibodies. WNV ELISA assays can cross-react with other flaviviruses and should be confirmed by antibody neutralization. IgM antibody does not cross the blood-brain barrier; therefore the presence of IgM in CSF strongly suggests CNS infection. WNV can be present in tissues, blood, serum, and CSF of infected humans or animals. A number of RT-PCR, TaqMan, and nucleic acid sequence–based amplification assays have been used for successful confirmation of WNV. There is no specific treatment for WNV infection, but in severe cases requiring hospitalization, supportive care, including IV fluids, respiratory support, and prevention of secondary infection, may be warranted.

Zika virus is an insect vector–borne disease that is most commonly transmitted through *Aedes* (*A. aegypti* and *A. albopictus*) mosquitoes. Zika virus can also be transmitted through exposure to infected blood or sexual contact. Less commonly, Zika virus can be transmitted from mother to child during pregnancy. Zika virus is typically endemic to parts of Africa and Asia; however, in 2015 through 2016, between 400,000 and 1.4 million cases were reported across South, Central, and North America, where the disease was previously unreported. Almost 500,000 cases of Zika virus infection have been reported in 38 countries throughout the Americas. Almost 50,000 of those cases have been laboratory confirmed and 360,000 cases are suspected to be positive. The CDC has confirmed more than 2000 cases of Zika virus infection across 46 states and three US territories with just under half of the cases acquired through travel and the rest transmitted by mosquitos. Symptoms of Zika virus infection in most people are similar to those of infections with other arboviruses, such as chikungunya virus (fever, headache, and fatigue, primarily). The recent epidemic in Brazil has been marked by the detection of the disease in fetal amniotic fluid and an increased reporting of cases of *microcephaly* (small head size) in newborns. This outbreak has resulted in the FDA looking to give fast approval for a PCR-based assay, allowing more rapid testing in pregnant women, coupled with serology testing for both IgG and IgM antibodies to help diagnose potential Zika virus exposure and/or infection.

Orthomyxoviridae

The influenza viruses are members of the family Orthomyxoviridae. These viruses are distinguished by using two major structural proteins—matrix protein (M) and nucleoprotein (NP). This places the influenza viruses into three genera within the family—*Influenzavirus A, Influenzavirus B,* and *Influenzavirus C.* Influenza viruses have a worldwide distribution and originate as zoonotic infections, carried by several different species of birds and mammals. The influenza season in the southern hemisphere is from May to October and in the northern hemisphere is from November to April.

Influenza A virus remains one of the most crucial health problems worldwide. In the pandemic of 1918 and 1919, influenza killed an estimated 20 million to 50 million people, including more than 500,000 in the United States. However, in the past 90 years, the world has only been able to react to the threat of influenza, rather than vanquish it. In six different years, from 1972 to 1995, influenza deaths in the United States exceeded 40,000. A typical influenza season in the United States hospitalizes almost 200,000 and kills almost 36,000 people.

Influenza viruses are enveloped, and types A and B have eight segments of ssRNA. Influenza A viruses are classified into subtypes using the two major surface glycoproteins hemagglutinin (H) and neuraminidase (N). The H antigen is used to bind to host cells, and the N antigen cleaves budding viruses from infected cells. There are 16 H antigens (H1 through H16), although human infections usually occur only with H1, H2, and H3. In total, there are nine N antigens (N1 through N9); human infections usually occur with N1 and N2.

The key to the persistence of influenza virus is its antigenic variation. Each year, antigenic drifts are caused by RNA replication errors of the virus. **Antigenic drift** is a minor change in antigenic structure as mutations accumulate. Antigenic drifts occur with all three influenza viruses—A, B, and C. The surface antigens sometimes can change drastically, causing an **antigenic shift**, resulting in a new H or N antigen. There are two mechanisms of antigenic shift. The first is genetic reassortment of the eight ssRNA strands of two separate influenza strains. Pigs have receptors for both avian and human influenza viruses as well as swine influenza viruses and can be co-infected with all three types of viruses. A reassortment occurs when the genomes of different influenza viruses combine into a single virion, resulting in a new strain of influenza virus. The second mechanism is an adaptive mutation, in which a novel virus slowly adjusts and becomes transmissible from a mammalian (including human) host. Shifts result in novel strains of influenza virus, so the human population is likely to have little or no historic exposure or resistance to the new strain, which greatly increases the risk of pandemics. Antigenic shift is associated only with influenza A virus.

Three major shifts occurred during the 20th century. Infection with influenza A virus (H1N1), the *Spanish flu,* appeared in 1918 to 1919. H1N1 was the predominant strain until a shift to influenza A virus (H2N2) occurred in 1957 to 1958. That shift resulted in the pandemic *Asian flu.* In 1968, another shift occurred, and a pandemic strain of influenza A virus (H3N2) resulted in the *Hong Kong flu.* The dominant strains of influenza A virus since 1977 have been influenza A viruses H1N1 and H3N2.

In 1998, an outbreak of infection with influenza A virus (H5N1), the *avian flu,* appeared in poultry in Hong Kong. At least 18 humans acquired the disease via contact with birds that year, and six deaths were reported. H5N1 is a highly pathogenic avian influenza (HPAI) virus; historically, most human infections are acquired after direct or close contact with infected birds. The H5N1 influenza virus appeared in flocks in parts of Asia in 2004 and again in 2005, resulting in large poultry losses in Japan, Cambodia, China, Vietnam, and nearby countries. Some humans were infected during these poultry outbreaks. Again, the incidence of human disease was low, but the mortality rate was high, although it is possible that some mild cases went undiagnosed.

Studies documented wide dissemination of influenza A virus (H5N1) throughout Asia as a result of migrating birds. By early 2006, influenza A virus (H5N1) had been isolated from birds in Turkey, Greece, Italy, Germany, Iran, Iraq, Nigeria, and many other countries. The potential for the virus to adapt to the human host, through genetic reassortment or adaptive mutation, remains an important concern for future influenza seasons. As of April 2013, the WHO had reported over 600 human cases of influenza A virus (H5N1) infections, with a fatality rate of about 60% in 15 countries. In April 2013, a novel avian flu caused by influenza A virus (H7N9) was reported in China. Only a few cases were reported initially, but 5 of the 11 human cases were fatal. Human-to-human transmission was not documented.

In the spring of 2009, a highly infectious novel form of influenza, *swine flu,* caused by influenza A virus (H1N1) emerged. It was first reported throughout Mexico and forced school closings and cancellation of sporting events. As of October 9, 2009, the WHO had received reports of more than 375,000 human cases and 4525 deaths. Because many countries stopped counting individual cases, the actual number was much larger. The outbreak quickly reached phase 6, which is the WHO definition of a pandemic. This was the first influenza pandemic in 40 years. From April 15, 2009, to July 24, 2009, the CDC reported a total of 43,771 confirmed and probable cases of H1N1 infection

in the United States. Of the reported cases, 5011 people were hospitalized, and 302 deaths occurred. In April of 2010, the WHO announced that the H1N1 influenza virus had moved into the postpandemic period.

H1N1 was found to contain RNA from avian, human, and porcine strains of the virus. Infection spreads in the same way as do typical seasonal influenza viruses, mainly through droplets from coughing and sneezing of infected individuals. Infection may also be spread by handling fomites. H1N1 infection causes a wide range of flulike symptoms, including fever, cough, sore throat, body aches, headache, chills, and fatigue. In addition, many people reported nausea, vomiting, and/or diarrhea.

More recently, there has been an increase in infections by the influenza variant H3N2v, which is a nonhuman influenza virus normally found in swine. When viruses that normally circulate in animals infect humans, they are termed *variant viruses*. This H3N2v virus was first detected in humans in January 2011, and it had genes from avian, porcine, and human viruses and the 2009 H1N1 pandemic virus M gene. Through August 2013, 340 cases had been reported in the United States. Most of the cases occurred in Indiana and Ohio and were linked to farm activities, livestock shows, or county fairs.

Because the H and N antigens of influenza A virus continually change, the CDC and the WHO make recommendations for the composition of the trivalent influenza vaccine several months before the influenza season begins. The vaccine usually contains two different strains of influenza A virus and a single strain of influenza B virus. Influenza B virus infections, which also can occur seasonally, are usually less common than influenza A virus infections, although epidemics of influenza B virus infections can occur every few years. It is highly recommended that persons with reduced immune status (those with HIV, diabetes, etc. and older adults) be vaccinated each year to prevent infection because it can be rapidly fatal. The use of a live attenuated quadrivalent intranasal vaccine has been discontinued because of lack of effect; however, it may be reformulated for future use.

Influenza C virus is capable of causing mild upper respiratory tract illness in humans. The virus is enveloped. Its genome consists of seven ssRNA segments, lacking the gene coding for neuraminidase, as in influenza A and B viruses. Studies have shown influenza C virus to be more stable genetically compared with influenza A virus, and although reassortment does occur in the former, it is less prone to major changes in infectivity.

Influenza viruses are spread through aerosol inhalation. The viruses attack the ciliated epithelial cells lining of the respiratory tract, causing necrosis and sloughing of the cells. The incubation period is 1 to 4 days. Although asymptomatic infections can occur, infections are usually characterized by rapid onset of malaise, fever, myalgia, and often a nonproductive cough. Temperature can be as high as 41° C. Infected patients are ill for as long as 7 days, and convalescence may require more than 2 weeks. Influenza can also cause a fatal viral pneumonia. Complications include secondary bacterial pneumonia.

The best specimens are nasopharyngeal swabs, washes, or aspirates collected early in the course of the disease. Flocked swabs (Copan Diagnostics, Corona, CA) are reported to collect significantly more epithelial cells from the nasopharynx compared with rayon swabs. Specimens should never be frozen. A number of rapid kits are commercially available for the diagnosis of influenza in about 30 minutes. Some of these kits are of low complexity and have waivers from the CLIA. A kit can detect and distinguish between influenza A and B viruses, detect both influenza A virus and influenza B virus but not distinguish between them, or detect only influenza A virus. Influenza virus can be identified in respiratory secretions by using DFA, EIA, and optical immunoassays. Influenza viruses grow in the amniotic cavity of embryonated chicken eggs and various mammalian cell culture lines, such as PMK and MDCK cells. Influenza-infected culture cells adsorb RBCs, a feature that can be used to detect positive cell cultures. Rapid culture assays can be performed using IF staining of infected monolayers grown in shell vials or flat-bottomed wells of microtiter plates. Nucleic acid–based assays are also used for the detection of influenza viruses, with the most common method being RT-PCR. This is sometimes used in conjunction with rapid antigen kits. The benefit of quick positive identification combined with appropriate antiviral therapy often outweighs cost considerations.

The antiviral drugs amantadine and rimantadine can prevent infection or reduce the severity of symptoms if administered within 48 hours of onset of infection. These antiviral drugs are effective only against influenza A virus. A newer class of antivirals, termed *neuraminidase inhibitors*, is available. These agents, such as zanamivir (Relenza) and oseltamivir (Tamiflu), are more expensive compared with amantadine but provide coverage against infections by both influenza A virus and influenza B virus. Current CDC guidelines highly recommend both zanamivir and oseltamivir as primary treatments in confirmed cases of influenza. There has been significant resistance to amantadine by influenza A virus (H3N2), so it is not recommended for treatment of infection by this virus. There have been some reports of resistance to oseltamivir by influenza A virus (H1N1), but use of this agent is still recommended as a primary treatment.

Paramyxoviridae

Parainfluenza Viruses

Several genera belong to the family Paramyxoviridae, including *Morbillivirus*, *Paramyxovirus*, *Pneumovirus*, and *Rubulavirus*. Four types (1 through 4) of PIVs can cause disease in humans. Human PIV-1 PIV-3 belong to the genus *Paramyxovirus;* PIV-2 and PIV-4 belong to the genus *Rubulavirus*. PIVs are enveloped helical RNA viruses with two surface antigens, hemagglutinin-neuraminidase (HN) antigen and fusion (F) antigen. HN antigen is the viral adhesion molecule; F antigen is responsible for the fusion of the virus to the cell and of one infected cell to another infected cell.

PIV-1 occurs most often in the fall every other year, and the incidence of PIV-2 is generally lower than that of PIV-1 and PIV-3. PIV-2 is seen every 2 years alternating with PIV-1. PIV-3 occurs almost every year in both spring and summer and can been seen yearlong in temperate climates. PIV-4 is seldom isolated, but routine testing is not readily available.

PIVs are a primary cause of respiratory disease in young children. PIV-1 and PIV-2 cause the most serious illnesses in children between 2 and 4 years of age. PIV-1 is the primary cause of croup (laryngotracheobronchitis) in children. PIV-3 causes bronchiolitis and pneumonia in infants and is second in importance only to RSV. PIV-4 generally causes mild upper respiratory tract infections. The viruses are spread through respiratory secretions, aerosol inhalation, and direct contact. Infection of the cells in the respiratory tract leads to cell death and an inflammatory reaction

in the upper and lower portions of the respiratory tract. Rhinitis, pharyngitis, laryngotracheitis, tracheobronchitis, bronchiolitis, and pneumonia may result.

The best specimens for viral culture are aspirated secretions and nasopharyngeal washes. Specimens for viral isolation should be taken as early in the illness as possible, kept cold, and inoculated into PMK cells or LLC-MK2 cells. The viruses can be identified by using hemadsorption, IF, or EIA techniques. Direct examination of nasopharyngeal secretions by IF can give rapid results. Serologic assays are more valuable for epidemiologic studies than for diagnostic purposes. Some newer nucleic acid assay panels now include this virus (PIV-1 to PIV-3), which aids in treatment and epidemiology efforts. Most of these newer PCR technologies are on panels that include several other respiratory pathogens. Aerosolized ribavirin can be used to treat infection. No vaccines are available, and infection control measures similar to those for RSV are used to prevent spread in health care facilities.

Mumps Virus

Mumps virus is related to PIVs and is in the genus *Rubulavirus*. It is an enveloped virus, with HN and F surface antigens. Mumps virus, which has a global distribution, is spread through droplets of infected saliva. It causes an acute illness producing unilateral or bilateral swelling of the parotid glands, although other glands, such as the testes, ovaries, and pancreas, can be infected. The virus infects primarily children and adolescents and usually results in long-lasting immunity. The primary infection of the ductal epithelial cells in the glands results in cell death and inflammation.

A vaccine effective in controlling the disease is available, with two doses being recommended for better immunity—the first at 12 to 15 months and the second at 4, 6, 11, or 13 years of age. Even with vaccination, from 2000 to 2003, about 250 cases of mumps were reported in the United States each year. A large multistate outbreak of almost 6000 cases of mumps from January to October 2006 was documented. Most of the cases were reported in the Midwestern states among previously vaccinated persons 18 to 25 years of age. The reason for the outbreak remains unknown. From 2009 to 2010, another outbreak, with 3502 cases in a New York camp, was reported and seemed to confirm waning immune protection by the vaccine. The mumps strain, genotype G, was the same one that had been circulating in the United Kingdom since 2004, where more than 70,000 cases have been reported. Most cases in the United Kingdom occurred in unvaccinated individuals.

The mumps virus can be isolated from infected saliva and swabbing of the Stensen duct, from 9 days before onset of symptoms until 8 days after parotitis appears. The virus, which is relatively fragile, can also be recovered from urine and CSF. Specimens may be examined directly by using IF and EIA methods. Studies have shown viral isolation using shell vial cultures of Vero or LLC-MK2 cells to be more successful than those with HEp2 or HeLa cell lines. Isolates can be identified by hemadsorption inhibition, IF, and EIA tests.

Paired sera can be tested for mumps antibody by EIA, IF, and hemagglutination inhibition tests. Paired sera taken at as small an interval as 4 to 5 days can demonstrate a diagnostic or fourfold rise in titer. Cross-reactions between soluble and viral antigens can confuse the interpretation of serologic results. Virus isolation is preferable, although physicians rarely have trouble recognizing mumps clinically.

Measles Virus

The measles virus is an enveloped virus classified in the genus *Morbillivirus*. It is found worldwide; in temperate zones, epidemics occur during winter and spring. At one time, measles (rubeola) was the most common viral disease in children in the United States. An average of 500,000 cases of measles were reported annually in the 1950s, with an average of 500 deaths. Immunization programs began in the United States in 1963, and the reported number of cases dropped to fewer than 1500 by 1983. A reemergence of measles occurred in 1989 through 1991, attributed to lack of vaccination and immigration. The decision to administer a second dose of vaccine to school-age children has drastically reduced the incidence of measles in the United States. On average, 70 cases per year were reported from 2001 to 2011. A spike of 131 cases in the first half of 2008 was blamed on the rapid spread of the virus from a few imported cases, mostly unvaccinated school-age children. A minor outbreak in Indiana, linked to two infected people who visited the Super Bowl Village in February 2012, sparked a debate on vaccination, immigration, and global travel. The WHO estimates that more than 30 million cases occur annually, with approximately 500,000 deaths in Africa alone.

Measles is highly contagious and spreads by aerosol. Initial replication takes place in the mucosal cells of the respiratory tract. The measles virus then replicates in the local lymph nodes and spreads systemically. The virus circulates in T and B cells and monocytes until eventually the lungs, gut, bile duct, bladder, skin, and lymphatic organs are involved. After an incubation period of 7 to 10 days, there is an abrupt onset, with symptoms of sneezing, runny nose and cough, red eyes, and rapidly rising temperature. About 2 to 3 days later, a maculopapular rash appears on the head and trunk. **Koplik spots**, lesions on the oral mucosa consisting of irregular red spots with a bluish-white speck in the center (Fig. 29.15), generally appear 2 to 3 days before the rash and are diagnostic. Complications such as otitis, pneumonia, and encephalitis may occur. A progressive, highly fatal form of encephalitis can occur but is rare. In developing countries with malnutrition and poor hygiene, measles can have a high fatality rate. Infection confers lifelong immunity. An effective attenuated vaccine is available and recommended for all children.

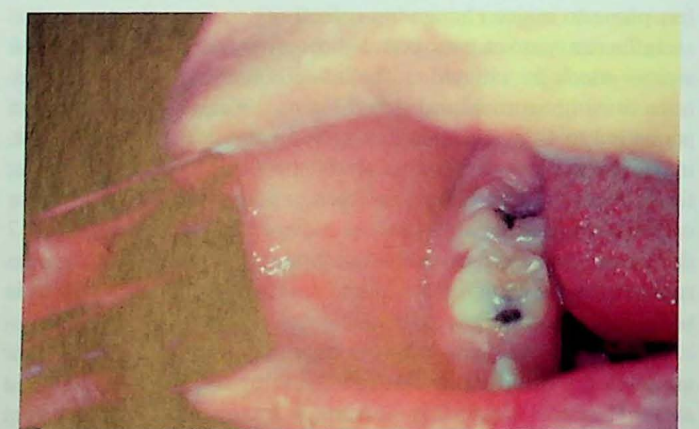

FIG. 29.15 Patient presenting on the third preeruptive day with Koplik spots indicative of the onset of measles. (Courtesy Centers for Disease Control and Prevention, Atlanta, GA.)

Measles is easily diagnosed clinically, so few requests for laboratory identification are made. The virus is fragile and must be handled carefully. The specimens of choice are from the nasopharynx and urine, but the virus can be recovered from these sources only in the early stages of infection. The virus grows on PMK cells, causing the formation of distinctive spindle-shaped or multinucleated cells. Virus isolates can be identified by using serum neutralization, EIA, or IF tests. Serologic diagnosis of measles is accomplished by demonstrating measles-specific IgM in the specimens collected during the acute phase of the disease. Nucleic acid testing should be considered for diagnostic use if IgM testing is compromised by the recent use of measles virus–containing vaccine as part of a routine vaccination or in response to a suspected outbreak.

Respiratory Syncytial Virus

RSV, a member of the genus *Pneumovirus*, causes croup, bronchitis, bronchiolitis, and interstitial pneumonia. It is the most common cause of severe lower respiratory tract disease among infants and young children worldwide. Almost half of all infants are infected by RSV during their first year of life, and by the age of 2 years, almost all have been exposed to RSV. Because infection does not confer complete immunity, multiple infections can occur throughout life and can be severe in older adults, the immunocompromised, and those with cardiac and respiratory problems. It is estimated that 24 of every 1000 children with RSV infection will be hospitalized. For that reason, health care–associated RSV is a problem in many medical facilities. Recommendations to reduce the risk of nosocomial spread include testing hospital personnel and infants with upper respiratory tract infections for RSV, isolating infants with RSV infection, following good handwashing and personal protective equipment practices, limiting visitation, and organizing patients and staff members into cohorts.

RSV can be a significant cause of morbidity and death in older patients. With a rapidly growing aging population, RSV in elder care facilities is becoming a significant problem in the United States. Unlike the bronchiolitis caused by RSV in children, pneumonia often develops in adults. The virus spreads mostly through large-particle droplets and contact with fomites rather than through inhalation of small aerosols. The virus may be carried in the nares of asymptomatic adults. RSV infections occur in yearly outbreaks that last 2 to 5 months and usually appear during winter or early spring in temperate zones.

RSV can be identified in specimens from nasopharyngeal swabs and washes by using DFA or EIA. Because the virus is extremely fragile, recovering it from cultures is difficult. Unfortunately, molecular methods have not been standardized, and there is high variability in samples used for each technique. Specimens must be kept cold but cannot be frozen. RSV grows readily in continuous epithelial cell lines, such as HEp2, forming syncytia. It also grows in PMK and human diploid fetal cells. Once the CPE is detected, RSV can be identified by using IF, EIA, and serum neutralization tests. Rapid antigen detection kits are also available for RSV. Several PCR-based molecular tests are available for RSV testing, with most taking less than an hour to provide the result. These are normally coupled with other respiratory viral markers. With the rise of RSV infection in older adults, molecular testing will become much more prevalent, facilitating more rapid treatment and isolation.

The antiviral compound ribavirin is approved for treatment of RSV infection. However, some controversy regarding the efficacy of ribavirin therapy developed recently and was still unresolved in 2016. A monoclonal antibody, palivizumab (PVZ), which blocks RSV entry into the host cell, is also approved for RSV treatment and prophylaxis in high-risk children. Often, ribavirin is recommended to be used in combination with PVZ. In 1996, RSV immunoglobulin was released for preexposure prophylaxis of susceptible patients; however, recommendations for its use are not clearly defined. No vaccine for RSV infection is available.

Human Metapneumovirus

hMPV was first described in children with previously virus-negative cultures. Infected children display many clinical symptoms similar to those of infections caused by RSV, influenza virus, and PIV. Often, these diseases are ruled out early in diagnosis, which leads to a presumptive identification of hMPV. Serologic and RNA sequence studies have shown that the virus is found almost all over the world and that most children have been exposed by the time they reach 5 years of age. Half the lower respiratory tract infections seen in children in the first 6 months of life are caused by this virus. Clinical disease ranges from mild upper respiratory tract infection to acute lower respiratory tract infection and includes fever, a nonproductive cough, sore throat, wheezing, congestion, shortness of breath, and lethargy. hMPV infections usually occur in the winter months, but outbreaks have been documented during summer.

hMPV infections usually occur in children. A survey in Finland among 1338 children younger than 13 years found that 47 (3.5%) with respiratory illness were positive for hMPV. The highest concentration of illness (7.6%) was seen in children younger than 2 years. Co-infections with another virus, including enterovirus, rhinovirus, influenza virus, and PIV, were detected in eight (17%) of the infected children. hMPV has also been documented to cause outbreaks in long-term care facilities. In the summer of 2006, hMPV affected 26 residents and 13 staff members in a 171-bed California long-term care facility. All the affected residents had an underlying medical condition; two were hospitalized, but none died. This outbreak indicates a year-round risk of infection in institutionalized older adults. Treatment for hMPV infection is mostly supportive in nature.

hMPV grows slowly in standard cell culture lines, such as monkey kidney and A549 cell lines. Specimens can be collected from the nostrils by using a swab, placed in transport media, and transported on ice to the laboratory for culture or molecular analysis. RT-PCR and fluorescent monoclonal antibodies are currently being used for identification. The respiratory viral panel assay from Luminex Molecular Diagnostics (Toronto, Canada) claims 100% sensitivity and 98.2% specificity for identification of hMPV in clinical specimens.

Picornaviridae

Picornaviridae is one of the largest families of viruses, with more than 280 members. It contains many important human and animal pathogens. Four genera with human clinical significance belong to the family Picornaviridae—*Enterovirus, Hepatovirus, Rhinovirus* and *Parechovirus*. The genus *Hepatovirus* includes hepatitis A virus (HAV). This virus is discussed in detail in the section on hepatitis viruses.

Enteroviruses

The enteroviruses found in the genus *Enterovirus* include the following:

- Enteroviruses 73 to 120 (hepatitis E virus [HEV] A, HEV B, HEV C, and HEV D)
- Rhinoviruses A to C
- Polioviruses 1 to 3
- Coxsackieviruses A1 to A24
- Coxsackieviruses B1 to B6
- Echoviruses 1 to 33

The genus *Parechovirus* currently contains 16 types, designated human parchoviruses (HPeV) 1-16.

These small naked viruses cause various conditions, including fever of unknown origin, aseptic meningitis, paralysis, sepsis like illness, myopericarditis, pleurodynia, conjunctivitis, exanthemas, pharyngitis, and pneumonia. Enteroviruses have also been implicated in early-onset diabetes, cardiomyopathy, and fetal malformations.

Most serotypes of the enteroviruses are distributed worldwide. In temperate zones, enterovirus epidemics occur in summer and early fall. Enterovirus infections are more prevalent in areas with poverty, overcrowding, poor hygiene, and poor sanitation. Viruses are spread via aerosol inhalation, the fecal-oral route, and fomites. The portal of entry is the alimentary canal via the mouth. The viruses replicate initially in the lymphoid tissues of the pharynx and gut. Viremia can result in the virus spreading from these locations to the spinal cord, heart, and skin. The clinical disease caused by enteroviruses can be neurologic, respiratory, or cardiac, depending on viral spreading and the immune status of the host. Enterovirus infections most often cause mild nausea and diarrhea in adults. However, disease can be much more severe in neonates because of the immaturity of their immune system.

The polioviruses tend to infect the CNS and can cause paralysis in a small percentage of infected individuals. The viruses destroy their host cells. In the intestines, damage is temporary because the cells lining the gut are rapidly replaced. In contrast, neurons are not replaced, which results in neuron death and permanent paralysis.

No vaccines are available for enteroviruses other than poliovirus. Good personal and hospital hygiene and proper sanitation can reduce the incidence of enterovirus infections. Poliovirus vaccines of attenuated or inactivated viruses are available. Since 1988, the polio vaccine program has been crucial to the WHO's effort to eradicate polio worldwide. In countries where polio is considered to have endemic rates of incidence, there has been a steady decrease since the program began. In 1988, 125 countries reported endemic rates; by polio vaccine 1999, the number of such countries had decreased to 30, and in 2013 to only three—Afghanistan, Nigeria, and Pakistan. Unfortunately, interruptions in vaccine programs that began in 2003 have resulted in the reemergence of polio, with 650 cases seen in 2011 to 2012. Because of this, the WHO has stepped up efforts to eradicate this disease in endemic areas.

Enteroviruses can be cultured from pharyngeal specimens immediately before the onset of symptoms and for 1 to 2 weeks afterward; the viruses can be isolated from feces for as long as 6 weeks thereafter. However, ideally, specimens should be obtained early in the course of the infection. Specimens from the throat, feces, rectum, CSF, and conjunctiva are recommended.

Polioviruses, type B coxsackieviruses, and echoviruses grow readily in a number of cell lines, including PMK, continuous human and primate, and human fetal diploid fibroblast lines. The high-numbered enteroviruses (68 to 71) require special handling. The CPE appears quickly and is readily identifiable. Enteroviruses have no group antigen, so they must be identified individually by a serum neutralization test. The WHO distributes pools of enterovirus antisera that allow identification by neutralization patterns in the antisera. The CPE and resistance to detergent, acid, and solvents constitute a presumptive diagnosis of enterovirus infection.

Hand, foot, and mouth disease (HFMD) is caused primarily by coxsackievirus types A5, A10, and A16 and occasionally by enterovirus type 71. HFMD is generally a disease that occurs in young children. Since 1997, large outbreaks of HFMD caused by EV71 have been reported, mostly in children in East and Southeast Asia, with some recent cases seen in Cambodia and China, resulting in the death of hundreds. It is spread by fomites or via the oral-fecal route. A mild prodromal phase may develop, with malaise, headache, and abdominal pains. Small painful sores suddenly appear on the tongue, buccal mucosa, and soft palate. Simultaneously, a maculopapular rash appears on hands, feet, and buttocks, followed by bullae on the soles of feet and the palms of hands. The lesions regress in about 1 week. If a rash develops, it is transient. The virus can be isolated from specimens from swabs of the mouth and bullae. Coxsackievirus A16 grows in PMK and human diploid fibroblast cells and can be identified by serum neutralization tests.

More than 150 serotypes of rhinoviruses exist, and they are the major cause of the common cold. Most people experience two to five colds each year, and almost 50% of these colds are caused by the rhinoviruses. Rhinovirus infections occur throughout the year, but their incidence increases in winter and spring. Transmission is primarily via aerosols, but contact with secretions and fomites can also cause infection.

Rhinoviruses infect the nasal epithelial cells and activate the mediators of inflammation. Symptoms include a profuse watery discharge, nasal congestion, sneezing, headache, sore throat, and cough. In severe cases, bronchitis and asthma may result. Unfortunately, no cure for the common cold has been found yet. Treating symptoms and reducing the spread of the virus in the household is the typical response. Both natural and recombinant interferons have been shown to be effective in preventing infection and illness when given intranasally over short periods. However, prolonged administration has resulted in adverse effects, such as nasal irritation, ulceration, and bleeding.

Retroviridae

The family Retroviridae contains several subfamilies, including Oncovirinae and Lentivirinae. The retroviruses have a unique mode of replication; they require an RNA-dependent DNA polymerase (reverse transcriptase) to synthesize DNA from the RNA genome. The human T-lymphotropic viruses HTLV-1, HTLV-2, and HTLV-5 belong to the subfamily Oncovirinae. These viruses are not cytolytic but are associated with several leukemias, sarcomas, and lymphomas.

HIV belongs to the subfamily Lentivirinae. Although some groups of individuals, mostly in West Africa, are infected by HIV-2, it is the impact of HIV-1 that continues to be felt around

the world. HIV causes AIDS. HIV-1 was identified first and is responsible for the AIDS pandemic, with about 34 million people identified as having HIV/AIDS at the end of 2011 and approximately 7400 new cases diagnosed each day. Approximately 1.7 million HIV/AIDS–related deaths occurred in 2011, with about 2.5 million new cases diagnosed. The region most severely affected by HIV/AIDS is sub-Saharan Africa, which has approximately 22.9 million patients with HIV infection, accounting for 67% of the total worldwide incidence. Children share this burden; in 2011, approximately 3.4 million children younger than 15 years had HIV/AIDS, and about 330,000 cases of newly infected children were reported. As of 2014, globally, approximately 36 million people were identified as having HIV infection.

The virus is transmitted via blood and exchange of other body fluids. HIV is cell associated, so fewer viruses are found in cell-free plasma than in whole blood, and even fewer viruses are found in saliva, tears, urine, or breast milk. HIV is not highly contagious, and normal, social, nonsexual contact poses no threat to individuals. High risk for contracting the virus includes unprotected sex with multiple partners, IV drug abuse, infusion of blood and blood products, and presence of the virus in a pregnant woman, who passes it to the fetus during pregnancy. Individuals with ulcerative sexually transmitted infections (STIs; e.g., syphilis, genital herpes, chancroid) are at greatest risk. Today, all donor blood and blood products are screened for HIV, which substantially reduces the risk.

It has been estimated that more than 1.2 million people had HIV/AIDS in the United States in 2016, with one in eight unaware that they were infected. Of this number, approximately 25% are women; the greatest number of persons who contract HIV/AIDS are MSM. As of 2014, in the United States, new cases of HIV were associated with the following:

- Heterosexual contact (24%)
- MSM (66%)
- IV drug use (6%)
- Heterosexual contact and IV drug use (3%)
- Transfusions or mother-to-infant transmission (1%)

HIV is a spherical virus, with a three-layer structure (Fig. 29.16). In the center are two identical copies of ssRNA and reverse transcriptase surrounded by an icosahedral capsid. The nucleocapsid is enclosed by a matrix shell to which an envelope of host cell origin is attached. Inserted into the viral envelope are viral glycoprotein (gp) trimers or spikes. The diagnostically important HIV antigens are the structural proteins p24, gp4l, gpl20, and gpl60.

Once HIV enters the body, the primary target cells are the $CD4^+$ T cells, monocytes, and macrophages. Acute infections are generally mild and can resemble infectious mononucleosis. The individual will enter a period of clinical latency, and even though the virus is replicating rapidly in lymphoid tissues, the virus is not detectable in the bloodstream, and the patient remains asymptomatic. Eventually lymphopenia results, with the greatest loss in the $CD4^+$ T-cell population. Healthy individuals have $CD4^+$ counts of at least $1000/mm^3$, whereas patients with HIV/AIDS can have counts lower than $200/mm^3$. Lymph nodes become enlarged and hyperplastic. The virus destroys the cells (T-helper cells) critical in host immune response to infectious agents. The patient begins experiencing several chronic and recurrent infections (Box 29.1). As the disease progresses, the $CD4^+$ cell count continues to decline, and the severity of opportunistic infections increases. The patient can also develop virus-induced cancers, such as KS. Death usually occurs as a result of opportunistic infections, although HIV-1 itself can directly cause encephalitis and dementia.

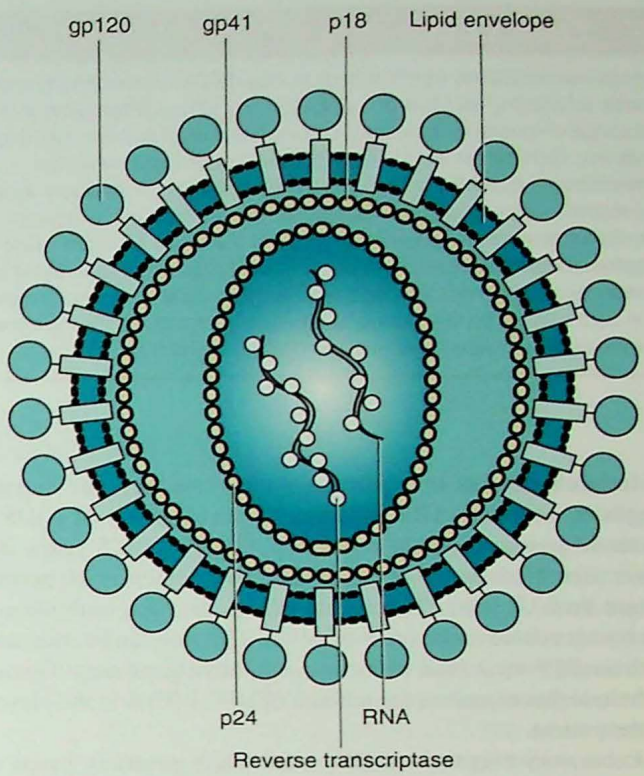

FIG. 29.16 Human immunodeficiency virus (HIV).

BOX 29.1 Common Opportunistic Infections and Cancers in Patients with Acquired Immunodeficiency Syndrome

- Candidiasis of the respiratory tract
- Coccidiomycosis
- Cryptococcal meningitis
- Cryptosporidiosis with persistent diarrhea
- Cytomegalovirus infections of organs other than the liver, spleen, or lymph nodes
- Histoplasmosis
- Persistent herpes simplex virus infections
- Kaposi sarcoma or lymphoma of the brain in patients older than 60 years
- Oral hairy leukoplakia
- Lymphoid interstitial pneumonia, pulmonary lymphoid hyperplasia, or both in children younger than 13 years
- *Mycobacterium avium* complex, *Mycobacterium kansasii,* or *Pneumocystis jirovecii* pneumonia
- Progressive multifocal leukoencephalopathy
- Recurrent pneumonia
- Toxoplasmosis of the brain in infants younger than 1 month
- Wasting disease

✓ Case Check 29.1

Clinical manifestations of HIV infection include CNS involvement, opportunistic infections, and tumors. CNS involvement is often seen in HIV-associated dementia complex but can manifest itself as other neurologic problems, such as loss of bowel or bladder control and weakness in the extremities. KS develops from the cells lining the lymph or blood vessels. The resulting lesions are red, purple, or brown blotches or tumors on skin. Other than their appearance, the skin lesions of KS often cause no symptoms. However, in some cases, they may cause painful swelling and be more painful when found in the legs, groin, or skin around the eyes. KS in such sites as the liver, lungs, or digestive tract may be life-threatening as a result of abnormal bleeding or difficulty breathing.

In adult patients living in developed countries, the average length of time from HIV infection to development of AIDS is about 10 years. About 20% develop AIDS within 5 years, and fewer than 5% have an asymptomatic HIV infection for periods longer than 10 years. The rate at which the virus multiplies in the host is related to the onset of AIDS. This rate can be measured with an HIV viral load assay, a quantitative gene amplification technique that measures the amount of HIV-1 RNA in the plasma of the patient.

Laboratory diagnosis of HIV infection is generally based on demonstration of anti-HIV antibodies and, in some cases, detection of viral antigens and RNA. HIV antibodies are normally produced within a few weeks after infection. A number of assays are commercially available as screening tests using different methods, including EIA and IF. The early diagnostic kits, referred to as *first-generation screening tests*, used purified viral lysate as antigens. The second-generation tests used recombinant viral proteins, thus improving performance. The third-generation tests relied on the double-antigen sandwich assay. In this procedure, viral antigen attached to a solid phase bound antibody to HIV from the patient's serum. Labeled HIV antigen was then added, captured by the patient's antibody, and measured. Fourth-generation kits detected antibody and p24 antigen. By detecting antigen, early infections could be identified before antibody is produced. The first fifth-generation assay is a multiplexed screening test that detects and differentiates all three HIV analyte markers: HIV-1 antibodies, HIV-2 antibodies, and the HIV-1 p24 antigen.

A number of rapid assays screen for HIV infection by using serum, plasma, and even saliva. By 2012, the FDA had approved six rapid tests for the diagnosis of HIV infection—OraQuick Advance rapid HIV-1/2 antibody test (Orasure, Bethlehem, PA); Uni-Gold Recombigen HIV test (Trinity Biotech, Wicklow, Ireland); Reveal G2 rapid HIV-1 antibody test (MedMira, Halifax, Canada); Multispot HIV-1/2 rapid test (Bio-Rad Laboratories, Hercules, CA); and Clearview HIV-1/2 and Clearview Complete HIV-1/2 (Inverness Medical Professional Diagnostics, Princeton, NJ). OraQuick for whole blood and oral fluid specimens, Clearview Complete for whole blood, and Uni-Gold for whole blood samples have waivers from the CLIA. Currently, the FDA has approved one home collection kit, Home Access HIV-1 test system (Home Access Health Corporation, Maria Stein, OH). In 2012, the first FDA-approved in-home HIV test kit was the OraQuick (OraSure Technologies, Inc. Bethlehem, PA), and by 2016, the FDA had approved multiple tests in each category.

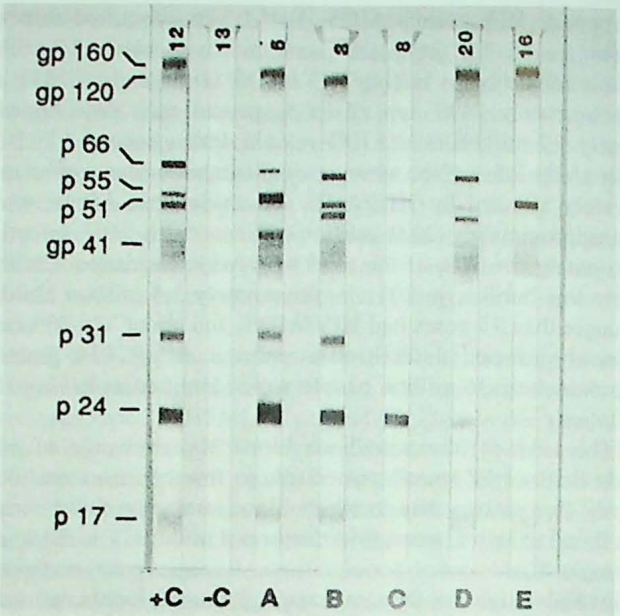

FIG. 29.17 Human immunodeficiency virus immunoblot. Reactive protein *(p)* bands appear as purplish lines across the strip. Proteins with higher molecular weights appear at the top of the strip. Structural and nonstructural proteins are given RNA structural genome codes: *GAG* for group-specific antigens; *POL* for polymerase; and *ENV* for envelope. *ENV* codes for glycoprotein (gp) precursors: gp160, gp120, and gp41 through gp43. POL codes for p65, p51, and p31. GAG codes for p55, p24, and p17. Results are negative, indeterminate, or positive based on the pattern on the strip. Positive corresponds to reactivity to two or more of the following antigens: p24, gp41 or gp120/gp160. Indeterminate corresponds to the appearance of one or more bands in a pattern that does not satisfy the positive criteria. Negative corresponds to the absence of any band on the strips. (Courtesy Patricia A. Cruse.)

Current CDC guidelines (published in 2014) recommend that any screening test detect HIV-1 and HIV-2 antibodies and p24 antigen. No further testing is required if the result of a test is negative. Any reactive result on a screening test, usually an EIA, is retested in duplicate. If both tests are reactive, the specimen is reported as repeatedly reactive and is submitted for confirmatory testing. This is usually performed by using the Western blot test or IF. The Western blot test for HIV is less sensitive than the screening tests and is prone to cross-reactivity. However, despite these flaws, the Western blot assay has remained the principal confirmatory assay for HIV antibody detection. The Western blot test detects antibodies specific to viral antigens, such as p24, p31, gp41, and gpl20/gpl60 (Fig. 29.17). If the confirmatory test is reactive, the HIV test result should be considered positive, and the patient is confirmed to have HIV infection. The presence of HIV antibodies is diagnostic, but a negative result simply means that no antibody was detected. It may take 6 weeks after infection before antibodies appear, and the antibody can disappear as immune complexes form in the late stages of the infection. Other immunologic markers of HIV infection are listed in Box 29.2. Newer guidelines recommend confirmation with an FDA-approved antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies. A reactive initial screen and a positive differentiating immunoassay are considered positive for HIV. Some

BOX 29.2 Important Immunologic Markers for Acquired Immunodeficiency Syndrome

- Steady decline in number of $CD4^+$ T cells
- Depression of the $CD4^+$-to-$CD8^+$ cell ratio to less than 0.9 (reference value, ≥1.5)
- Functional impairment of monocytes and macrophages
- Decreased natural killer cell activity
- Anergy to recall antigens in skin tests

clinical laboratories continue to add the Westernblot test to this algorithm, but it is not strictly required according to the 2014 CDC guidelines.

Because these viruses readily develop resistance to drugs, HIV infection is often treated with combination therapy. Highly active antiretroviral therapy (HAART) involves aggressive combination therapy soon after HIV infection is diagnosed. In hospital exposures, a similar strategy is used if a health care worker is exposed to the virus accidentally. Aggressive therapy is initiated after the exposure, and this significantly reduces the risk of contracting the infection. Despite aggressive therapy, a cure is not generally achieved. However, in 2013, physicians in the United States reported that a newborn was cured of HIV infection in 2010, the second documented case of a cure. The first was a middle-aged man living in Berlin. At the time of delivery, the infant's mother had a diagnosis of HIV infection. Blood tests on the infant revealed about 20,000 copies of viral RNA per milliliter. This titer is considered low for a baby, but because tests so soon after birth were positive, it suggested that the infection occurred in the womb rather than during delivery. The infant received aggressive, three-regimen antiviral therapy, starting about 30 hours after birth, a method not commonly used. By 1 month of age, virus was not detectable in blood samples. Treatment continued for 18 months, when the mother stopped coming to the clinic. Some scientists question whether the infant was truly infected. If the case can be confirmed, this could affect treatment strategies worldwide.

Several classes of antiviral drugs are approved for treatment. Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of retroviral drugs developed and are incorporated into viral DNA; these include adefovir, azidothymidine, dideoxyinosine, d4T (stavudine), 3TC (lamivudine), and tenofovir. NRTIs inhibit the conversion of nucleoside analogues in the body to nucleotide analogues. Non-NRTIs attach to reverse transcriptase, preventing conversion of RNA to DNA; examples include delavirdine, nevirapine, and efavirenz. Other classes of antiviral drugs include protease inhibitors (e.g., ritonavir, saquinavir, indinavir, amprenavir) and fusion inhibitors (initially called T-20, enfuvirtide). HAART includes combinations, such as two NRTIs combined with a protease inhibitor.

HIV viral load assays can predict therapeutic efficacy. In different studies, suppression of HIV RNA levels to less than 5000 copies of RNA per milliliter for up to 2 years was correlated with an increase in $CD4^+$ cell counts, up to 90/mm^3. In contrast, patients with HIV RNA loads of more than 5000 copies of RNA per milliliter generally showed declines in $CD4^+$ cell counts. Usually, these assays are performed monthly so that therapy can be adjusted on an individual basis.

✓ Case Check 29.2

In HIV infection, the immune system is severely compromised, giving rise to many opportunistic infections caused by viruses, bacteria, fungi, and protozoa, as well as neoplastic disease, wasting syndrome, progressive multifocal leukoencephalopathy, and HIV encephalopathy. These complications often result in death. Meningitis occurs frequently in patients with HIV infection and can be caused by a variety of agents. In the Case in Point, the patient was treated with acyclovir for a presumed HSV meningitis and/or encephalitis. Acyclovir is an inactive nucleoside analogue that is metabolized by viral thymidine kinase into an active form, which results in premature termination of DNA during synthesis.

Rhabdoviridae

Rabies is caused by several strains of viruses belonging to the genus *Lyssavirus*. The fear associated with rabies virus infection is justified. It is estimated that 55,000 persons die as a result of rabies worldwide annually, with 40% of those being children less than 15 years of age. Dogs are implicated in 99% of rabies deaths among humans. Cleaning the wound and immunization within a few hours after contact can prevent the disease; each year, more than 15 million people worldwide receive postexposure preventive treatment. This is estimated to prevent 327,000 rabies deaths annually. In the United States, human cases of rabies are rare, with approximately two per year. However, rabies is an emerging infection in animals, with all mammals being susceptible. Most rabies infections 40 years ago occurred in dogs, with some occurring in cats, foxes, and skunks. However, in the United States, rabies is now more closely associated with wild animals (92%), such as raccoons, skunks, foxes, and bats. Programs to vaccinate domestic animals have reduced the number of rabies cases in dogs and cats, which has, in turn, decreased the risk to humans.

Humans usually acquire the rabies virus when they are bitten or scratched by animals with rabies. With the number of endemic areas increasing among wildlife, the risk of human exposure to rabies increases because of the increased likelihood of encountering a wild animal with rabies or a domestic animal that has contracted rabies from wildlife. Humans infected with the rabies virus experience a brief prodromal period of pain at the exposure site and have vague flulike symptoms. Mental status changes, such as anxiety, irritability, and depression, may also become evident. After the prodromal period, patients suffer additional CNS changes, including hallucinations, paralysis, excessive salivation, hydrophobia, bouts of terror, seizures, respiratory and cardiac abnormalities, and hypertension. These symptoms are followed ultimately by coma and death. In 2004, a Texas hospital encountered five cases of rabies. This occurrence was a result of transplanting organ and tissues from a person who was later discovered to have been a victim of a bat bite into four patients, all of whom subsequently died as a result of the disease. Rabies virus is not considered a bloodborne pathogen; therefore it is likely that the spread was caused by infected nerve tissue that came with the new organs.

Laboratory diagnosis of rabies involves determining whether an animal that has bitten a human has rabies. The animal is killed, and its head is removed and sent to a reference laboratory. The fastest and most sensitive method of identifying rabies virus in a specimen is by using a direct IF technique. Impression smears should be made from various areas of the brain, primarily the

hippocampus, pons, cerebellum, and medulla oblongata. In living patients suspected of having rabies, skin biopsy, especially at the hairline at the back of the neck, and an impression of the cornea may be performed. The presence of rabies virus in these specimens is diagnostic, but its absence merely means that no virus is present in those specific specimens, not that the patient does not have rabies. Rabies virus can be grown in suckling or young adult mice, murine neuroblastoma, or related cell lines. EIAs are currently the most sensitive assays to use for serologic tests.

Rabies cannot be successfully treated once symptoms appear. However, postexposure prophylaxis is 100% effective in preventing the disease if the bite victim is treated immediately after exposure. Postexposure prophylaxis includes vigorously cleaning the wound site, providing human rabies immunoglobulin, and administering a three-injection series of the rabies vaccine. Two approved human vaccine preparations are available in the United States and can be given to persons who may have been exposed to rabies, such as veterinarians, laboratory personnel, people who explore caves, and those visiting high-risk countries for longer than 30 days. Only one unvaccinated person with rabies has ever survived. A female teenager in Wisconsin developed rabies about 1 month after being bitten by a rabid bat. She was put into a coma and treated with several antiviral compounds. However, the reason for her survival is still not completely understood.

Togaviridae

The family Togaviridae contains the genera *Alphavirus, Rubivirus,* and *Arterivirus.* No member of the genus *Arterivirus* is known to infect humans. Many of the viruses in the genus *Alphavirus* are mosquito-borne and cause encephalitis. Eastern equine encephalitis (EEE), which causes disease in horses and humans, occurs primarily in the eastern half of the United States. About 250 human cases have been reported since 1964, and these infections have a mortality rate of about 40%. Infections can cause a range of effects, from mild flulike symptoms to encephalitis. Of those who survive, almost 50% suffer permanent damage to the CNS. Birds are the natural reservoirs of the virus, which is spread to humans and horses via bites of mosquitoes. Because horses and humans are dead-end hosts, EEE in horses can be a predictor of human EEE cases.

The western equine encephalitis (WEE) virus also causes disease in humans and horses. WEE virus causes a milder disease compared with EEE virus, and patients develop an asymptomatic or mild infection consisting of fever, headache, nausea, and mental status changes. Of young children and infants who survive, about 30% will suffer permanent CNS damage. Mortality is about 3%. Since the period from 1964 to 2012, there have been 640 cases reported in the United States.

Venezuelan equine encephalitis (VEE) has caused large outbreaks of human and equine encephalitis in the Americas. In 1995, VEE caused encephalitis in an estimated 75,000 to 100,000 people in Venezuela and Colombia. Death is much less common in patients with VEE than in patients with WEE or EEE. Infected adults often develop a flulike illness, whereas encephalitis is more commonly seen in children with VEE.

Rubella virus is an enveloped virus belonging to the genus *Rubivirus.* It causes the disease rubella, or German measles, a mild febrile illness accompanied by an erythematous, maculopapular, discrete rash with postauricular and suboccipital lymphadenopathy. Like measles, rubella occurs most frequently in winter and spring. The diseases are so similar that as many as 50% of suspected measles cases are diagnosed as rubella. The rubella virus is transmitted via droplets. The virus is present in the nasopharyngeal specimens or any secretion or tissue of infected infants, who shed the virus in large amounts for long periods. A rash starts on the face and spreads to the trunk and limbs. No rash appears on the palms and soles. About 50% of those infected with rubella virus are asymptomatic. Transient polyarthralgia and polyarthritis can occur in children and are common in adults.

Rubella would be of little concern if it did not cross the placenta and spread to fetal tissues, which results in congenital rubella syndrome. The syndrome can cause effects ranging from birth of a normal infant to birth of a severely impaired infant, fetal death, or spontaneous abortion. Because the rubella virus halts or slows cell growth, the impact on the embryo is worse when the infection develops in the earliest stages of pregnancy.

An effective attenuated vaccine is available and should be administered to all children and to young women before they become sexually active. Since 2003, the incidence of rubella in the United States has dropped dramatically, from 364 cases in 1998 to approximately 10 cases annually. In 2004, rubella was declared no longer endemic in the United States. Direct examination of specimens with IF or EIA is recommended because isolation procedures are cumbersome. Serologic procedures are effective because any rubella antibody is presumed to be protective. The most sensitive serologic assays are the solid-phase and passive hemagglutination tests. Latex agglutination and antigen-coated RBC tests are useful but less sensitive. Several molecular tests are being evaluated, and PCR-based assays are being evaluated in clinical research trials conducted by the U.S. Army. The CDC currently performs RT-PCR and end-point RT-PCR to detect rubella RNA, and these protocols can be adapted in routine clinical laboratories providing the guidelines are followed, but they are not FDA approved.

Hepatitis Viruses

The hepatitis viruses are grouped together, not because of their structural or genetic similarities but because they share the same tissue tropism, which is the liver. Before the 1970s, patients with hepatitis were classified as having infectious hepatitis or serum hepatitis. Infectious hepatitis was transmitted from person to person via the fecal-oral route, and serum hepatitis resulted from transfusion of infected blood and blood products. During the past 30 years, at least eight different hepatitis viruses (Table 29.7) have been recognized—HAV, HBV, HCV, delta hepatitis virus (or hepatitis D virus; HDV), hepatitis E virus (HEV), hepatitis G virus (HGV), SEN virus, and transfusion-transmitted virus (TTV). HAV and HEV are transmitted via the fecal-oral route; HBV, HCV, HDV, HGV, SEN virus, and TTV are transmitted via transfusion of infected blood and blood products. HBV, TTV, and SEN virus have DNA genomes, whereas the others have an RNA genome.

Despite the biological and morphologic differences among the hepatitis viruses, many of the clinical symptoms caused by them are similar. Therefore differentiation based on clinical findings should not be relied on for diagnosis. The most common symptoms are fatigue, headache, anorexia, nausea, vomiting, abdominal pain (right upper quadrant or diffuse), jaundice, and dark-colored urine.

TABLE 29.7 Clinical and Epidemiologic Differences among HAV, HBV, HDV, and HCV

Clinical Features	Hepatitis A (HAV)	Hepatitis B (HBV)	Hepatitis D (HDV)	Hepatitis C (HCV)
Incubation (days)	15–45	30–120	21–90	40–50
Type of onset	Acute	Insidious	Usually acute	Insidious
Mode of Transmission				
Fecal-oral	Usual	Infrequent	Infrequent	
Parenteral	Increasing	Usual	Usual	Likely
Other	Foodborne, waterborne	Intimate contact, transmucosal transfer	Intimate contact, less efficient than for hepatitis B virus	Vertical transmission Intranasal cocaine use
Sequelae				
Carrier	No	5%–10%	Yes	Yes
Chronic hepatitis	No	Yes	Yes	Yes
Mortality (%)	0.1–0.2	0.5–2.0	30 (chronic form)	0.2–0.3

Hepatitis A Virus

HAV is a small, icosahedral, naked ssRNA virus, the sole member of the genus *Hepatovirus* in the family Picornaviridae. HAV infects people of all ages. In the United States, children between the ages of 5 and 14 years have the highest rate of infection, with almost 30% of all cases occurring in children less than 15 years of age. Reported cases have declined from an average of 25,000 per year in the 1990s to an average of 3000 per year since 2006. In 2011, the rate decreased to about 1 case per 100,000 persons, with only 1398 total reported cases in the United States. The WHO estimates that 1.5 million clinical cases of HAV infection occur each year.

HAV is almost always transmitted via the fecal-oral route and is usually acquired through close personal contact or via contaminated food. The risk factors for HAV infection include sexual or household contact with an infected person, daycare contacts, foodborne or waterborne outbreaks, IV drug use, and international travel. However, almost 50% of the cases in the United States have no established risk factor. The virus is shed in large amounts in feces during the incubation period and early prodromal stage, and food and water contamination can result. The incubation period for HAV infection is approximately 1 month. After infection, individuals experience a transient viremia, after which the virus reaches the liver and replicates in hepatocytes. The virus passes into the intestine, and viral shedding begins and can persist for months.

Infections in more than 90% of children less than 5 years of age tend to be asymptomatic. In adults, symptoms can range from mild to severe prolonged hepatitis. The onset is abrupt, and patients experience fever, chills, fatigue, malaise, aches, pains, and, in some cases, jaundice. The infection is self-limiting, with convalescence possibly lasting weeks. Complete recovery can take months. HAV infection has a low mortality rate and no persistence and does not cause chronic liver damage.

The most common method for laboratory diagnosis of HAV infection is to demonstrate IgM to HAV (Fig. 29.18). Isolation of HAV is not practical because it is difficult to grow in culture and tends to mutate drastically. Safe, effective vaccines for HAV are available. A newer RT-PCR is available from reference laboratories that can detect HAV infection from many different specimen types. Studies comparing antibody detection with RT-PCR have shown that HAV RNA can be detected much earlier after infection. Vaccination of children has the potential to reduce the incidence of HAV infection. Other vaccination target groups include people who travel to countries with endemic HAV, MSM, drug abusers, and patients with chronic liver disease. Persons who have not been vaccinated and have been exposed to HAV can receive immunoglobulin therapy, which is 80% to 90% effective in preventing infection when administered soon after exposure. Immunoglobulin therapy can also be used as preexposure prophylaxis.

Hepatitis B Virus

HBV is an enveloped, partially dsDNA virus that belongs to the family Hepadnaviridae. The virus contains the hepatitis B surface antigen (HBsAg), which circulates in the bloodstream as 22-nm particles. The whole virus has a total diameter of about 45 nm. The virion also contains a core antigen (HBcAg) and hepatitis B e antigen (HBeAg). Eight genotypes of HBV have been identified (A to H), and several studies have shown a difference in clinical outcome based on the genotype.

Almost half the world's population lives in areas with endemic HBV, and more than 8% of the population is positive for HBsAg. About 350 million people worldwide are long-term carriers. In the United States, there are more than 1 million long-term carriers. However, the incidence of acute hepatitis has declined by more than 81% in the United States since the mid-1980s, mostly because of aggressive screening and vaccination programs. In 2011, the total number of confirmed cases of HBV infection was 2890, a rate of 1.1 per 100,000 persons. This was a 29% decrease from the previous year in the United States. Even with successful vaccination programs worldwide, approximately 50 million new cases occur each year.

HBV is primarily a bloodborne pathogen. Infected individuals can have as many as 1 million infectious particles per milliliter of blood. Lower concentrations of virus appear in semen, vaginal fluid, and saliva. Many other body fluids (e.g., tears, urine, sweat, breast milk) contain HBsAg but do not seem to be infective. The main modes of transmission are through sexual, perinatal, and parenteral routes. In the United States, heterosexual and male homosexual contacts are the most common routes of transmission. High-risk groups include IV drug abusers, MSM, individuals from endemic areas, persons with household or sexual contacts with

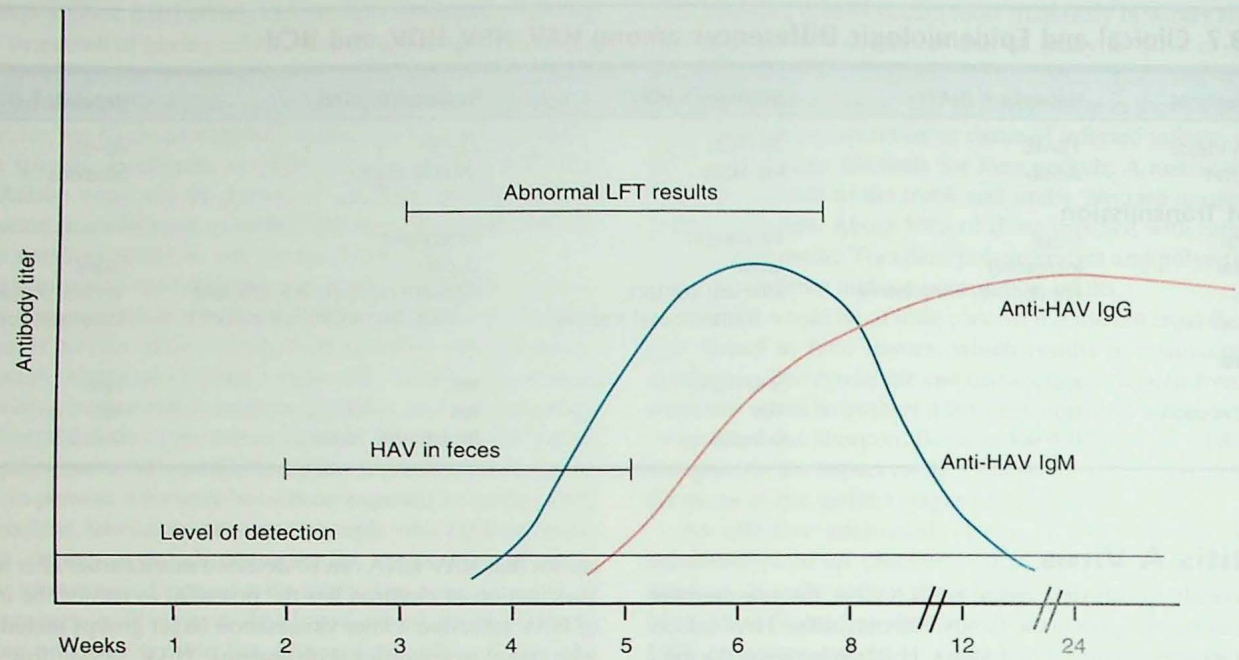

FIG. 29.18 Serologic evaluation of hepatitis A virus *(HAV)* infection showing the rise and fall of detectable antibodies. *IgG,* Immunoglobulin G; *IgM,* immunoglobulin M; *LFT,* liver function test.

HBV carriers, health care personnel, people with tattoos or body piercings, and infants born to HBV-positive mothers. Almost one third of the patients who become infected, however, have no known risk factor.

The human cost of the infection is high. As many as 1 million deaths per year worldwide are related to HBV infection. Once HBV enters the host, it travels from blood to the liver and infects the hepatocytes. Cytotoxic T cells then attack the HBV-infected hepatocytes. The incubation period for HBV infection ranges from 2 to 6 months, with an insidious onset that includes symptoms of fever, anorexia, and hepatic tenderness. Jaundice occurs in only about 10% of children who are less than 5 years of age and is much more common in older children and adults (32%–54%).

As the immune response is activated, the virus is slowly cleared from the system, and most patients become noninfectious. In adults, about 50% of infections are asymptomatic; 20% to 30% of patients exhibit clinical jaundice but have a benign resolution of the infection. Therefore about 80% of infections do not cause serious sequelae. The risk for chronic infection is inversely proportional to the age at the time of infection, with approximately 90% of infants and only 3% of adults developing chronic infection. Individuals with chronic infection have a higher risk of liver disease, such as cirrhosis or hepatic carcinoma. A safe and effective recombinant vaccine is available for preventing HBV infection. The U.S. Advisory Committee on Immunization Practices recommends that the series begins at birth and be completed by 6 to 18 months.

Diagnosis of HBV infection is based on clinical presentation and demonstration of specific serologic markers for HBV (Box 29.3). Serum aminotransferase levels also increase in infected patients. The presence of HBsAg in a patient's serum indicates that the patient has an active HBV infection, is a long-term carrier, or is in an incubation period. IgM anti-HBc appears early in the course of the disease and indicates an acute infection. In patients in whom HBsAg is not detected and anti-HBs has not yet appeared, detection of IgM anti-HBc confirms the diagnosis of acute HBV infection. The period between the inability to detect HBsAg and the detection of anti-HBs antibodies is often referred to as the *core window.* The detection of anti-HBs in the serum indicates convalescence or immune status.

When the infection resolves, IgG anti-HBc and anti-HBs become detectable in the patient's serum. The presence of HBsAg after 6 months of acute infection is a strong indication that the patient is a long-term carrier; the appearance of HBeAg in this case is indicative of a chronic infection and high infectivity. Table 29.8 shows the interpretation of HBV serologic markers. Fig. 29.19 depicts the increase and decrease in the levels of detectable serologic markers during acute HBV infection and resolution and presentation of chronic HBV infection. There are several molecular assays available that detect viral DNA that provide a short TAT and very sensitive detection.

BOX 29.3 Serologic Markers for the Diagnosis of Hepatitis B Virus Infection

- HBsAg—hepatitis B surface antigen, the envelope protein consisting of three polypeptides
- Anti-HBs—antibody to hepatitis B surface antigen
- Anti-HBc—antibody to hepatitis B core antigen
- HBeAg—antigen associated with the nucleocapsid, also found as soluble protein in serum
- Anti-HBe—antibody to hepatitis B e antigen

TABLE 29.8 Interpretation of Hepatitis B Serologic Markers

HBsAg	HBeAg	Anti-HBc	Anti-HBc IgM	Anti-HBs	Anti-HBe	Interpretation
–	NA	–		–	NA	No previous infection with HBV or early incubation
–	NA	+	–	±	NA	Convalescent or past infection
–	NA	–	–	±	NA	Immunization to HBsAg
+	–	–	±	–	–	Acute infection
+	+	±	+	–	–	Acute infection, high infectivity
+	–	±	+	–	+	Acute infection, low infectivity
+	+	+	–	–	–	Chronic infection, high infectivity
+	–	+	–	–	+	Chronic infection, low infectivity

–, Negative; +, positive; ±, positive or negative; *anti-HBc,* antibody against hepatitis B core antigen; *anti-HBe,* antibodies against hepatitis B envelope antigen; *anti-HBs,* antibody against hepatitis B surface antigens; *HBeAg,* hepatitis Be antigen; *HBsAg,* hepatitis B surface antigen; *IgM,* immunoglobulin M; *NA,* not applicable.

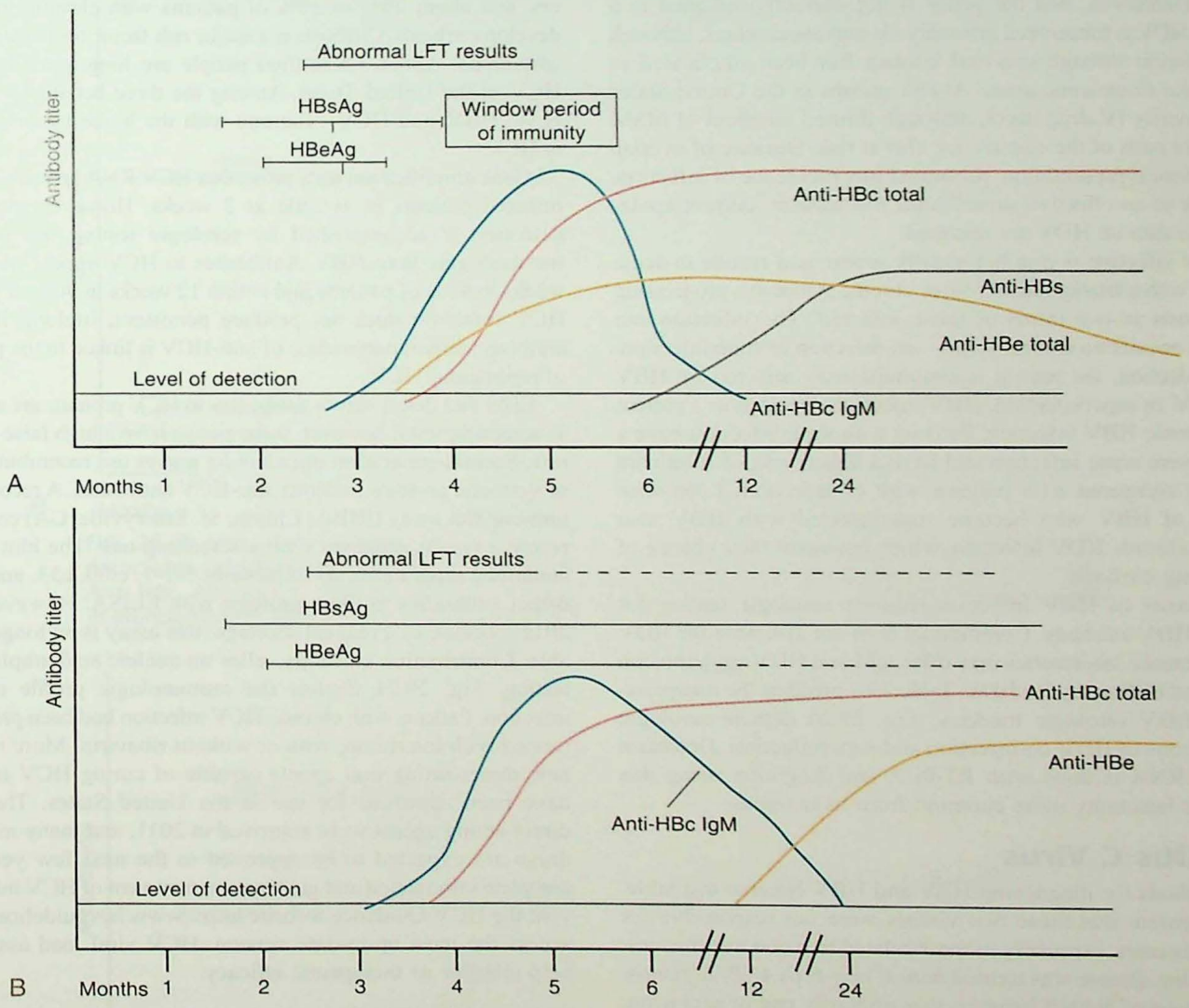

FIG. 29.19 Serologic evaluation of hepatitis B virus infection showing the increase and decrease of the levels of detectable antibodies. **A,** Serologic presentation in acute hepatitis infection with resolution. **B,** Serologic presentation in chronic hepatitis infection with late seroconversion. *Anti-HBc,* Antibodies against hepatitis B core antigen; *anti-HBc,* antibodies against hepatitis B core antigen; *anti-HBs,* antibodies against hepatitis B surface antigen; *HBeAg,* hepatitis B e antigen; *HBsAg,* hepatitis B surface antigen; *LFT,* liver function test.

TABLE 29.9 Interpretation of Hepatitis D Virus (HDV) Infection Serologic Markers

	Serologic Markers			
Clinical Variant	Anti-HBc IgM	HBsAg	Anti-HDV	Anti-HDV IgM
Co-infection	+	+	+	+
Superinfection	–	+	+	NA

Anti-HBc, antibody to hepatitis B core antigen; *HBsAg,* hepatitis B surface antigen; *HDV,* hepatitis D virus; *IgM,* immunoglobulin M; *NA,* not applicable.

Hepatitis D Virus

HDV, also known as the *delta hepatitis virus,* is a defective 1.7-kb ssRNA virus that requires HBV for replication. HDV requires the HBV HBsAg for its envelope. HDV is the sole member of the genus *Deltavirus,* and the genus is not currently assigned to a family. HDV is transmitted primarily via parenteral routes, although transmission through mucosal contact has been implicated in epidemics in endemic areas. At-risk groups in the United States are primarily IV drug users, although limited numbers of MSM in certain parts of the country are also at risk. Because of overlap in the clinical presentation, presumed low incidence of infection, and lack of an effective surveillance mechanism, current epidemiologic data on HDV are minimal.

HDV infection is rare but usually severe and results in acute disease, with a fatality rate of 5%, or chronic symptoms progressing to cirrhosis in two thirds of those infected. The infection can occur in one of two clinical forms—co-infection or superinfection. In co-infection, the patient is simultaneously infected by HBV and HDV. In superinfection, HDV infection develops in a patient with chronic HBV infection. Patients with superinfection have a more severe acute infection and have a higher risk of fulminant hepatitis compared with patients with co-infection. Long-term carriers of HBV who become superinfected with HDV also develop chronic HDV infection, which increases their chance of developing cirrhosis.

Diagnosis of HDV infection requires serologic testing for specific HDV antibody. Commercial tests are available for HDV IgG. Reference laboratories may offer IgM and HDV agglutination testing and PCR assay for HDV. Table 29.9 presents the interpretation of HDV serologic markers; Fig. 29.20 depicts serologic presentations of HDV co-infection and superinfection. Detection of HDV RNA is done with RT-PCR and diagnosis using this method is becoming more common from liver tissue.

Hepatitis C Virus

After methods for diagnosing HAV and HBV became available, it was apparent that these two viruses were not responsible for all hepatitis cases, especially in those related to blood transfusions. The resulting disease was termed *non-A, non-B (NANB) hepatitis.* The diagnosis of NANB hepatitis was primarily one of exclusion. In 1974, without any direct evidence, scientists predicted that a type C hepatitis virus must exist. Then, 15 years later, with the aid of molecular and cloning techniques, the genomic sequence of HCV was determined before the virus was ever seen with an electron microscope.

HCV is an ssRNA virus in the genus *Hepacivirus,* family Flaviviridae; it accounts for about 90% of all previous cases of NANB hepatitis. Currently, fewer than 1000 new cases occur annually in the United States, with only 850 seen in 2012. However, because of its long incubation period, it is estimated that approximately 20,000 acute infections occur each year. Throughout the 1980s, the estimated number of annual infections hovered around 200,000. This was ultimately reduced because of such factors as safer use of needles by IV drug abusers and reduction of posttransfusion infections as result of better testing. Worldwide, as many as 170 million new cases may develop each year. Although perinatal and sexual transmission of infection occur and parenteral transmission has been identified as a major route for infection, HCV antibody has been detected in patients in whom the routes of transmission are poorly understood or who have no evidence of identifiable risk factors.

Symptoms may be subtle and may take time to become apparent. About 50% of HCV-positive patients become long-term carriers, and about 20% to 30% of patients with chronic infections develop cirrhosis. Cirrhosis is a major risk factor for hepatocellular carcinoma. About 3.5 million people are long-term carriers of HCV in the United States. Among the three hepatitis viruses—HAV, HBV, and HCV—the one with the highest mortality rate is HCV.

Gene amplification tests prove that HCV RNA appears in newly infected patients in as little as 2 weeks. However, most virus detection is accomplished by serologic testing. HCV is less immunogenic than HBV. Antibodies to HCV appear in about 6 weeks in 80% of patients and within 12 weeks in 90% of patients. HCV infection does not produce persistent, lifelong levels of antibody; rather, persistence of anti-HCV is linked to the presence of replicating HCV.

EIAs that detect serum antibodies to HCV proteins are available as screening tests; however, these assays have a high false-positive rate. Second-generation immunoblot assays use recombinant and/or synthetic proteins to detect anti-HCV antibodies. A recombinant immunoblot assay (RIBA; Chiron, St. Emeryville, CA) confirmed reactive results obtained with a screening test. The blot or strip contained separate bands of proteins 5-1-1, c100, c33, and c22 to detect antibodies to these proteins with ELISA. However, since 2012, because of a reagent shortage, this assay is no longer available. Confirmation currently relies on nucleic acid amplification testing. Fig. 29.21 depicts the immunologic profile of HCV infection. Patients with chronic HCV infection had been previously treated with interferon, with or without ribavirin. More recently, new direct-acting oral agents capable of curing HCV infection have been approved for use in the United States. The initial direct-acting agents were approved in 2011, and many more oral drugs are expected to be approved in the next few years. For complete information and guidance on treatment of HCV infections, visit the HCV Guidance website http://www.hcvguidelines.org to access the most up-to-date version. HCV viral load assays can help monitor its therapeutic efficacy.

Hepatitis E Virus

HEV is a small (32–34 nm), naked, ssRNA virus classified in the genus *Hepevirus,* family Hepeviridae. HEV is transmitted via the fecal-oral route, particularly through contaminated drinking water. HEV has been identified as the cause of epidemics of enterically

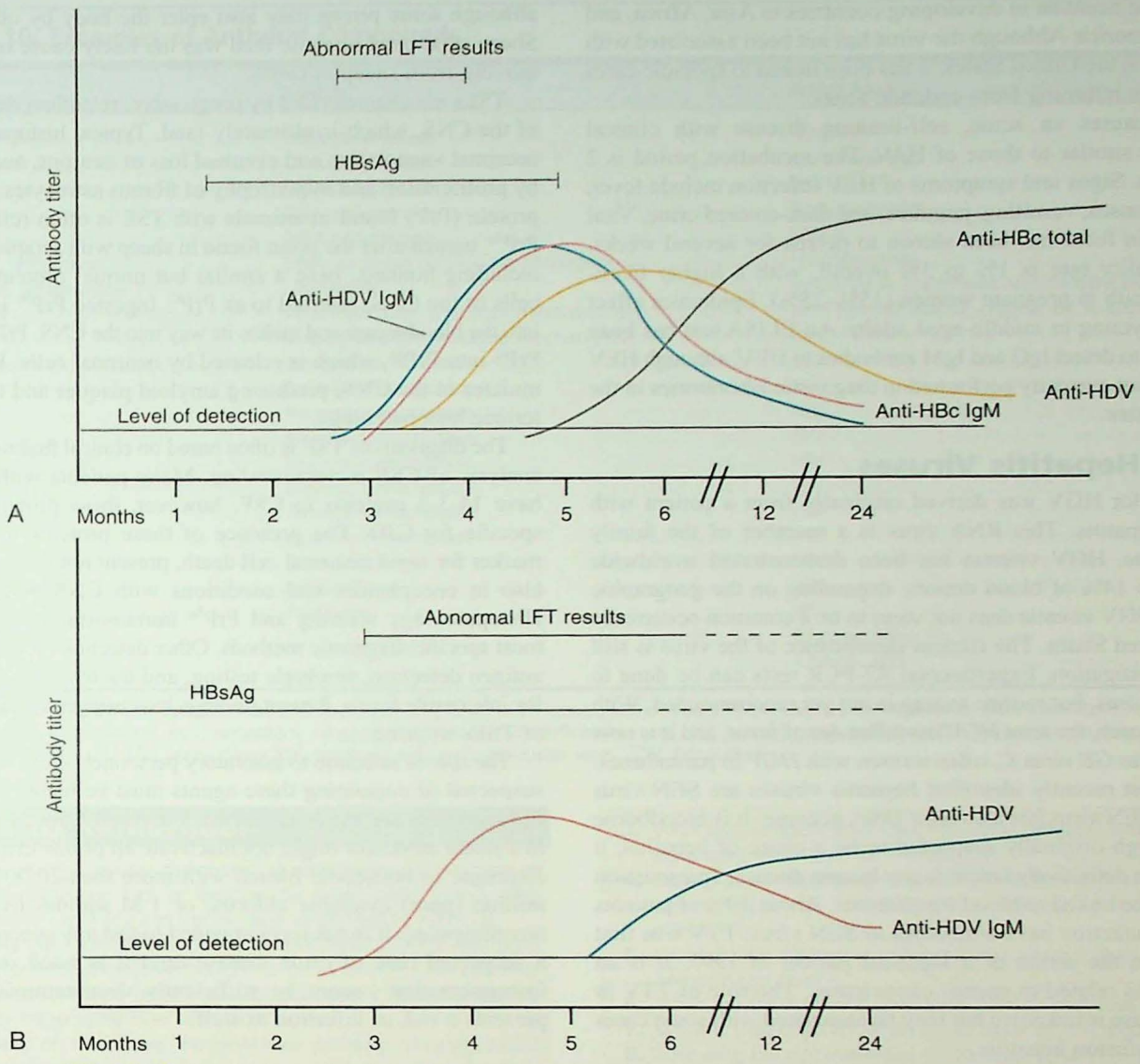

FIG. 29.20 Serologic evaluation of hepatitis D virus *(HDV)* infection showing the persistence of detectable antibodies, **A,** Hepatitis B virus (HBV)-HDV co-infection. **B,** HBV-HDV superinfection. *Anti-HBc,* Antibodies against hepatitis B core antigen; *HBsAg,* hepatitis B surface antigen; *IgM,* immunoglobulin M; *LFT,* liver function test.

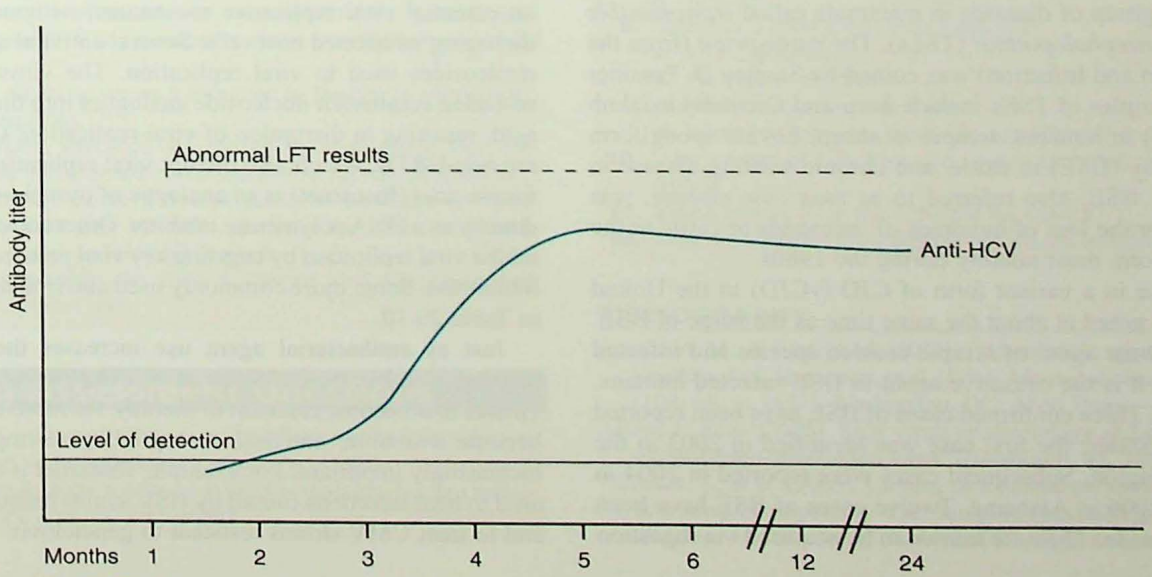

FIG. 29.21 Serologic evaluation of hepatitis C virus *(HCV)* infection showing the persistence of detectable antibodies, indicating the presence of replication HCV. *LFT,* Liver function test.

transmitted hepatitis in developing countries in Asia, Africa, and Central America. Although the virus has not been associated with outbreaks in the United States, it has been linked to sporadic cases in travelers returning from endemic areas.

HEV causes an acute, self-limiting disease with clinical symptoms similar to those of HAV. The incubation period is 2 to 9 weeks. Signs and symptoms of HEV infection include fever, malaise, nausea, vomiting, jaundice, and dark-colored urine. Viral shedding in feces has been shown to persist for several weeks. The mortality rate is 1% to 3% overall, with a higher likelihood of death in pregnant women (15%–25%). Epidemics affect primarily young to middle-aged adults. An ELISA test has been developed to detect IgG and IgM antibodies to HEV, although HEV testing is not currently performed in diagnostic laboratories in the United States.

Other Hepatitis Viruses

Evidence for HGV was derived originally from a patient with NANB hepatitis. This RNA virus is a member of the family Flaviviridae. HGV viremia has been demonstrated worldwide in 0.6% to 14% of blood donors, depending on the geographic location. HGV viremia does not seem to be a common occurrence in the United States. The clinical significance of the virus is still under investigation. Experimental RT-PCR tests can be done to detect the virus, but routine testing is not yet recommended. With further research, the term *HGV* has fallen out of favor, and it is now referred to as *GB virus C*, often written with *HGV* in parentheses.

The most recently identified hepatitis viruses are SEN virus and TTV. SEN virus has a circular DNA genome. It is bloodborne and, although originally suspected to be a cause of hepatitis, it has not been definitively linked to any human disease. Transmission appears to be linked to blood transfusions. About 30% of patients with HIV infection have antibodies to SEN virus. TTV was first identified in the serum of a Japanese patient in 1997. It is an ssDNA virus related to animal circoviruses. The role of TTV in human disease is unknown but may be associated with some cases of posttransfusion hepatitis.

Prions

Prions are not viruses but are proteinaceous infectious particles that cause a group of diseases in mammals called *transmissible spongiform encephalopathies* (TSEs). The name *prion* (from the words **pro**tein and **in**fection) was coined by Stanley B. Prusiner in 1982. Examples of TSEs include kuru and Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease in deer and elk. BSE, also referred to as *mad cow disease,* was responsible for the loss of hundreds of thousands of cattle in the United Kingdom, most notably during the 1980s.

An increase in a variant form of CJD (vCJD) in the United Kingdom was noted at about the same time as the surge of BSE. It appears that the agent of scrapie crossed species and infected cows and that it is the causative agent in BSE-infected humans, causing vCJD. Three confirmed cases of BSE have been reported in the United States; the first case was identified in 2003 in the state of Washington. Subsequent cases were reported in 2004 in Texas and in 2006 in Alabama. Twelve cases of BSE have been reported in Canada. TSEs are known to be acquired via ingestion although some prions may also enter the body by other routes. Sheep offal used in cattle feed was the likely cause of the prion moving from sheep to cattle.

TSEs are characterized by progressive, relentless degeneration of the CNS, which is ultimately fatal. Typical histopathology is neuronal vacuolation and eventual loss of neurons, accompanied by proliferation and hypertrophy of fibrous astrocytes. The prion protein (PrP) found in animals with TSE is often referred to as PrP^{Sc}, named after the prion found in sheep with scrapie. Animals, including humans, have a similar but normal protein found on cells of the CNS, referred to as PrP^{C}. Ingested PrP^{Sc} is absorbed into the bloodstream and makes its way into the CNS. PrP^{Sc} converts PrP^{C} into PrP^{Sc}, which is released by neuronal cells. PrP^{Sc} accumulates in the CNS, producing amyloid plaques and the characteristic histopathology.

The diagnosis of TSE is often based on clinical findings. Routine analysis of CSF is nonrevealing. Many patients with CJD will have 14-3-3 proteins in CSF; however, these proteins are not specific for CJD. The presence of these proteins in CSF is a marker for rapid neuronal cell death, present not only in CJD but also in encephalitis and conditions with CNS hemorrhaging. Histopathology staining and PrP^{Sc} immunostaining remain the most specific diagnostic methods. Other detection methods include antigen detection, serologic testing, and nucleic acid sequencing for inheritable forms. Recent findings have suggested the excretion of TSEs in urine.

The risk of infection to laboratory personnel is low, but material suspected of containing these agents must be handled carefully. Prion proteins are extremely resistant to inactivation; even 2 hours in a steam autoclave might not inactivate all prions in a specimen. Exposure to household bleach with more than 20,000 parts per million (ppm) available chlorine or 1 M sodium hydroxide is recommended. It is not recommended to test any specimens from a suspected case of prion disease until it is ruled out because instrumentation cannot be sufficiently decontaminated and it presents a risk of infection to staff.

Antiviral Therapy

Some viral infections are treatable, especially if the laboratory can rapidly identify the pathogen. Antiviral compounds must target an essential viral replicative mechanism without destroying or damaging uninfected host cells. Several antiviral agents resemble nucleosides used in viral replication. The viruses insert these so-called counterfeit nucleoside analogues into their own nucleic acid, resulting in disruption of viral replication. Other antivirals are non-NRTIs, which also disrupt viral replication. Phosphonoformic acid (foscarnet) is an analogue of pyrophosphate that acts directly as a DNA polymerase inhibitor. Other antiviral compounds inhibit viral replication by targeting key viral proteins (e.g., protease inhibitors). Some more commonly used antiviral agents are given in Table 29.10.

Just as antibacterial agent use increases the risk of drug resistance in bacteria, the use of antiviral agents can result in viruses that become resistant to therapy. As more antiviral agents become available, antiviral susceptibility testing will become increasingly important. For example, foscarnet is currently being used to treat infections caused by HSV strains resistant to acyclovir and to treat CMV strains resistant to ganciclovir.

TABLE 29.10 Examples of Antiviral Compounds

Antiviral	Inhibits	Active Against
Acyclovir	DNA polymerase	HSV, VZV
Cidofovir	DNA polymerase	CMV (retinitis)
Famciclovir	DNA polymerase	HSV-2
Ganciclovir	DNA polymerase	CMV (retinitis)
Valacyclovir	DNA polymerase	HSV-2
Idoxuridine, trifluridine	DNA synthesis (DNA base analogue)	HSV (keratitis)
Amantadine, rimantadine	Uncoating	Influenza A (treatment and prophylaxis)
Interferon-α	Viral replication (multiple mechanisms)	HPV (genital warts); chronic HCV, Kaposi sarcoma
Ribavirin	Viral replication (multiple mechanisms)	RSV; CCHF
AZT or ZDV	Reverse transcriptase	HIV
ddI	Reverse transcriptase	HIV
ABC	Reverse transcriptase	HIV
3TC	Reverse transcriptase	HIV
d4T	Reverse transcriptase	HIV
ddC	Reverse transcriptase	HIV
FTC	Reverse transcriptase	HIV
TDF	Reverse transcriptase	HIV
Indinavir	Proteases	HIV
Nelfinavir, ritonavir	Proteases	HIV
Saquinavir	Proteases	HIV
Lamivudine		Chronic HBV
Adefovir		Chronic HBV

ABC, Abacavir sulfate; *AZT,* azidothymidine; *d4T,* stavudine; *CCHF,* Crimean-Congo hemorrhagic fever; *CMV,* cytomegalovirus; *ddC,* zalcitabine; *ddI,* didanosine; *FTC,* emtricitabine; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HPV,* human papillomavirus; *HSV,* herpes simplex virus; *RSV,* respiratory syncytial virus; *3TC,* lamivudine; *TDF,* tenofovir; *VZV,* varicella-zoster virus; *ZDV,* zidovudine.

Points to Remember

- Clinical virology services can consist of simple and rapid antigen or antibody detection kits, or they can be more sophisticated, with cell culture capability and nucleic acid amplification methods.
- Clinically significant viruses can be isolated from patients with signs and symptoms commonly thought to be associated with bacterial infections, including pneumonia, GI disorders, cutaneous lesions, sexually transmitted infections, and sepsis.
- Members of the family Herpesviridae produce life-long latent infections.
- Most cases of cervical cancer are linked to HPV, the causative agent of genital warts.
- Some viruses mutate rapidly, resulting in new strains, which can be challenging to contain or treat.
- Retroviruses (e.g., HIV) replicate with the enzyme reverse transcriptase, which uses viral RNA as a template to make a complementary DNA strand.
- Arboviruses are those viruses transmitted by the bite of arthropods, such as mosquitoes.
- Many emerging infections are caused by viral agents that are unexpectedly transplanted into a susceptible human population.
- The hepatitis viruses are a diverse collection of viruses grouped together because they all infect primarily the liver. Laboratory diagnosis is based on serologic markers.
- Antiviral compounds can treat numerous viral infections, but resistance has been seen.

Learning Assessment Questions

1. Which opportunistic infections or conditions are used as indicators of acquired immunodeficiency syndrome (AIDS)?
2. Which immunologic markers are used to diagnose human immunodeficiency virus (HIV) infection?
3. What disease does Epstein-Barr virus (EBV) produce? What complications may result from EBV infections?
4. How is acute hepatitis B virus (HBV) infection differentiated from chronic infection? Which markers indicate resolution of the infection?
5. What are the differences between classic dengue fever and dengue hemorrhagic fever?
6. What are the methods commonly used to diagnosis rabies?
7. What is fifth disease? What is the cause of this disease?
8. Following the administration of the vaccine for HBV, which of the following serologic markers should be positive?
   a. HBeAg
   b. HBcAg
   c. Anti-HBs
   d. Anti-HBc
9. Rotavirus is most often associated with:
   a. Infant diarrhea
   b. Paralysis in children
   c. Infant respiratory infections
   d. Infectious mononucleosis–like symptoms in young adults
10. Which types of infections are caused by human papillomavirus (HPV)?
11. Which viruses have the potential for latency?
12. Why are vaccines for influenza not always effective?

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