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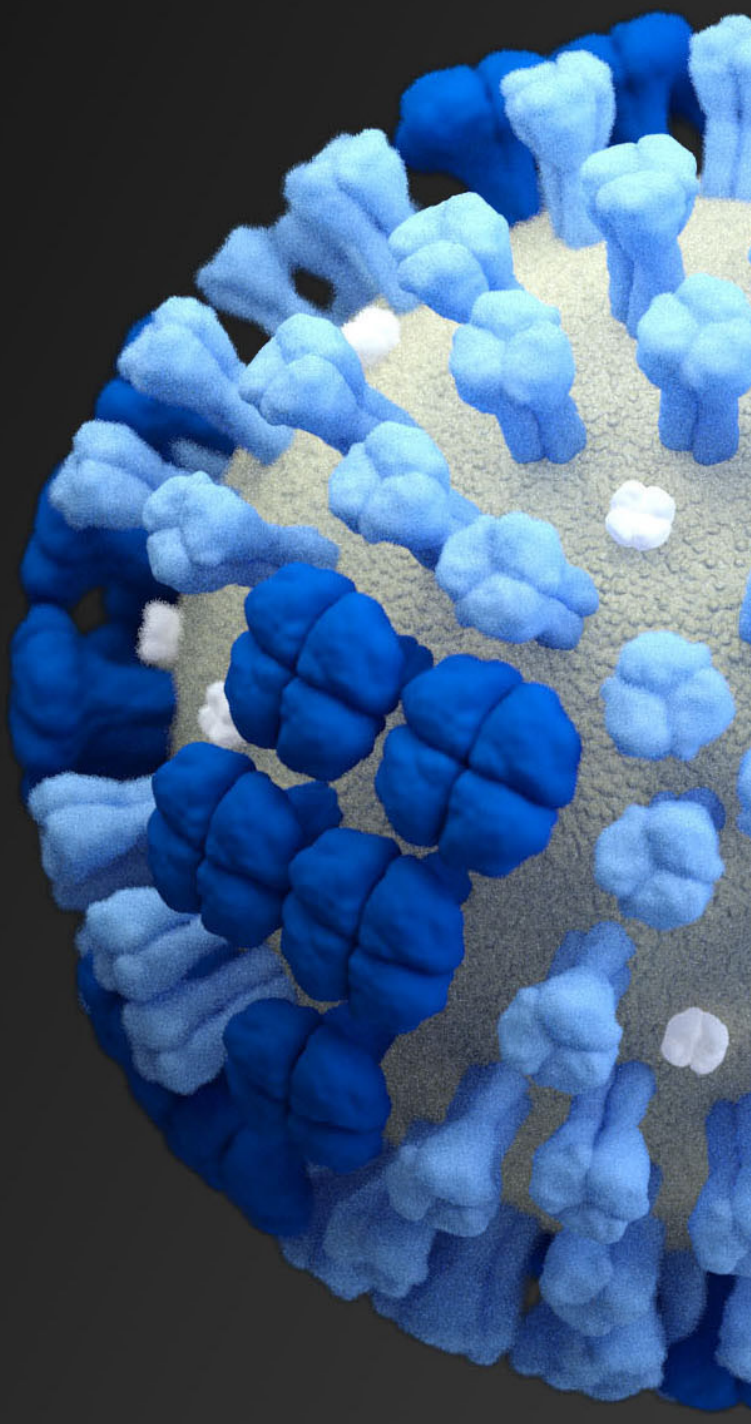
# Influenza

## Sampler



**Presenting sample chapters on influenza from the *Manual of Clinical Microbiology, 12th Edition,***

Chapter 86 “Influenza Viruses”
by Robert L. Atmar
This chapter discusses seasonal influenza strains as well as novel swine and avian influenza strains that can infect people and have pandemic potential.

Chapter 83 “Algorithms for Detection and Identification of Viruses”
by Marie Louise Landry, Angela M. Caliendo, Christine C. Ginocchio, Randall Hayden, and Yi-Wei Tang
This chapter outlines technological advances for the diagnosis of viral infections.

Chapter 113 “Antiviral Agents”
by Carlos A.Q. Santos and Nell S. Lurian
This chapter reviews antiviral agents approved by FDA and their mechanism(s) of action.

Photo Credit: CDC/ Douglas Jordan, Dan Higgins

# Influenza Viruses*

ROBERT L. ATMAR

# 86

## TAXONOMY

The influenza viruses are members of the family *Orthomyxoviridae*. Antigenic differences in two major structural proteins, the matrix protein (M) and the nucleoprotein (NP), and phylogenetic analyses of the virus genome are used to separate the influenza viruses into four genera within the family: *Influenzavirus A*, *Influenzavirus B*, *Influenzavirus C*, and *Influenzavirus D*. Members of these four genera are also referred to as influenza type A, B, C, and D viruses, respectively. The influenza A viruses are further classified into subtypes based upon characteristics of the two major surface glycoproteins, the hemagglutinin (HA) and neuraminidase (NA). Subtypes are recognized by the lack of cross-reactivity in double immunodiffusion assays with animal hyperimmune sera corresponding to each antigen (1). Currently, 18 HA subtypes and 11 NA subtypes are recognized (2). Within a subtype, strains may be further subclassified into lineages or clades based upon phylogenetic analysis of gene sequences. An example is the classification of the Eurasian lineage of highly pathogenic H5N1 strains into clades and the further subdivision of circulating viruses into second-, third-, fourth- and fifth-order clades (3). Influenza B viruses do not have subtypes, but they are subdivided into two antigenically distinct lineages: B/Victoria and B/Yamagata.

The following information is used in the naming of individual virus strains: type, species of origin (if nonhuman), geographic location of isolation strain, laboratory identification number, year of isolation, and subtype (influenza A viruses only). Thus, an example of a human strain of influenza is A/Texas/50/2012 (H3N2), while A/quail/Vietnam/36/2004 (H5N1) is an example of an avian strain isolated in an epizootic in Asia.

## DESCRIPTION OF THE AGENTS

Orthomyxoviruses are enveloped, single-stranded RNA viruses with segmented genomes of negative sense. Influenza A and B viruses have eight RNA segments, while influenza C and D viruses have only seven segments. Gene segments range from ~800 to ~2,500 nucleotides in length, and the entire genome ranges from 10 to 14.6 kb. The segmented genome of influenza viruses allows the exchange of one or more gene segments between two viruses when both infect a single cell. This exchange is called genetic reassortment and results in the generation of new strains containing a mix of genes from both parental viruses. Genetic reassortment between human and avian influenza virus strains led to the generation of the 1957 H2N2 and 1968 H3N2 pandemic strains, and it also played a role in the emergence of the pandemic 2009 H1N1 virus and in H7N9 avian strains from China that are causing infections in people (4).

Influenza viruses are spherical and pleomorphic, with diameters of 80 to 120 nm after serial passage in culture. Filamentous forms also occur and may be up to several micrometers in size. The lipid envelope is derived from the host cell membrane through which maturing virus particles bud, and HA and NA form characteristic rod-like spikes (HA) and spikes with globular heads (NA) on the virus surface. As its name implies, the HA can agglutinate red blood cells from both mammalian (e.g., human [type O], guinea pig, and horse) and avian (e.g., chicken and turkey) species by binding to sialic acid residues. The HA protein is the major antigenic determinant and is used to identify viruses with immune sera. The lipid envelope surrounds the nucleocapsid, which has helical symmetry and consists of the genomic RNA segments, several copies of the polymerase proteins, and the NP. The matrix-1 (M1) protein is present between the nucleocapsid and the envelope, and the matrix-2 (M2) protein forms an ion channel across the envelope in influenza A viruses.

## EPIDEMIOLOGY AND TRANSMISSION

Influenza A and B viruses cause annual epidemics in areas with temperate climates, but in tropical climates seasonality is less apparent and influenza viruses can be isolated throughout the year. In the temperate regions of the Northern Hemisphere, epidemics generally occur between December and March, and in the Southern Hemisphere, the epidemic period is usually between May and August. Epidemics are characterized by a sudden increase in febrile respiratory illnesses and absenteeism from school and work, and within a community the epidemic period usually lasts from 3 to 8 weeks. A single subtype (A) or type (B) of influenza virus usually predominates, but epidemics have

*This chapter contains information from chapter 84 by Robert L. Atmar and Stephen E. Lindstrom in the 11th edition of this *Manual*.

occurred in which both A and B viruses or two influenza A virus subtypes were isolated. Global epidemics, or pandemics, occur less frequently and are seen only with influenza A viruses. Pandemics occur following the emergence of an influenza A virus that carries a novel HA and that can be readily transmitted from person to person. The pandemic strain may develop because of genetic reassortment following coinfection of a susceptible host with human and animal influenza viruses or through gradual adaptation of an avian strain to mammalian hosts. Influenza C viruses cause asymptomatic or mild respiratory disease in people. Influenza D viruses infect swine and cattle, but they do not infect people (5).

Influenza viruses are transmitted from person to person primarily via droplets generated by sneezing, coughing, and speaking. Direct or indirect (fomite) contact with contaminated secretions and small-particle aerosols is another potential route of transmission that has been noted. The relative importance of these different routes has not been determined for influenza viruses (6). As for human infections caused by avian strains of influenza virus, direct contact with infected birds has been the most common factor of transmission, and direct inoculation into the pharynx or gastrointestinal tract may lead to infection (7, 8).

The pandemic potential of avian strains of influenza has been a concern since at least 1997, when several human cases of infection with H5N1 viruses occurred in Hong Kong in association with a large poultry outbreak. The outbreak was controlled by slaughtering all poultry in Hong Kong, but H5N1 viruses again caused outbreaks in poultry in China in 2003. By late 2005, the virus had spread to other parts of Asia and to parts of Europe, Africa, and the Middle East. Human cases of H5N1 infection have been directly associated with outbreaks in poultry, and as of 2017 more than 850 human infections have been documented. Most cases have occurred in southeastern Asia, but cases have also been documented in the Middle East and in northern Africa. Most human cases have been due to direct contact with infected birds, but limited human-to-human transmission has also occurred (7). Several mutations in influenza virus genes are required for avian influenza viruses to replicate efficiently in mammalian cells and to transmit by droplet aerosol between ferrets, an animal model of human infection (9, 10). H5N1 viruses continue to evolve and increase diversity, raising the possibility that they may acquire the ability to spread efficiently among humans.

Other avian influenza A virus subtypes are also of concern. An outbreak of H7N7 virus in commercial poultry farms in the Netherlands in 2003 was associated with respiratory illness in >400 persons, although only a single person died (11). Since 2013, H7N9 viruses have emerged in poultry markets in China, with more than 1,200 persons having been infected (12). Sporadic infection of humans with other avian subtypes is occasionally observed. The greatest risk for infection has been exposure to infected poultry, similar to what has been observed with human cases of H5N1.

Swine are another source of novel influenza virus strains that can infect people. In 2009 a novel influenza A/H1N1 virus (pdm09) was initially identified as a cause of significant febrile respiratory illnesses in Mexico and the United States, and it rapidly spread to many countries around the world, which prompted the World Health Organization (WHO) to declare an influenza pandemic. The new strain subsequently replaced previously circulating seasonal H1N1 strains. Other infections with swine virus and antigenically distinct HAs (e.g., variant H3N2) have been identified in the United States (13). Fortunately, most cases are associated with direct or indirect contact with swine, and these variant strains have not spread among the population like the H1N1 pdm09 strain and can be suspected based upon epidemiologic exposures. The transmission of influenza viruses to people from avian and swine species highlights the need for vigilant surveillance for such events.

## CLINICAL SIGNIFICANCE

Influenza A and B virus infections typically cause a febrile respiratory illness characterized by fever, cough, upper respiratory tract symptoms (including sore throat, rhinorrhea, and nasal congestion), and systemic symptoms (including headache, myalgia, and malaise). This constellation of symptoms is called influenza, although other clinical presentations, ranging from asymptomatic infection to viral pneumonia, also occur. Illness begins abruptly after a 1- to 5-day incubation period (average, 2 days). Fever generally lasts for 3 to 5 days, but symptoms of dry cough and malaise may persist for several weeks. Complications include otitis media in children, sinusitis, viral pneumonia, secondary bacterial pneumonia, exacerbation of underlying cardiac or pulmonary disease, myositis (including rhabdomyolysis), neurologic problems (seizures, acute encephalitis/encephalopathy, and postinfectious encephalopathy), Reye syndrome (associated with aspirin use), myopericarditis, and death (14–16). In contrast, influenza C viruses cause mild respiratory illnesses that clinically are not distinguishable from common colds.

Influenza A(H5N1) and A(H7N9) viruses also cause a febrile respiratory illness, although lower respiratory tract illness is more prevalent. Upper respiratory tract symptoms may be absent, and gastrointestinal symptoms (watery diarrhea, vomiting, and abdominal pain) occur in some patients (7, 17). Acute encephalitis may occur. H5N1 infection is associated with a high mortality (~60%), with most patients dying of progressive pneumonia. Although overall severity of infection with H7N9 viruses is lower than for H5N1 strains, mortality is still at least 30% (17). Patient age and the presence of underlying diseases have been different among hospitalized patients dying from H5N1 or H7N9 infection (18). Viral replication may be prolonged, and levels of several inflammatory mediators (e.g., interleukin-6, interleukin-8, and interleukin-1beta) in plasma have been higher in fatal cases than in nonfatal cases. Surviving patients develop measurable serum antibody responses 10 to 14 days after symptom onset.

Influenza A and B virus infections spread rapidly through the community, with clinical attack rates having been documented to be as high as 70% following a common source exposure in an enclosed space. Epidemic disease is associated with an increase in hospitalization rates, especially in young children and in the elderly, and an increase in mortality rates in the elderly. Mortality rates have been higher in epidemics caused by influenza A/H3N2 viruses than in those caused by H1N1 or B viruses in the past 20 years. Additional information on the clinical presentation, manifestations, and complications of the diseases can be found in clinical textbooks (14, 15).

There are five licensed antiviral medications available for the treatment of influenza virus infection. Amantadine and rimantadine are adamantanes that block the M2 ion channel. The adamantanes have no activity against influenza B viruses, and unfortunately the currently circulating influenza A viruses have developed resistance so that the adamantanes are not clinically useful as monotherapy for these viruses either. Zanamivir, oseltamivir, and peramivir are NA inhibitors and are active against both influenza A

and B viruses. Clinically significant resistance can occur following treatment of immunocompromised patients. Treatment with any of these medications should be initiated within 2 days of symptom onset to have demonstrable clinical benefit, although initiation of treatment of virus-positive hospitalized patients has been recommended at any time during the illness (19). These drugs have also been used for prophylaxis, but annual immunization with a trivalent or quadrivalent influenza vaccine is the primary means of prevention of influenza.

Inactivated influenza vaccines (IIVs), live attenuated influenza virus (LAIV) vaccine, and a recombinant hemagglutinin vaccine (RIV) are licensed in the United States (20). The IIVs are derived from viruses grown in cell culture (ccIIV) or embryonated chicken eggs that are harvested and then inactivated. Viral proteins are partially purified and standardized to contain 15 μg of HA per dose. The IIVs may be trivalent (IIV3), containing influenza A/H1N1, A/H3N2, and B virus strains, or quadrivalent (IIV4), containing influenza B virus strains from two lineages (B/Victoria and B/Yamagata), A/H1N1, and A/H3N2. A high-dose IIV3 containing 60 μg of each HA and an MF59-adjuvanted IIV3 containing 15 μg of each HA are also licensed for adults 65 years of age and older. The RIV3 and RIV4 vaccines contain 45 μg of baculovirus-expressed, recombinant hemagglutinin for an A/H1N1, A/H3N2, and one or two B strains. LAIV4 vaccine is quadrivalent and contains the same strains recommended for IIV4. A reassortant vaccine virus for each strain to be included is derived to contain six internal genes from a parental attenuated influenza (A or B) virus and the HA and NA from the WHO-recommended vaccine strain. It is given topically into the nose, and the virus replicates in the upper respiratory tract (21). The vaccine is licensed in the United States for use in persons 2 to 49 years of age, although 3 years of poor effectiveness against influenza A viruses led the American Committee on Immunization Practices (ACIP) to withdraw its recommendation for its use for 2 years (20). In February 2018, the ACIP recommendation for LAIV use was renewed based upon changes made to correct the poor replicative fitness of the H1N1 component that was identified as the putative cause of LAIV's low effectiveness. The latest guidance for the use of influenza vaccines in the United States can be found online at https://www.cdc.gov/flu/protect/keyfacts.htm.

Due to constant virus evolution causing gradual antigenic changes in the HA protein, viruses included in the influenza vaccines must be updated periodically. The strains to be included in the vaccine are selected twice annually by WHO. Vaccine strains for Northern Hemisphere countries are selected in January and February to make vaccine for use in September. New vaccine alternatives, including those given by other routes and in combination with adjuvants, are undergoing clinical studies.

## COLLECTION, TRANSPORT, AND STORAGE OF SPECIMENS

Influenza viruses infect the respiratory epithelium and can be found in respiratory secretions of all types. The level of virus shedding parallels the severity of clinical symptoms in uncomplicated influenza and is maximal in the first several days of illness. Samples should be collected during this time (first 2 to 3 days) to maximize the likelihood of virus detection. A variety of upper respiratory tract samples alone or in combination are routinely used for virus identification, including nasal aspirates, nasal wash fluids, nasal or nasopharyngeal swabs, throat swabs, and throat wash fluids. Virus titers tend to be lower in samples collected from the throat, so assays of these samples alone tend to be less sensitive (22, 23). However, reports of human infection caused by H5N1 and H7N9 strains suggest that throat samples and lower respiratory tract samples may have better diagnostic yields than samples collected from the nose (7, 24). Lower respiratory tract samples, including sputa, tracheal aspirates, and bronchoalveolar lavage fluids, may yield virus and can be assayed when available; some studies have found higher yields with sputum than with upper respiratory samples (25, 26). Virus can occasionally be identified in nonrespiratory clinical samples (7).

Once collected, the clinical samples should be placed in viral transport medium. A number of transport media are suitable for influenza viruses, including veal infusion broth, Hanks balanced salt solution, tryptose phosphate broth, sucrose phosphate buffer, and commercially available cell culture medium. All these media are supplemented with 0.5% bovine serum albumin or 0.1% gelatin to stabilize the virus and antimicrobials (antibiotics and antifungals) to inhibit the growth of other respiratory biota. However, the use of transport medium may interfere with the test performance for certain commercially available virus detection assays; the package inserts of these assays should be consulted if they are to be used for diagnosis (Tables 1 and 2). Influenza virus infectivity is maintained for up to 5 days when samples are placed in transport media and maintained at 4°C (27). Clinical samples should be transported to the diagnostic laboratory as rapidly as possible after collection under these conditions. If a sample cannot be cultured during this time frame, it should be stored immediately at −70°C; storage at higher temperatures (e.g., −20°C) leads to the loss of virus viability. Immediate transport and processing of samples after collection are necessary for immunofluorescence detection of virus antigen in exfoliated epithelial cells.

## DIRECT DETECTION

### Microscopy

Influenza viruses have been detected in clinical specimens by direct and indirect visualization of their typical morphological appearance by electron microscopy (EM). Immune EM has been the most sensitive EM method and allows differentiation of virus type and subtype when specific hyperimmune sera are used in the assay (28). However, large numbers of viruses ($>10^5$ to $10^6$ per ml) must be present in the clinical sample for successful detection using this diagnostic approach. Because of the need for an experienced microscopist and access to an electron microscope, the relatively high costs of assay performance, and the greater sensitivity of other diagnostic approaches, EM is not routinely used for the diagnosis of influenza virus infection.

### Antigen Detection

Antigen detection assays are used in a variety of formats to rapidly detect influenza viruses in clinical specimens and to confirm the identity of isolates grown in culture. These assays are based upon detection of the interaction of viral proteins with specific antibodies. A variety of different formats have been used, including direct and indirect fluorescent antibody (FA) staining, enzyme immunoassay, immunochromatographic assay, and fluoroimmunoassay.

FA assays identify viral antigens present on, or in, infected, exfoliated epithelial cells present in respiratory secretions. Cells are collected on swabs or in aspirates or

**TABLE 1** Commercially available kits for detection of influenza A or B viruses by fluorescent antibody staining[a]

| Assay format | Kit name (Manufacturer) | Acceptable clinical samples for direct detection; cell culture confirmation | Comments | Influenza virus types detected | Assay sensitivity and specificity for direct detection per mfg. brochure | Assay sensitivity and specificity for isolate identification per mfg. brochure | Other viruses detected |
|---|---|---|---|---|---|---|---|
| DFA | D$^3$ Ultra DFA Respiratory Virus Screening & ID kit (Diagnostic Hybrids, Inc.) | NA, NPA, NW; cell culture | Virus-specific MAbs provided mixed for screen and individually for identification | A and B | A: 100%, 100%<br>B: 100%, 98.7–100% | A: 100%, 100%<br>B: 100%, 100% | Ad, P1, P2, P3, RSV |
| DFA | D$^3$ Duet DFA Respiratory Virus Screening kit (Diagnostic Hybrids, Inc.) | NA, NPA, NPS, NS; cell culture | Distinguishes influenza A from other respiratory viruses; these viruses (including influenza B) must be identified with reagents from the Ultra kit | A and B | A: 99%, A and B 100%<br>B: 100%, 100% | A: 100%, 100%<br>B: 100%, 100% | Ad, P1, P2, P3, RSV |
| DFA | D$^3$ FastPoint DFA Respiratory Virus Screening kit (Diagnostic Hybrids, Inc.) | NA, NPA, NPS, NS, NW | Distinguishes influenza A from influenza B | | A: 82.9–100%, 97.5–100%<br>B: 66.7–100%, 99.7–100% | N/A | |
| DFA | Imagen Influenza Virus A and B (Thermo Fisher Scientific) | NPA; cell culture | Virus-specific MAbs provided mixed for screen and individually for identification | A and B | A: 96.2%, 100%<br>B: 86.7%, 99.5% | A: 100%, 100%<br>B: 100%, 100% | None |
| DFA | PathoDx Respiratory Virus Panel (Oxoid) | Cell culture only | Not approved for direct use on clinical specimens | A and B | N/A | A: 100%, 100%<br>B: 100%, 100% | Ad, P1, P2, P3, RSV |
| DFA | Light Diagnostics Simulfluor Viral Diagnostic Screen (Millipore) | Cell culture | Does not distinguish influenza A or influenza B from Ad, P1, P2, or P3 | A and B | N/A | A: 95.8–100%, 99.6–100%<br>B: 100%, 100% | Ad, P1, P2, P3, RSV |
| DFA | Light Diagnostics Simulfluor Flu A/Flu B (Millipore) | BAL, NPA, NPS, NS, NW, TS; cell culture | Distinguishes influenza A from influenza B | A and B | A: 58.8–80%, 98.3–98.6%<br>B: 43.2–50%, 98.3–100% | A: 97.8–100%, 100%<br>B: 100%, 100% | |
| DFA | Light Diagnostics Simulfluor RSV Flu A (Millipore) | Cell culture | Distinguishes influenza A from RSV | A | N/A | A: 95.8–100%, 99.6–100% | RSV |
| IFA | Imagen Respiratory Screen (Thermo Fisher Scientific) | NPA; cell culture | Does not distinguish between different viruses in kit | A and B | 96.7%, 89.6%[b] | A: 100%, 100%<br>B: 100%, 100% | Ad, P1, P2, P3, RSV |
| IFA | Light Diagnostics Respiratory Viral Screen IFA (Millipore) | Cell culture | Does not distinguish influenza A or influenza B from Ad, P1, P2, P3, or RSV | A and B | A: 100%, 100%<br>B: 100%, 100% | A: 100%, 100%<br>B: 100%, 100% | Ad, P1, P2, P3, RSV |

[a]DFA, direct fluorescent antibody; IFA, indirect fluorescent antibody; NA, nasal aspirate; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; NS, nasal swab; NW, nasal wash; TS, throat swab; N/A, not applicable; Ad, adenovirus; P1, parainfluenza type 1; P2, parainfluenza type 2; P3, parainfluenza type 3; RSV, respiratory syncytial virus.

[b]Sensitivity and specificity for all viruses tested; unable to determine assay parameters for each virus.

**TABLE 2** Commercially available, antigen-based RIDT kits for rapid (≤30 minutes) detection of influenza A or B viruses[a,b]

| Assay format | Kit name (Manufacturer) | Acceptable clinical samples | Sample collection restrictions | Assay performance time (min) | Assay complexity[c] (510K number) |
|---|---|---|---|---|---|
| Dipstick chromatographic immunoassay | Alere Influenza A & B (Alere) | NS | Use the swabs provided in the kit | 10 | CLIA waived (K092349) |
| Dipstick chromatographic immunoassay | QuickVue Influenza A+B Test (Quidel Corporation) | NPS, NS | Limited transport media supported | 10 | CLIA waived (K031899) |
| Lateral flow chromatographic immunoassay | Biosign Flu A+B (Princeton BioMeditech Corporation); Consult Immunoassay Influenza A&B (McKesson); ImmunoCard STAT! Flu A&B (Meridian Bioscience, Inc.); OraSure Quick Flu Rapid Flu A+B Test (OraSure Technologies, Inc.); OSOM Ultra Flu A&B (Sekisui Diagnostics); Status Flu A&B (Life Sign LLC) | NS, NPS, NPA, NW | Use only swabs supplied with the kit | 10–15 | CLIA waived (NS, NPS); moderate (NPA, NW) (K083746)[d] |
| Lateral flow chromatographic immunoassay | Xpect Flu A&B (Thermo Fisher Scientific) | NS, NW, TS | For swab samples, use synthetic-tipped (Dacron or nylon) swabs with aluminum or plastic shafts; cotton tips and wooden shafts not recommended; do not use calcium alginate | 15 | CLIA moderate (K031565) |
| Lateral flow chromatographic immunoassay with a reader | Alere BinaxNOW Influenza A&B Card 2 (Alere)[e] | NPS, NS | Swabs included in the kit | 15 | CLIA waived (K162642) |
| Lateral flow chromatographic immunoassay with a reader | BD Veritor (Becton Dickinson)[e] | NPS, NS | — | 10 | CLIA waived (K112277) |
| Lateral flow chromatographic immunoassay with a reader | BD Veritor (Becton Dickinson)[e] | NA, NW, NPS in transport media | — | 10 | CLIA moderate (K121797) |
| Lateral flow fluorescent immunoassay with a reader | Sofia Influenza A+B FIA (Quidel Corporation)[e] | NPA, NPS, NS, NW | Use nylon-flocked swab for NPS and kit swab for NS | 15 | CLIA waived (K162438) |

[a]Additional information on rapid tests can be found at the following website: http://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm.
[b]NA, nasal aspirate; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; NS, nasal swab; TS, throat swab; NW, nasal wash.
[c]CLIA (Clinical Laboratory Improvement Amendments): CLIA-waived laboratory assays employ methodologies that are so simple and accurate as to render the likelihood of erroneous results negligible. CLIA-moderate complexity assays require some knowledge, training, reagent preparation, processing, proficiency, ability to troubleshoot or interpret, and judgment in the performance of the test. CLIA-waived assays may be used as point-of-care tests; some when used in the laboratory are reclassified as moderate complexity.
[d]Several kits with different names are distributed under the same 510K number.
[e]Requires a reader for assay interpretation.

wash fluids and are washed in cold buffer to remove mucus before being applied and fixed to a microscope slide. Use of cytocentrifugation for application of the cells to slides can improve the number and morphology of cells for evaluation and enhance the accuracy of interpretation. Virus-specific antibodies are applied to the fixed cells; monoclonal antibodies directed against viral proteins that are conserved and expressed in large quantities (e.g., M and NP) are used because of their greater specificity compared to polyclonal sera and are available from a number of manufacturers. A fluorochrome is conjugated to the virus-specific antibody in direct FA (DFA) assays, and it is conjugated to a second antibody that reacts with the virus-specific antibody in indirect FA (IFA) assays. Antibody staining of cells is detected with a fluorescent microscope. Contaminating mucus can cause nonspecific fluorescence that can be reduced by treating the samples with N-acetylcysteine or dithiothreitol and by centrifuging cells through Percoll. DFA and IFA assays take 2 to 4 h to perform, although some diagnostic laboratories batch samples and do not perform tests as soon as the sample is received, delaying the availability of results. In theory, IFA assays should be more sensitive and less specific than DFA assays, but there is significant overlap, noted in published reports, in the sensitivities (50% to 90%) and

specificities (generally >90%) of these assays (29). Lower sensitivities may be the result of suboptimal laboratory expertise or malfunctioning equipment (30). An advantage of FA assays is that sample quality can be determined by observing whether an adequate number of epithelial cells are present. In addition, kits are available to screen for other respiratory viruses (e.g., respiratory syncytial virus, parainfluenza viruses, and adenovirus) as well as for influenza A and B viruses (Table 1). These multiplex assays allow for efficient screening for other viral causes of febrile respiratory disease. Disadvantages include the need for specialized equipment (a fluorescent microscope) and the effect of technician expertise on assay performance characteristics (i.e., sensitivity and specificity). Each laboratory should establish its own performance characteristics compared to those of cell culture.

Several immunoassays that use different reporter formats (colorimetric, fluorometric, and chromatographic) have been developed for the detection of influenza virus antigen in clinical specimens. Many of these assays take at least 2 h to perform and have 50% to 80% sensitivity compared to culture methods. RIDT kits that use immunoassay formats for rapid (≤30-min) detection of influenza A and B viruses in clinical specimens are used much more commonly than other antigen detection immunoassay formats (Table 2). The kits use monoclonal antibodies to detect the presence of the influenza A or B nucleoprotein by chromatographic immunoassay. All the kits provide results within 30 min, and some of them can be used as point-of-care tests (i.e., those classified by the Clinical Laboratory Improvement Amendments [CLIA] as waived). The types of specimens that are appropriate for testing vary among the kits, and specific instructions for sample collection and processing must be followed for optimal results. Assay performance characteristics in clinical settings are affected by the age of the patient (generally lower sensitivity in adults), by the amount of virus in the clinical sample, and by the type of specimen analyzed. The sensitivity of antigen detection-based RIDTs for identification of infection was noted to be quite poor in some circumstances during the 2009 H1N1 pandemic (31, 32). The lower sensitivity associated with many of the antigen detection-based RIDTs led the Food and Drug Administration to establish minimum sample sensitivity requirements with appropriate culture or molecular methods as the gold standard (Table 3), to monitor device performance over time to evaluate its ability to identify contemporary strains (available annually from the Centers for Disease Control and Prevention), and to require provisions for evaluating an antigen detection-based RIDT's ability to detect newly emerging influenza virus strains (33). As a result, several previously marketed kits are no longer available, and others have been modified to enhance their performance. Even with these new requirements, a negative RIDT result should not prevent prescription of antiviral treatment for a patient with suspected influenza, especially when influenza is prevalent in the community, and follow-up testing with culture or RT-PCR should be considered (19).

**TABLE 3** FDA minimal performance requirements for antigen-based RIDts (28)

| | Comparator = molecular assay | Comparator = culture |
|---|---|---|
| Sensitivity minimal point estimate | | |
| Influenza A | 80% | 90% |
| Influenza B | 80% | 80% |
| Sensitivity, 95% CI lower bound | | |
| Influenza A | ≥70% | ≥80% |
| Influenza B | ≥70% | ≥70% |
| Specificity, minimal point estimate | | |
| Influenza A and B | 95% | 95% |
| Specificity, 95% CI lower bound | | |
| Influenza A and B | ≥90% | ≥90% |

## Nucleic Acid Analyses

Molecular methods are commonly being used both for the detection and characterization (see below) of influenza viruses. The most commonly used molecular method is reverse transcription (RT)-PCR. Viral nucleic acids are first extracted from clinical samples. The use of guanidinium thiocyanate with silica particles or commercial kits based upon this approach reliably removes inhibitors of the enzymatic amplification that are often present in clinical specimens. Automated extraction instruments decrease the amount of time personnel must spend in sample preparation while increasing the reproducibility of the procedure compared to the use of manual extraction methods, and several commercial assays are licensed to be used in combination with an automated extraction procedure. Reverse transcriptase is used to synthesize cDNA from viral RNA by random hexamers or virus gene-specific oligonucleotides. The cDNA is then amplified by use of virus gene-specific oligonucleotides as primers and a heat-stable DNA polymerase. Resulting amplicons are identified as virus specific by a variety of different methods (e.g., identification by size, hybridization, restriction enzyme mapping, and sequencing).

Many different RT-PCR assays have been developed since the initial description in 1991 of an RT-PCR method to detect and distinguish influenza A, B, and C viruses (34). Assays that identify and distinguish different influenza virus types have targeted conserved genes, such as the matrix gene, and subtype-specific assays have amplified a portion of the HA gene (Table 4). Nested PCR assays have been developed to improve assay sensitivity, but the inherent problem of carryover contamination associated with the use of this assay format limits its utility for most diagnostic laboratories. Real-time RT-PCR assays, which are less vulnerable to cross-contamination, can directly and rapidly detect influenza viruses in clinical specimens with a sensitivity approaching or exceeding that of culture (35). Multiplexed assays able to identify influenza viruses and other respiratory viruses have been developed and have performance characteristics that meet or exceed those of cell culture (36, 37). A variety of methodologies are used to detect amplified products, and different equipment is needed based upon each assay's characteristics. Multiplexed respiratory virus panels may be less sensitive than monoplex molecular assays that target a single virus (36, 37). Genetic drift among circulating viruses can result in mutations in primer and probe target regions, resulting in decreased assay sensitivity, as has been noted for some assays targeting H3N2 viruses in recent years (38). The availability, and FDA clearance, of such assays and their ability to identify multiple other respiratory pathogens (Table 4) has led many diagnostic laboratories to use these assays for respiratory virus diagnosis in place of the more time-consuming cell

**TABLE 4** FDA-cleared molecular detection assays for influenza viruses[a,b]

| Assay format | Kit name (Manufacturer) | Instrumentation | Acceptable clinical samples | Virus type(s), (subtypes) detected | Influenza target gene(s) | Assay performance time (min) | Assay complexity (510K number) | Other viruses detected | Reference(s) |
|---|---|---|---|---|---|---|---|---|---|
| Isothermal nucleic acid amplification assay—nicking enzyme amplification reaction (NEAR) | Alere i Influenza A&B; Alere i Influenza A&B 2 (Alere) | Alere i | NPS, NS; direct or in VTM | A, B | PB2 (A); PA (B) | <15 | CLIA waived (K141520, K171792); Moderate (K163266, K111387) | None | 42, 78 |
| Isothermal nucleic acid amplification assay—reverse transcriptase-helicase-dependent amplification (RT-HDA) | Solana Influenza A+B assay (Quidel Corporation) | Solana Instrument | NPS, NS | A, B | Matrix (A, B) | ~45 | Moderate (K161814) | None | 79 |
| Multiplex real-time RT-PCR assay | cobas Liat Influenza A/B (Roche Molecular Diagnostics) | cobas Liat system | NPS | A, B | Matrix (A); NSP (B) | ~20 | CLIA waived (K111387) | None | 78 |
| Multiplex real-time RT-PCR assay | cobas Liat Influenza A/B & RSV (Roche Molecular Diagnostics) | cobas Liat system | NPS | A, B | Matrix (A); NSP (B) | ~20 | CLIA waived (K153544) | RSV | 80 |
| Multiplex real-time RT-PCR assay | Panther Fusion Flu A/B/RSV (Hologic, Inc.) | Panther Fusion system | NPS | A, B | Matrix (A, B) | ~150 | High (K171963) | RSV | 81 |
| Real-time RT-PCR | CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (CDC) | ABI 7500 Fast DX Real-Time PCR instrument | BAL, BW, NA, NPS, NS, NW, TA, TS, sputum, lung | A (H1, H3, 2009 H1, H5), B (Yamagata lineage [B/Yam], Victoria [B/Vic] lineage) | Matrix (A); NP (A/swine); NSP (B); HA (H1, H3, 2009 H1, H5, B/Yam, B/Vic) | ~240 | High (K132508) | None | 82 |
| Real-time RT-PCR | Joint Biologic Agent Identification Diagnostic System (JBAIDS) Influenza A&B (U.S. Army) | JBAIDS instrument | NPS in VTM, NW | A, B | Matrix (A); NSP (B) | ~240 | High (K111775) | None | 83 |
| Real-time RT-PCR | JBAIDS Influenza A/H5 (Asian lineage) Detection Kit (U.S. Army) | JBAIDS instrument | NPS, TS in VTM | A/H5 | HA (H5, Asian lineage) | ~240 | High (K100287) | None | None |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Real-time RT-PCR | JBAIDS Influenza A Subtyping Kit (U.S. Army) | JBAIDS instrument | NPS in VTM, NW | A/H1, A/2009 H1, A/H3, A/swine | HA (H1, 2009 H1, H3); NP (A/swine) | ~240 | High (K111778) | None | 83 |
| Multiplex RT-PCR with colorimetric visualization | Accula Flu A/B Test (Mesa Biotech, Inc.) | Accula Dock instrument | NS | A, B | PB2 (A), Matrix (B) | ~30 | CLIA waived (K171641) | None | None |
| Multiplex RT-PCR | artus Influenza A/B RG Kit (Qiagen GmbH) | Rotor-Gene Q MDx instrument | NPS in VTM | A, B | Matrix (A, B) | ~240 | High (K113323) | None | 84 |
| Multiplex real-time RT-PCR | Cepheid Xpert Flu Assay | Cepheid GeneXpert Instrument Systems | NA, NPS, NW in VTM | A, B | Matrix (A); HA (2009 H1); ? for B | 75 | Moderate (K123191) | None | 85, 86 |
| Multiplex real-time RT-PCR | Cepheid Xpert Flu/RSV XC Assay | Cepheid GeneXpert Instrument Systems | NA, NPS, NW in VTM | A, B | Matrix (A, B), PB2 (A); PA (A); NSP (B) | 60 | CLIA waived, Moderate (K142045) | RSV | 87 |
| Multiplex real-time RT-PCR | Cepheid Xpert Xpress Flu Assay | Cepheid GeneXpert Instrument Systems | NPS in VTM | A, B | Matrix (A, B), PB2 (A), PA (A), NSP (B) | ~30 | CLIA waived (K171552), Moderate (K162456) | None | None |
| Multiplex real-time RT-PCR | Cepheid Xpert Xpress Flu/RSV Assay | Cepheid GeneXpert Instrument Systems | NPS in VTM | A, B | Matrix (A, B), PB2 (A), PA (A), NSP (B) | ~30 | Moderate (K162331) | RSV | 88, 89 |
| Multiplex RT-PCR with probe detection using voltammetry | ePlex Respiratory Virus Panel (GenMark Diagnostics, Inc.) | ePlex Instrument | NPS in VTM | A (H1, 2009 H1, H3), B | Matrix (A); PB1 (B); HA (H1, H3, 2009 H1) | ~105 | Moderate (K163636) | RSVA, RSVB, PIV1, PIV2, PIV3, PIV4, hMPV, Ad, HRV/Ent, CoV | 90 |
| Multiplex RT-PCR with probe detection using voltammetry | eSensor Respiratory Virus Panel (GenMark Diagnostics, Inc.) | eSensor XT-8TM System | NPS in VTM | A (H1, 2009 H1, H3), B | Matrix (A); PB1 (B); HA (H1, H3, 2009 H1) | ~480 | High (K113731) | RSVA, RSVB, PIV1, PIV2, PIV3, hMPV, Ad, HRV/Ent | 39, 91 |
| Multiplex RT-PCR with endpoint melt curve analysis | FilmArray Respiratory Panel (BioFire Diagnostics) | FilmArray Instrument, FilmArray System 2.0 or FilmArray Torch System | NPS in VTM | A (H1, 2009 H1, H3), B | Matrix (A); HA (H1, 2009 H1, H3, B) | ~60 | Moderate (K110764, K160068) | RSV, PIV1, PIV2, PIV3, PIV4, hMPV, HRV/Ent, Ad, OC43, HKU1, 229E, NL63 | 39, 90 |
| Multiplex RT-PCR with endpoint melt curve analysis | FilmArray Respiratory Panel 2.0 (BioFire Diagnostics) | FilmArray System 2.0 or FilmArray Torch System | NPS in VTM | A (H1, 2009 H1, H3), B | Matrix (A); HA (H1, 2009 H1, H3, B) | ~45 | Moderate (K170604) | RSV, PIV1, PIV2, PIV3, PIV4, hMPV, HRV/Ent, Ad, OC43, HKU1, 229E, NL63 | 92 |

*(Continued on next page)*

**TABLE 4** FDA-cleared molecular detection assays for influenza viruses[a,b] *(Continued)*

| Assay format | Kit name (Manufacturer) | Instrumentation | Acceptable clinical samples | Virus type(s), (subtypes) detected | Influenza target gene(s) | Assay performance time (min) | Assay complexity (510K number) | Other viruses detected | Reference(s) |
|---|---|---|---|---|---|---|---|---|---|
| Multiplex RT-PCR with endpoint melt curve analysis | FilmArray Respiratory Panel EZ (BioFire Diagnostics) | FilmArray Instrument | NPS in VTM | A (H1, 2009 H1, H3), B | Matrix (A); HA (H1, 2009 H1, H3, B) | ~60 | CLIA waived (K152579) | RSV, PIV[c], hMPV, HRV/Ent, Ad, CoV[c] | None |
| Multiplex RT-PCR with electrospray ionization-mass spectrometry (ESI-MS) | PLEX-ID Flu (Abbott Laboratories) | PLEX-ID system | NPS | A (H1, H3), B | PB1, NP (A), Matrix (A), PA (A), PB (A), PB2 (B), NS1 (A), HA (2009 H1), NA (2009 H1) | ~480 | High (K121003) | None | 93 |
| Multiplex real-time RT-PCR | ProFast+ Assay (Hologic, Inc.) | Cepheid Smart-Cycler II | NPS in VTM | A (2009 H1, seasonal H1, H3) | HA | ~240 | High (K101855) | None | 93, 94 |
| Multiplex real-time RT-PCR | ProFlu+ Assay (Hologic, Inc.) | Cepheid SmartCycler II | NPS in VTM | A, B | Matrix (A), NSP (B) | ~240 | High (K110968, K132129, K153219) | RSV | 88, 94 |
| Multiplex RT-PCR | Lyra Influenza A+B (Quidel) | ABI 7500 Fast Dx Real-Time PCR Instrument, QuantStudio Dx Real-Time PCR Instrument, Cepheid SmartCycler II | NS, NPS in VTM | A, B | Matrix (A), NA (B) | <75 | Moderate (K112172, K113777, K131728) | None | None |
| Multiplex real-time RT-PCR | Simplexa Flu A/B & RSV (Focus Diagnostics) | 3M Integrated Cycler | NPS in VTM | A, B | Matrix (A, B) | <240 | High (K102170) | RSV | 95, 96 |
| Multiplex real-time RT-PCR | Simplexa Flu A/B & RSV Direct (Focus Diagnostics) | 3M Integrated Cycler | NPS in VTM | A, B | Matrix (A, B) | ~60 | Moderate (K120413) | RSV | 97 |
| Multiplex real-time RT-PCR | Simplexa Influenza A H1N1 (2009) Kit (Focus Diagnostics) | 3M Integrated Cycler | NPA, NPS, NS in VTM | A (2009 H1) | Matrix (A), HA (2009 H1) | <240 | High (K100148) | None | 98 |
| Multiplex real-time RT-PCR with melt curve analysis | ARIES Flu A/B & RSV Assay (Luminex Corporation) | ARIES System, ARIES M1 System | NPS in VTM | A, B | Matrix (A, B) | <120 | Moderate (K161220) | RSV | 87 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Multiplex real-time RT-PCR, target-specific primer extension, fluidic microbead microarray | xTAG Respiratory Virus Panel (Luminex Molecular Diagnostics) | Thermal cycler plus Luminex 100 or 200 system | NPS in VTM | A (H1, H3), B | Matrix (A), HA (H1, H3, B) | ~450 | High (K063765, K112199) | RSV A, RSV B, PIV1, PIV2, PIV3, hMPV, HRV, Ad | 36, 39 |
| Multiplex real-time RT-PCR, target-specific primer extension, fluidic microbead microarray | xTAG Respiratory Virus Panel Fast (Luminex Molecular Diagnostics) | Thermal cycler plus Luminex 100 or 200 system | NPS in VTM | A (H1, H3), B | Matrix (A), HA (H1, H3, B) | ~360 | High (K103776) | RSV, hMPV, HRV, Ad | 96 |
| Multiplex real-time RT-PCR, target-specific primer extension, fluidic microbead microarray | NxTAG Respiratory Virus Panel (Luminex Molecular Diagnostics) | MAGPIX instrument | NPS in VTM | A (seasonal H1, H3), B | Matrix (A, B), HA (H1, H3) | ~225 | High (K152386) | RSV A, RSV B, PIV1, PIV2, PIV3, PIV4, hMPV, HRV, Ad, OC43, 229E, NL63, HKU1, Boca | 99 |
| Multiplex RT-PCR with microarray hybridization | Verigene Respiratory Pathogens Flex Nucleic Acid Test (Luminex Corporation) | Verigene System | NPS in VTM | A (H1, H3, 2009 H1), B | Matrix (A), HA (2009 H1, H3), NSP (B) | ~120 | Moderate (K143653) | RSV A, RSV B, PIV1, PIV2, PIV3, PIV4, hMPV, HRV, Ad | 100 |

[a]Additional information on rapid tests can be found at the following website: http://www.cdc.gov/flu/professionals/diagnosis/molecular-assays.htm.

[b]BAL, bronchoalveolar lavage; NA, nasal aspirate; NPS, nasopharyngeal swab; NS, nasal swab; TS, throat swab; BW, bronchial wash; NW, nasal wash; TA, tracheal aspirate; VTM, viral transport medium; NP, nucleoprotein; RSV, respiratory syncytial virus; PIV, parainfluenza virus; hMPV, human metapneumovirus; HRV, human rhinovirus; Ent, enterovirus; Ad, adenovirus; Boca, bocavirus; OC43, 229E, NL63, HKU1, human coronavirus (CoV) variants; HA, hemagglutinin; NSP, nonstructural protein; ?, not reported.

[c]Does not differentiate type.

culture methods, and the improved sensitivity of molecular methods is replacing culture methods as the gold standard for influenza virus detection (36, 37, 39).

A number of isothermal molecular amplification assays are undergoing evaluation for direct detection of influenza viruses in clinical samples. These include nucleic acid sequence-based amplification (NASBA), reverse transcription-loop mediated amplification (RT-LAMP), RT-helicase dependent amplification (RT-HDA), and RT-nicking enzyme amplification reaction (RT-NEAR) (40, 41). Nucleic acid amplification occurs at a single temperature without requiring the cycling associated with PCR. All these assays require initial synthesis of complementary DNA with a reverse transcriptase. NASBA uses T7 RNA polymerase to generate RNA amplicons while the other listed methods use a DNA polymerase to produce DNA amplicons. For the DNA-based methods, separation of double-stranded DNA occurs enzymatically rather than as a result of the heat denaturation used in PCR reactions. Successful amplification is detected with a variety of different methods, including molecular beacon probes, turbidity assays (RT-LAMP), and probe hybridization using electrochemical readouts. As with RT-PCR assays, these isothermal amplification methods are more sensitive than culture or immunofluorescent-antibody staining for the diagnosis of influenza virus infection.

The time to a result for molecular assays varies widely depending on the assay used, but it can exceed 4 hours (Table 4). However, some assays provide results in <30 minutes, and there are also several CLIA-waived assays available (Table 4). These assays have improved sensitivity compared to results obtained with rapid antigen detection tests and can be used as point-of-care tests, improving patient care in outpatient settings (32, 42).

## ISOLATION PROCEDURES

Influenza virus isolation procedures should be performed under biosafety level 2 (BSL-2) conditions. When the clinical sample comes from a patient suspected to be infected with a highly pathogenic avian influenza (HPAI) virus strain or other avian influenza A viruses with the potential to cause severe human disease, attempts at virus isolation should be performed under BSL-3 or higher conditions (43). Human clinical samples should be processed in separate laboratories and by staff members other than those handling clinical material from swine or birds (44).

### Cell Culture

Influenza viruses can be grown in a number of different cell lines, including primary monkey kidney cells, Vero cells, human diploid lung fibroblasts, mink lung epithelial cells, human lung adenocarcinoma (A549) cells, and Madin-Darby canine kidney (MDCK) cells (41, 45, 46). Although some variability can be seen from season to season, MDCK and primary monkey kidney cell lines have similar isolation frequencies (45), and MDCK cells are more sensitive than Vero or diploid lung fibroblast cells (46). Thus, MDCK cells (CCL 34; American Type Culture Collection, Manassas, VA), a continuous polarized cell line, are the most common cell line used for isolation of influenza viruses and will support the growth of type A, B, and C strains. Continuous cell lines do not produce proteases that will cleave the viral HA, a step necessary to produce infectious viral progeny, so exogenous protease must be added to the maintenance medium. L-(Tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin at a concentration of 1 to 2 μg/ml provides the necessary proteolytic activity and is the recommended protease for virus isolation. Chymotrypsin cleavage of the HA prevents the trypsin-mediated enhancement of viral infectivity, and TPCK treatment inactivates chymotrypsin activity, which may contaminate pancreatic extracts of trypsin.

MDCK cells are propagated in growth medium that contains 5% to 10% fetal calf serum (FCS). FCS contains inhibitors that prevent the production of infectious virus, so the FCS must be removed prior to inoculation of the clinical sample (47, 48). The inhibitory effects of FCS can be prevented by washing the cell sheet with Hanks buffer or serum-free medium sufficiently to remove the protein-containing growth medium and then adding serum-free medium to cover the cell sheet. The clinical sample is then inoculated into the medium. After a 2-h incubation, the inoculum-medium mixture is removed and replaced with serum-free medium supplemented with TPCK-treated trypsin. Alternatively, the sample can be inoculated directly onto cells with serum-free medium supplemented with TPCK-treated trypsin and incubated overnight prior to changing of the medium the next day. The cultures are maintained at 33°C to 34°C and monitored for virus growth.

The replication of influenza viruses typically leads to cytopathic effects (CPE) and destruction of the cell sheet within a week after inoculation. CPE may be inapparent or absent in the presence of viral replication, but viral replication can be identified by the ability of the viral HA to bind to sialic residues on the erythrocytes of different animal species. Cultures should be screened every 2 to 3 days by hemadsorption (binding of erythrocytes to the viral HA of infected cells) or hemagglutination (cross-linking of erythrocytes by virus in the culture medium) for evidence of viral replication. To evaluate hemadsorption of cells grown in a tissue culture tube, the monolayer is first examined for CPE (Fig. 1A), and the medium is removed and stored. The cell sheet is rinsed three times with 1 ml of 0.05% guinea pig red blood cells. One milliliter of 0.5% guinea pig red blood cells is then added, and the tube is stored at 4°C for 20 min, with the red blood cell suspension covering the cells. The tube is then shaken, and adherence of red blood cells to the cell sheet is determined microscopically (Fig. 1B). If cytopathic changes are scored as less than 4+ (i.e., less than 75% of cell sheet with CPE), the tissue culture tubes are rinsed with phosphate-buffered saline and re-fed with culture medium. The media collected initially from tubes with 4+ cytopathic changes can be used for further characterization. All procedures are performed in a BSL-2 safety cabinet, and care must be taken to prevent cross-contamination between cultures. Guinea pig red blood cells are more sensitive for detection of influenza virus than are avian cells, but influenza C virus does not agglutinate guinea pig red blood cells. Chicken red blood cells can be used in agglutination assays to identify influenza C viruses. Although most isolates will demonstrate growth within 1 week after inoculation, virus from samples with low infectious titers may require extended culture incubation for 10 to 14 days and additional blind passaging of negative cultures. Presumptive isolates are characterized further, as outlined below.

A disadvantage of traditional cell culture methods is the time needed to obtain a positive result (average, 4 to 5 days). More rapid methods have been developed by inoculating samples onto cell culture monolayers maintained in shell vials or multiwell plates. This approach can use either cell lines employed in traditional cell culture for identification of influenza virus (e.g., MDCK cells) or

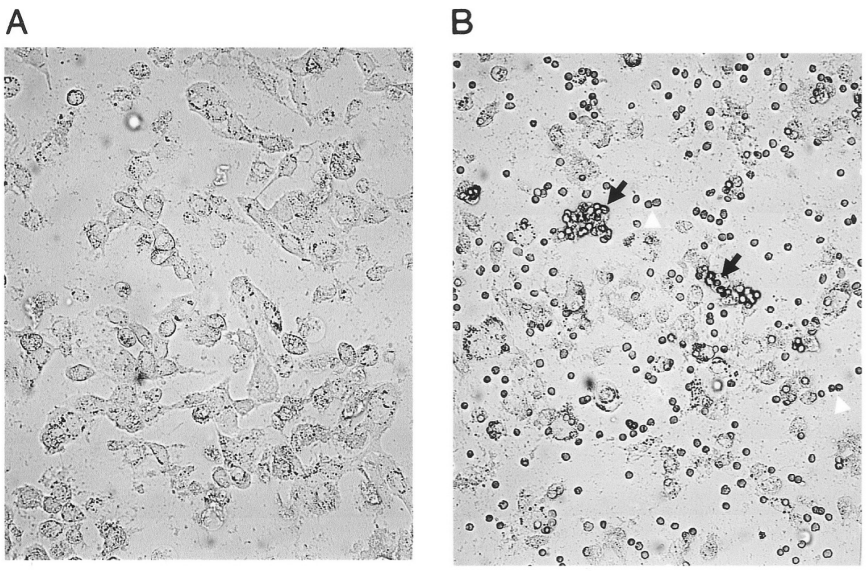



**FIGURE 1** Influenza virus-infected MDCK cells. (A) Cytopathic changes. (B) Hemadsorption with guinea pig red blood cells. Red blood cells adsorb to both infected cells (black arrows) and the plastic previously occupied by infected cells and where residual hemagglutinin protein is still present (white arrowheads).

mixed cell cultures (e.g., A549 cells plus mink lung cells) to screen for multiple respiratory viruses (R-Mix Fresh-Cells; Quidel, San Diego, CA), which are reported to detect seasonal influenza virus strains as well as strains with novel hemagglutinins (31, 49). The cells are fixed after 24 to 72 h, and type-specific monoclonal antibodies are used to detect viral antigen. Sensitivity can be lower than that achieved by standard isolation methods, although R-mix cells have been reported to have 82% to 100% sensitivity for detection of influenza A and B viruses (31, 49). Shell vial assays have the disadvantage of not producing virus for additional studies (e.g., antigenic characterization). Screening for viral antigen by immunofluorescence also can be used at the end of the 10- to 14-day incubation period for standard culture prior to discarding of cells (50). This step is usually not necessary if screening by hemadsorption or hemagglutination is being performed, but it may detect virus in the absence of cytopathic changes if other strategies for virus detection are not used.

### Isolation from Embryonated Chicken Eggs

The amniotic and allantoic cavities of 10- to 11-day-old embryonated chicken eggs are inoculated with the clinical sample for isolation of influenza A and B viruses. Seven- to 8-day-old eggs are used for isolation of influenza C viruses, although these viruses are also isolated with 10- to 11-day-old eggs. Embryonated eggs have endogenous proteases that can cleave the viral HA to yield infectious virus, so exogenous administration of proteases is not necessary. Inoculated eggs are incubated at 33°C to 34°C for 2 to 3 days (5 days for influenza C viruses), and then both amniotic and allantoic fluids are collected and assayed for hemagglutination activity. Influenza A and B viruses can grow both in cells lining the allantoic cavities and in those lining the amniotic cavities, whereas influenza C virus grows only in cells lining the amniotic cavities of embryonated eggs. If no hemagglutination activity is detected, influenza viruses may still be recovered by performing one or two blind passages. A pool containing equal volumes of the amniotic and allantoic fluids is inoculated into eggs as described above (47).

Isolation and passaging of influenza viruses in eggs can lead to adaptive mutations that include alterations in glycosylation sites in the viral hemagglutinin (51, 52). Such alterations can adversely affect the immunogenicity of egg-passaged viruses used in vaccines, which leads to decreased vaccine effectiveness, as has been observed for egg-passaged inactivated influenza vaccines targeting A/H3N2 viruses (52).

## IDENTIFICATION AND TYPING SYSTEMS

A variety of methods are used to identify and characterize influenza virus isolates. The most common are shown in Table 5 and are based upon immunologic or molecular approaches. The initial step is to identify the isolate as an influenza virus and to distinguish it from other respiratory viruses that have the ability to agglutinate or adsorb red blood cells (e.g., parainfluenza viruses and mumps virus). In many instances, it is sufficient to identify the virus by type, and this may be accomplished by immunofluorescent or immunoperoxidase stains or an enzyme-linked immunosorbent assay (ELISA) using commercially available, type-specific antibodies targeting the viral NP or M proteins. These assays are particularly useful for working with cell culture isolates. The rapid immunochromatographic assays described in Table 2 may be able to identify isolates and type them, but there are limited data on the use of these assays for this purpose, and these assays are not approved for this use. Importantly, the immunochromatographic assays may give false-negative results when the quantity of virus in a cell culture harvest is low.

Hemagglutination inhibition (HAI) assays have been performed for more than 75 years and are still used for identification (44, 53). HAI assays can be type, subtype, or strain specific, and they are particularly useful for examining antigenic relationships among strains of the same subtype. HAI is the WHO gold standard for antigenic

**TABLE 5** Methods to identify and characterize influenza virus isolates

| Assay | Advantages | Limitations |
|---|---|---|
| Assays using type- or subtype-specific antisera | | |
| ELISA | Standard assay with known performance characteristics; most labs experienced with assay format | For subtyping of influenza A strains, need to update sera periodically to detect circulating strain |
| Hemagglutination inhibition | Standard assay with known performance characteristics; no special equipment needed; gold standard for antigenic characterization | For subtyping of influenza A strains, need to update sera periodically to detect circulating strain; many clinical labs not experienced with this method |
| Immunofluorescence or immunoperoxidase staining of infected cells | Standard assay with known performance characteristics; many labs experienced with assay format; monoclonal antibodies commercially available | For subtyping of influenza A strains, need to update monoclonal antibodies periodically to detect circulating strain |
| Molecular methods | | |
| RT-PCR | Very sensitive assays | Potential for carryover contamination; need for stringent laboratory controls |
| Amplicon size | Ease of performance | Potential for false-positive results due to nonspecific amplification |
| Probe hybridization | Most commonly used approach for confirmation of PCR results; real-time formats eliminate need for post-amplification processes | Depending on hybridization format used, may add time to performance of assay |
| Restriction analysis | Ease of performance | Need to know specific sequence; requires specific nuclease site; increased handling of post-PCR samples |
| Genetic sequence | Highest level of identity; sequence data that may be used in other studies | Need for specialized equipment; technically complex; increased cost |
| Microarray analysis | Potential to analyze multiple genetic sequences simultaneously | Investigational; limited experience |

characterization of influenza isolates and vaccine strain selection. Immune sera are usually produced in ferrets, sheep, or chickens. The hemagglutination activity of the virus is quantitated, and a standard amount of viral HA (4 HA units) is mixed with serial 2-fold dilutions of the immune serum and turkey or guinea pig red blood cells. A 4-fold or greater difference in HAI activities between the isolate and the reference strain is an indication that the isolate may be an antigenic variant. Because the HA undergoes antigenic change over time, subtype-specific antisera for interpandemic strains must be prepared and standardized periodically. Thus, subtype identification by HAI is usually performed only as part of surveillance activities or investigation of a case in which there is a strong epidemiologic suspicion of infection with a non-human strain.

Molecular assays can be used for virus identification and characterization. The same RT-PCR assays used for detection of viruses in clinical samples also can be used to identify clinical isolates. An advantage that molecular assays have over immunology-based assays is that the molecular assays can identify influenza A virus subtypes even after significant antigenic variation has occurred because there are well-conserved regions of the HA gene that serve as targets for the primers and probes used for identification. Multiplex assays can also be used to distinguish influenza A and B viruses or to identify HA and NA subtypes (54). Results are determined by identification of amplicon size, by hybridization to type- or subtype-specific probes, and by direct sequencing of the amplicons. If the sequences of different variants are known, it may be possible to identify unique differences by digesting amplified DNA with restriction endonucleases that generate restriction fragment length polymorphisms (RFLP) unique to each strain. For example, this method was used to distinguish two H3N2 variants that cocirculated during a single season (55). Influenza A/Wuhan/359/95 (H3N2) virus-like variants generated amplicons that could be digested with the BstF5I restriction enzyme, whereas amplicons from influenza A/Sydney/05/97 (H3N2) virus-like variants could be digested by HindIII. Given the difficulty to design and perform RFLP analysis and the reduced cost and time required to perform DNA sequencing, direct sequencing of amplicons, or the entire HA gene, has become a more common way to track and characterize specific strains. Electrospray ionization-mass spectrometry is another method that can be used to analyze virus-specific PCR amplicons and to identify novel variants and reassortants when the viral genomic sequence is unknown, as was done with the initial identification of the 2009 H1N1 virus as a likely swine-origin virus (56).

DNA microarrays are being used increasingly in diagnostics for identification of specific pathogens. Oligonucleotide probes are arrayed on a chip or membrane, and hybridization of virus-specific sequences is then detected. The viral sequences can be generated by cDNA synthesis from viral genomic RNA or by amplification of fragments of genomic RNA by RT-PCR. Microarray analysis strategies have been developed that distinguish influenza virus types (A versus B) and subtypes (57, 58) but at the present time they are too costly for most individual laboratories to develop.

Next-generation sequencing methods are being applied to influenza for the analysis of the entire influenza genome (59, 60). The sequence of each segment is determined, which allows a more detailed evaluation of reassortment and evolution of viral genes. This technology has the promise of being able to more fully characterize strains in surveillance studies.

## SEROLOGIC TESTS

Influenza virus infections are also identified by serologic methods. Most persons have been infected previously with influenza viruses, so detection of virus-specific

immunoglobulin M or other immunoglobulin subclasses has not been particularly useful (61). An exception may be detection of immunoglobulin M responses to novel HAs from avian strains (62). Instead, paired acute- and convalescent-phase serum samples collected at least 10 days apart are needed to detect a significant (4-fold or greater) increase in serum antibody levels. The requirement for paired sera to identify infection makes serology an impractical method for identification of influenza virus infection in the acutely ill individual. Instead, serology is used primarily in surveillance and in epidemiologic studies. The most widely used assay formats include complement fixation, HAI, neutralization, and enzyme immunoassay. Complement fixation identifies type-specific antibodies to the NP, but it is not as sensitive as the other commonly used serologic assays in detecting significant rises in antibody levels. HAI and neutralization antibodies in serum are functionally significant in that higher serum antibody levels correlate with protection from infection and illness, and these antibody levels are used to measure responses to vaccination and to identify infection. HAI antibodies block the binding of the viral HA to sialic acid residues on red blood cells and thus inhibit hemagglutination. Each of the components in the HAI assay may affect the outcome of the test. Human and animal sera may contain nonspecific inhibitors of hemagglutination, but methods to remove these inhibitors have been developed (47). The source of the viral antigen can affect results in that virus initially isolated in cell culture may detect a greater frequency of antibody rises than egg-grown virus (63). The species from which the red blood cells are derived can affect assay results. Chicken and turkey red blood cells are commonly used to measure HAI antibody to human strains of influenza viruses, but they may fail to detect HAI antibodies to avian strains (such as H5N1). Substitution of horse red blood cells can improve HAI assay sensitivity for detection of antibodies to avian influenza virus strains (64). Neutralizing antibodies block viral infectivity and provide a more sensitive assay for detection of antibodies to influenza A and B viruses (65). Neutralization assays are the preferred method for the detection of antibodies to HPAI virus strains (66). Consensus approaches have been developed to allow comparable results to be obtained between laboratories (67). Neutralization assays require the use of live virus, so their use with HPAI virus strains is restricted to those laboratories with BSL-3 or higher facilities. Enzyme immunoassays are also used for detection of antibody responses to whole-virus antigen or to specific viral proteins. The conjugate and the antigen used in the assay are factors that affect the performance characteristics (sensitivity and specificity) of these assays. Enzyme immunoassays are used to measure specific immunoglobulin responses in a variety of clinical specimens (serum samples and respiratory secretions). Serologic assays targeting influenza are not used to manage individual patients clinically, but such tests are useful in vaccine evaluation and in epidemiological and other research studies.

## ANTIVIRAL SUSCEPTIBILITIES

Plaque inhibition assays are the "gold standard" for measuring susceptibility to amantadine and rimantadine, but the assays are cumbersome and time-consuming to perform. ELISA methods have also been used to measure decreases in the expression of viral antigens in the presence of these drugs. These assays can be used in combination with genotypic characterization of the M2 gene since *in vitro* and *in vivo* resistance to these drugs is associated with specific M2 gene mutations (68). RT-PCR amplification followed by restriction fragment length polymorphism analysis or direct sequencing of amplicons is a genotypic method used to identify resistant viruses (69). Amplification of the influenza A M2 gene followed by pyrosequencing is a rapid, high-throughput method that allows the rapid and reliable identification of adamantane (amantadine and rimantadine) mutations (70).

Cell culture assays do not reliably identify antiviral susceptibility to the NA inhibitors zanamivir and oseltamivir. Instead, NA enzyme inhibition assays with chemiluminescent or fluorescent substrates are used to identify resistance (71). Several commercially available diagnostic assays (e.g., NA-Star, NA-Fluor, and NA-XTD, Applied Biosystems) are available for *in vitro* screening of influenza virus isolates (72). The results of these assays also correlate with mutations in the NA gene that can be identified by sequencing (73). Molecular approaches can be used to identify known NA gene mutations associated with NAI resistance (e.g., E119V and R292K in A/H3N2, H274Y in A/H1N1, R152K in influenza B) (74). Both traditional terminal deoxynucleotide (Sanger) sequencing and pyrosequencing of the NA gene can successfully identify these mutations. Another strategy to quickly screen a large number of isolates is application of a real-time RT-PCR assay that uses a probe that recognizes wild-type (susceptible) NA sequence. This approach identified all A/H1N1 strains with a H274Y NA gene mutation (75).

Mutations in the HA gene may also lead to a resistance phenotype through decreased binding affinity of HA to cell surface receptors and decreased reliance on NA function to release budding viruses from infected cells. No reliable cell culture system currently exists for identifying HA resistance mutations, so identification relies upon sequencing of the receptor binding site of the HA gene.

## EVALUATION, INTERPRETATION, AND REPORTING OF RESULTS

The results of a diagnostic test must be considered in the context of the overall setting in which the test is ordered. Clinicians play a critical role in assessing the plausibility of a test result, but the laboratory also can contribute to this appraisal. Seasonal, epidemiologic, and clinical factors are elements that must be evaluated in addition to the type of assay used. Unexpected laboratory results can be recognized by the laboratory as well as by the clinician. For example, a positive influenza test result when influenza is not recognized to be circulating in the community should prompt an assessment as to whether epidemiologic (e.g., travel history) or clinical (e.g., immunocompromised host) factors support the diagnosis of influenza virus infection. Similarly, a negative result, especially with a less sensitive assay (e.g., a RIDT), should not preclude prescription of antiviral treatment to a patient with signs and symptoms of influenza. Close interactions between the laboratory and clinician are a vital component of a quality control program.

No diagnostic assay has 100% sensitivity and specificity, so false-negative and false-positive results can be expected to occur. Many factors that contribute to lowered sensitivity and specificity are known and can be addressed in ongoing quality control programs. False-negative results may be due to poor quality or inappropriate clinical sample collection, delays in sample transportation or processing, inadequate sample storage (e.g., wrong temperature or transport medium), the time of sample collection during the clinical illness (e.g., later in the illness than recommended, when

viral shedding has decreased), the performance characteristics of the diagnostic assay (i.e., lower sensitivity), and the infecting strain (e.g., swine or avian influenza). False-positive results may also be due to other characteristics of the diagnostic assay (i.e., nonspecific reactions), cross-contamination within the laboratory, mislabeling of specimens, and microbial contamination. Standard operating procedures in the collection, transportation, and processing of clinical samples should be established and followed to minimize the occurrence of inaccurate test results. Reagents should be standardized, and periodic assessments of assay performance should be performed with known positive and negative controls. The timing of these assessments will be based upon the type and number of tests being performed and the sources of reagents.

Each laboratory must decide upon the goals of its influenza virus diagnostic program when selecting the diagnostic assays to be performed. Rapid and sensitive assays can favorably affect patient management by allowing the prescription of targeted antiviral therapy and the institution of appropriate infection control isolation procedures. Positive test results may form the basis for offering prophylactic therapy to close contacts of infected patients, especially those contacts with high-risk medical conditions. Early and rapid laboratory diagnosis also can be important for evaluating influenza-like illnesses in the setting of a nosocomial outbreak, at the beginning of the influenza season (before influenza is recognized to be circulating in the community), and in persons with a history of contact with pigs or birds or travel to an area where influenza virus is circulating. Confirmation of swine- or avian-origin virus strains can be accomplished by submission of suspect samples or isolates to a public health laboratory for evaluation. The laboratory's expertise, staffing, and available equipment also will influence test selection. For example, a fluorescent microscope and an experienced technician are necessary for the performance of immunofluorescence assays, and a thermal cycler along with other equipment are needed for RT-PCR assays. If the clinical specimen being tested comes from a patient who may be infected with an HPAI virus strain (e.g., H5N1), nonculture-based assays are currently recommended for laboratories that do not meet the BSL-3 or higher conditions recommended for growth of these strains (43). Commercially available antigen detection assays or the more sensitive H5- and H7-specific RT-PCR assays may be performed with BSL-2 work practices. In the United States, influenza A virus-positive samples from patients meeting the clinical (febrile [>38°C] respiratory illness [cough, sore throat, or dyspnea]) and epidemiologic (contact with poultry or domestic birds or with a patient with known or suspected H5N1 or H7N9 virus infection in a country with endemic transmission of avian influenza) parameters for suspected avian influenza virus infection are referred to the CDC for further evaluation. Selected negative samples may also be sent to the CDC for analysis in consultation with the local public health department (39).

As new strains of influenza virus emerge, the sensitivities of established methods to detect these strains may change. For example, cell lines may have diminished sensitivity to new strains, or the ability to detect influenza virus antigen in infected tissue culture cells (e.g., by hemadsorption) may decrease (50). Thus, it is prudent to reevaluate periodically the performance characteristics of established methods, especially if results do not correlate with those expected based upon clinical and epidemiologic criteria.

Influenza diagnosis is also performed for reasons other than patient management. On the local level, knowledge that influenza is circulating in a community allows diagnosis of influenza based upon clinical symptoms (febrile respiratory illness with cough) with a sensitivity (60% to 80%) similar to that of many rapid antigen tests (76). Influenza viruses isolated in national and global surveillance systems are characterized antigenically and genetically to identify variants. Information gained from these surveillance activities is used in the annual selection of strains for inclusion in updated trivalent influenza vaccines. Surveillance and characterization of isolates also allow the identification of infection with novel subtypes, as has occurred with influenza A/H5N1 and A/H7N9 viruses in Southeast Asia and A/H7N7 strains in the Netherlands (77).

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# Algorithms for Detection and Identification of Viruses

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# 83

Virology remains a dynamic field. Since the first edition of the *Manual of Clinical Microbiology* in 1970, virology has firmly established itself in the mainstream of clinical laboratory practice. When traditional virologic methods (namely, conventional cell cultures, neutralization tests with antisera for virus identification, manual serologic techniques, and light and electron microscopy) were the mainstay, diagnostic virology was a distinct discipline that was practiced primarily in public health, research, and academic settings. Time to result was slow, and it was often said that the patient was dead or better by the time the result was received.

## ADVANCES IN DIAGNOSTICS

Driven by effective antiviral therapies, diagnostic advances have transformed the field, allowing accurate results in a clinically useful time frame. Early technological improvements in the laboratory included enzyme immunoassays, IgM class capture assays, monoclonal antibodies for identification, rapid centrifugation cultures, and direct detection of viral antigens in clinical specimens by immunofluorescence. At the point of care (POC), lateral flow immunochromatography tests were introduced to detect viral antigens or antibodies in 10 to 20 minutes without equipment or reagent additions, allowing immediate impact on clinical decisions. The most transformative, however, has been the introduction of nucleic acid amplification tests (NAATs), which are both rapid and sensitive, can be automated, high-throughput, or random access, and can detect viruses not amenable to routine culture.

Initially, NAAT was confined to a limited number of specialized molecular laboratories, using multistep, technically demanding laboratory-developed methods, and required separate assays optimized for each pathogen. For decades, only a handful of FDA-cleared or -approved commercial NAATs were available. With each new edition of the *Manual*, the transition to molecular methods has accelerated, due to advances in technology, real-time amplification methods, and user-friendly, FDA-approved or -cleared devices.

For years, culture was considered the gold standard because it could detect a variety of pathogens and reveal an unexpected virus. With NAAT syndromic viral panels, first for respiratory viruses and then for meningitis/encephalitis and gastrointestinal pathogens, the relevance of viral culture to clinical management has receded further. Not only are these panels faster, requiring less than 1 hour to a few hours to generate a result, they also detect more viruses than culture and often include nonviral pathogens that can have a similar presentation. They also require less skill than culture. Some require the simple addition of an unprocessed sample into a device and then insertion into the instrument, with approximately 2 minutes of hands-on time. When a more limited diagnosis is sought, another option is a multiplexed minipanel for two or three key pathogens.

For quantitative monitoring of viral load in blood, additional tests have been FDA approved, and substantial effort has been invested in the development and implementation of international quantitative standards that will permit cross-institutional comparisons and interpretive guidelines (e.g., for cytomegalovirus, Epstein-Barr virus, polyomavirus BK, and parvovirus B19). As a result, standardization and commutability have been gradually improved between laboratories. Quantitative NAATs have also required batch testing, often with a limited batch size, and sometimes differing nucleic acid extraction steps for RNA and DNA viruses, as well as separate extraction and amplification instruments. Recent innovations include the ability to accommodate multiple assays in a flexible and automated manner, elimination of the need for separate RNA or DNA extraction, and shorter assay times.

Since the last edition of this *Manual*, rapid influenza virus immunoassays have been reclassified by the FDA as class II (moderate potential harm) and must meet new requirements for minimum performance, including reporting annual reactivity testing of circulating strains. Innovative solutions to improve sensitivity are expected if rapid immunoassays are to remain competitive. If successful, these changes will greatly benefit other POC immunoassays, which, due to their simplicity and low cost, are especially useful in limited-resource settings.

Another paradigm shift occurred in 2015, when the first NAAT was approved for POC use, providing results for influenza virus in 15 min, or in as little as 2 to 5 minutes for some respiratory syncytial virus (RSV) positives. Subsequently, additional CLIA-waived NAATs have been introduced that require the simple addition of a sample to a device, which is then inserted into an instrument. Results are available in 20 to 30 min for influenza virus A and B

**TABLE 1** Methods for detection[a] and identification of viruses

| Virus | Applicability of detection method[b] | | | | | Comments[c] |
|---|---|---|---|---|---|---|
| | Nucleic acid | Antigen | Virus isolation | Antibody | Pathology | |
| Adenoviruses | A | A | B | B | B | NAAT is most sensitive for detection, but tests vary in ability to detect diverse types. Quantitative NAAT is used to monitor viral load in compromised hosts. Antigen assays are used for ocular, enteric, or respiratory adenoviruses but are less sensitive than culture or NAAT. |
| Arboviruses | A, C | B | C | A, C | D | NAAT and IgM are useful in acute infection, depending on day of illness and clinical disease. However, NAAT is not commercially available for most neurotropic arboviruses, except WNV. Serologic cross-reactivity is problematic, especially for Zika and dengue viruses; more specific PRNT is available at CDC. Rapid antigen tests are available for dengue virus. Most arboviruses are readily cultured but may require BSL3 or -4 facilities. |
| Bocaviruses | A | D | D | D | D | NAAT is the only test available for diagnosis. Included in some multiplex respiratory panels. Clinical relevance awaits further investigation. |
| Coronaviruses OC43, 229E, NL63, HKU1 | A | D | D | D | D | NAAT is used for respiratory CoV as part of multiplex panels. |
| Coronaviruses SARS, MERS | A, C | C | C | C | D | NAAT and antibody tests are available only in public health or research laboratories. |
| Cytomegalovirus | A | B | B | A | B | NAAT is most sensitive and can determine viral load. pp65 antigenemia is used to determine viral load in blood, but NAAT is much more widely used. Culture can be used for nonblood specimens. IgG antibody is used to determine immune status, and IgM to screen for recent infection. CMV-specific gamma interferon release assay is available to measure cell-mediated immunity. |
| Enteroviruses and parechoviruses | A | D | B | D | D | NAAT is more sensitive and strongly preferred for CNS infection. Parechovirus requires separate NAAT. |
| Epstein-Barr virus | A | B | D | A | B | Serology is test of choice for diagnosis of primary infection. NAAT is useful for monitoring viral load in blood. IHC or ISH is used on tissue biopsy specimens. |
| Filoviruses and arenaviruses | C | C | C | A, C | C | NAAT is key to rapid diagnosis. BSL4 facility is needed for culture, except for LCMV. Patients with severe disease may die without developing antibody. LCMV is diagnosed primarily by serology. |
| Hantaviruses | C | C | C | A | D | NAAT and serology are equally useful for diagnosis. IHC is used in fatal cases. BSL4 facility is needed for culture. Isolation is difficult. |
| Hepatitis A virus | D | D | D | A | D | Serology is the standard diagnostic test. False-positive IgM is problematic in low-prevalence areas. |
| Hepatitis B virus | A | A | D | A | D | Detection of specific viral antigens and antibodies allows diagnosis and monitoring the course of infection. NAAT is used to monitor therapy and determine genotype. |
| Hepatitis C virus | A | B | D | A | D | Serology is used for diagnosis. NAAT is used to confirm active infection and monitor response to therapy. Genotyping helps determine drug regimen and duration of therapy. Antigen testing is a low-cost POC alternative in low-resource areas. |
| Hepatitis D virus | A | A | D | A | D | Testing is confined to reference laboratories. Diagnosis is relevant only in the presence of hepatitis B infection. IHC of biopsy tissue is useful for diagnosis. |

*(Continued on next page)*

**TABLE 1** Methods for detection[a] and identification of viruses *(Continued)*

| Virus | Applicability of detection method[b] | | | | | Comments[c] |
|---|---|---|---|---|---|---|
| | Nucleic acid | Antigen | Virus isolation | Antibody | Pathology | |
| Hepatitis E virus | A, C | D | D | A | D | Serology is the standard diagnostic test, but tests vary in sensitivity and specificity. False-positive IgM is problematic in low-prevalence areas. NAAT is required for accurate diagnosis in transplant patients. Genotyping is performed at CDC for autochthonous cases. |
| Herpes simplex virus | A | B | B | B | B | NAAT is test of choice, especially for CSF infection. IFA can be used for rapid detection in skin and mucous membrane lesions. Serology is used to determine immune status. |
| Herpesviruses 6A and 6B | A | D | D | B | D | NAAT is test of choice for diagnosis. Serology can document primary infection in children. Interpretation of HHV-6 NAAT can be complicated by chromosomal integration of virus. |
| Herpesvirus 7 | B | D | D | B | D | NAAT is test of choice but not routinely available. |
| Herpesvirus 8 | A | B | D | A | A | Serology is used to identify infected persons. NAAT of blood may be useful in diagnosis posttransplant and monitoring therapy. IHC is preferred for tissue. |
| Human immunodeficiency virus | A | A | C | A | D | Serology is primary diagnostic method. Antigen-antibody combination tests reduce seronegative window in acute infection. Quantitative RNA tests are used to guide therapy and monitor response. Proviral DNA tests are useful for diagnosis of neonatal infection. |
| Human metapneumovirus | A | A | B | D | D | NAAT is the test of choice for diagnosis. IFA and shell vial culture are less sensitive options. Conventional culture is difficult. |
| Human T-cell lymphotropic virus | B | D | D | A | B | Serology is primary diagnostic method. NAAT is qualitative only; useful if serology is indeterminate. |
| Influenza viruses | A | A | B | D | D | NAAT is most sensitive and can provide subtype. Rapid antigen tests are lower in sensitivity and specificity. IFA and rapid culture are more accurate. Serology is useful for epidemiological studies or retrospective diagnosis. |
| Measles viruses | A, C | C | C | A | D | Serology is used for diagnosis and determination of immunity. NAAT is best for acute infection. Isolation can be useful if attempted early (prodromal period to 4 days postrash). |
| Mumps virus | A, C | C | B | A | D | Serology is used most commonly for diagnosis and determination of immunity. NAAT is useful for diagnosing infection especially among vaccinated individuals. |
| Noroviruses | A | C | D | D | D | NAAT is test of choice but challenging due to strain variability. |
| Parainfluenza viruses | A | A | B | D | D | NAAT is more sensitive than isolation. IFA is most common rapid detection method. |
| Papillomaviruses | A | D | D | D | A | NAAT is test of choice for detection and genotype differentiation. Cytopathology is useful for diagnosis. |
| Parvovirus B19 | A | C | D | A | B | Serology is used to diagnose B19 in immunocompetent individuals. NAAT is test of choice for immunocompromised hosts, early in infection before antibody, and for B19-exposed fetuses. |
| Polyomaviruses | A | B | D | B | A | NAAT is test of choice, but genetic variability can lead to falsely low or negative results. JC virus DNA detection in CSF is useful for presumptive diagnosis of PML. JC virus antibody is used to predict risk for PML. BK virus DNA quantification in plasma/urine is used for preemptive diagnosis of PVAN. IHC and EM are useful for biopsy tissues. |

*(Continued on next page)*

**TABLE 1** Methods for detection[a] and identification of viruses *(Continued)*

| Virus | Applicability of detection method[b] | | | | | Comments[c] |
|---|---|---|---|---|---|---|
| | Nucleic acid | Antigen | Virus isolation | Antibody | Pathology | |
| Poxviruses | A, C | C | C | A, C | A | NAAT allows virus inactivation and rapid detection. Electron microscopy is very useful for rapid diagnosis but has limited availability. Smallpox isolation requires BSL3 or -4 and should be attempted only in WHO Collaborating Centers. Vaccinia virus requires BSL2 and grows readily in cell culture. |
| Rabies virus | C | C | C | A | A, C | For human rabies, testing is done at CDC. NAAT and culture used for saliva, CSF, and tissue; IFA for skin biopsy; serology for CSF and serum. Serology available at commercial laboratories used to monitor antibody titers in vaccinated professionals. |
| Respiratory syncytial virus | A | A | B | D | D | NAAT is most sensitive. Rapid antigen tests, especially IFA, can be useful in pediatric patients. Serology is useful only for epidemiological studies. |
| Rhinoviruses | A | D | B | D | D | NAAT is much more sensitive than culture; cross-reaction with enteroviruses can occur. |
| Rotaviruses | A | A | D | D | D | Antigen detection has been standard test for diagnosis. Rotavirus is now in NAAT gastroenteritis panels. EM is useful if available. |
| Rubella virus | C | D | C | A | D | Serology is used for diagnosis and immune status. NAAT is used for acute infection. Isolation is useful for postnatal rubella if attempted early (prodromal period to 4 days postrash). In CRS, virus can be isolated for weeks to months after birth. |
| Transmissible spongiform encephalopathy agents | B | B | D | D | A | Histology is most useful diagnostic test. Surrogate markers popular but lack specificity. Western blot for PrP is performed in specialized laboratories. Real-time quake-induced conversion is used to detect $PrP^{Sc}$. Human genome sequencing is useful for diagnosis of genetic disorders. |
| Varicella-zoster virus | A | A | B | B | B | NAAT is most sensitive and increasingly used. IFA on skin lesions is more sensitive than culture. Culture is slow and not sensitive. Serology is most useful for determination of immunity and can be useful in CNS vasculopathy. |

[a]Viral nucleic acids (DNA or RNA) can be detected by amplification methods such as PCR. Viral antigens can be detected by a variety of immunoassays. Virus isolation includes conventional cell culture and rapid centrifugation culture with detection of viral antigens by immunostaining. Antibody detection involves measurement of total or class-specific immunoglobulins directed at specific viral antigens. Pathology involves the visualization of virus-induced changes in tissue or cytology smears, including inclusions, multinucleated cells, immunohistochemistry, or *in situ* hybridization, or the visualization of viral particles by electron microscopy.

[b]A, test is generally preferred for routine clinical diagnosis; B, test alternative whose utility may be limited to specific indications, forms of infection, or sample types, as delineated in the rightmost column and in the text of the individual chapters; C, test is limited to public health laboratories, such as CDC, due to specialized testing or biosafety concerns; D, test is not available, is not generally useful, or is used only in research.

[c]Abbreviations: WNV, West Nile virus; PRNT, plaque reduction neutralization test; CDC, Centers for Disease Control and Prevention; BSL, biosafety level; CoV, coronavirus; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; CNS, central nervous system; LCMV, lymphocytic choriomeningitis virus; IHC, immunohistochemistry; ISH, *in situ* hybridization; PML, progressive multifocal leukoencephalopathy; PVAN, polyomavirus-associated nephropathy; IFA, immunofluorescence assay; EM, electron microscopy; CRS, congenital rubella syndrome; PrP, prion protein; CMV, cytomegalovirus; HHV, human herpesvirus.

with or without RSV using real-time PCR and in 60 min for 14 respiratory viruses and 3 bacterial pathogens using nested PCR. As an indication of how far the field has come, these POC tests are as sensitive as the best laboratory-performed assays. Thus, any hospital laboratory, emergency department, clinic, or doctor's office can now implement state-of-the-art molecular testing. The main obstacle is no longer lack of technical expertise and laboratory facilities, but cost, of equipment, service contracts, and reagents. Additionally, the expertise of the clinical virologist with regard to interpreting results may be lost, as tests are now performed outside the laboratory setting. For many pathogens, this may not be required, but for some results, such as the detection of latent herpes viruses in cerebrospinal fluid, interpretation can require both clinical and laboratory expertise. Going forward, linking best-practice guidelines to specific test results should be encouraged if the full benefits of an accurate rapid diagnosis are to be realized.

## CHALLENGES AND FUTURE PERSPECTIVES

In addition to the advantages of molecular testing, some pitfalls have become apparent as the tests are more widely used. For example, the sensitivities and specificities to

detect the same virus often vary for different assays. In addition, despite the fact that the tests target conserved regions of the genome, strain variability and mutations can lead to underquantification of viral load, or even falsely negative results for both qualitative and quantitative assays. Furthermore, as tests become more sensitive, low levels of clinically irrelevant or nonviable viruses may be detected and can be misleading to clinicians. Similarly, interpreting the clinical relevance of multiple viral pathogens in the same sample, especially when relative quantification is not available, is problematic.

Thus, with progress have come new challenges. Laboratories need to choose which platforms and tests to offer. Selecting the appropriate test will depend on the virus(es) sought, sample site, clinical presentation, clinical purpose (e.g., screening, confirmation, diagnosis, or monitoring), patient characteristics, and disease prevalence. Performance characteristics, staff expertise, and cost will also impact that choice. Laboratories must recognize the uses and also the limitations of each test in order to guide clinicians in test selection and in interpreting the results. This *Manual* should serve as a key resource for accomplishing these tasks. The choices available for each virus differ and continue to evolve. Table 1 provides a concise overview for each virus group; however, the reader is referred to the specific chapters for more detailed discussions.

Next-generation sequencing for resistance testing, outbreak management, and characterization and surveillance of pathogens, as well as metagenomics to discover unexpected etiologies of disease, is the next wave of technological advances beginning to move from the research laboratory to the clinical arena. As with other molecular assays, these techniques will provide an impetus to bring virology closer to the rest of clinical microbiology practice. However, there remain a number challenges in the implementation of next-generation sequencing for routine diagnosis, including the technical expertise required, cost of instrumentation, time to results, bioinformatics, and result interpretation. While the pace of change can be daunting for laboratories, it is extremely gratifying to witness the impact of state-of-the-art testing on patient care. As we move forward, it is critical that laboratorians communicate with each other to address problems, including the optimization and standardization of methods, and, in addition, encourage input and feedback from clinicians. Due to the speed of methodological change and the continuing discovery of new viruses and new therapies, keeping abreast of the most recent literature is strongly recommended.

# Antiviral Agents*

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# 113

The use of antiviral agents for the treatment of viral diseases continues to expand. Most of the agents currently approved by the Food and Drug Administration (FDA) are active against one or more of the following viruses: human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), hepatitis viruses B and C (HBV and HCV), the human herpesviruses, and influenza A and B viruses. This chapter is organized according to these virus groups with cross-referencing for agents with activity against more than one group of viruses. The major targets of these agents are viral replication enzymes, proteases, and entry/exit pathways (1–4). In a few cases, approved drugs for the above families of viruses have also been used to treat viruses in other families. The expanded spectrum of drug usage is discussed in the individual drug sections.

## AGENTS AGAINST HIV-1 AND HIV-2

There are now five classes of antiviral agents for the treatment of HIV-1: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs), (ii) nonnnucleoside reverse transcriptase inhibitors (NNRTIs), (iii) protease inhibitors (PIs), (iv) entry/fusion inhibitors, and (v) integrase strand transfer inhibitors (INSTIs). Current information on each drug is available through the AIDSinfo website (http://AIDSinfo.nih.gov), which has separate guidelines for the use of approved antiretroviral agents in adolescents and adults, children, and pregnant HIV-1-infected women (5–7). These guidelines describe the agents along with dosage, adverse effects, and drug interactions. Working groups for each of these patient populations regularly update the guidelines. Additional information can be obtained from the package inserts available from the pharmaceutical company websites. Changes in recommended drug doses as well as observed adverse effects and drug interactions occur frequently, making it necessary to consult the most up-to-date sources.

Antiretroviral agents are administered in combinations of different drug classes termed combined antiretroviral therapy (cART) to maximize efficacy and to minimize the induction of drug resistance. cART is now generally regarded as any combination regimen designed to achieve the goal of complete virus suppression. These regimens comprise a minimum of three drugs, which are usually NNRTI based (two NRTIs and/or NtRTIs plus one NNRTI), PI based (two NRTIs and/or NtRTIs plus one or more PIs), or more recently, INSTI based (two NRTIs and/or NtRTIs plus an INSTI) (5).

There are currently 25 approved antiretroviral drugs (1) with numerous possible combinations for treatment regimens. Recommended regimens for adults and adolescents are given in the guidelines (5) for treatment-naive and treatment-experienced patients. The large number of drugs creates a tremendous potential for drug interactions among the different classes as well as interactions with other types of drugs prescribed for conditions associated with HIV infection. Close monitoring of these complex interactions is required to avoid detrimental changes in drug levels and/or toxicity.

Table 1 summarizes the structure, mechanism of action, and major adverse effects of the individual drugs and drug combinations approved by the FDA. The drug interactions described below for each drug are only highlights of potential interactions. Frequent updates and more-comprehensive information can be obtained from the AIDSinfo website listed above.

### Nucleoside and/or Nucleotide Reverse Transcriptase Inhibitors

The NRTI-NtRTI class of drugs is not active as administered but must be phosphorylated by cellular kinases to the nucleoside triphosphate form, which may lack a 3′hydroxyl group for DNA chain elongation. The NRTIs require triphosphorylation, while the NtRTIs require only diphosphorylation (1). These antiviral agents act as competitive inhibitors of the viral reverse transcriptase (RT), which results in chain termination. They are active against both the HIV-1 and HIV-2 RTs, and they are used as dual-combination backbones in regimens with NNRTIs, PIs, and INSTIs (5). Several of them also are active against the HBV DNA polymerase, which has RT activity (see "Agents against Hepatitis B Virus" below) (3). Lactic acidosis with hepatic steatosis is a rare but very serious adverse effect associated with all members of this class. These toxic effects of NRTIs and NtRTIs appear to be the result of inhibition of the mitochondrial DNA polymerase γ (8).

*This chapter contains information presented by Aimee C. Hodowanec, Kenneth D. Thompson, and Nell S. Lurain in chapter 110 of the 11th edition of this *Manual*.

**TABLE 1** Antiviral agents for HIV therapy[a]

| Antiviral agent (abbreviation) | Trade name (pharmaceutical company[b]) | Mechanism of action/ route of administration | Major adverse effects[c] |
|---|---|---|---|
| Nucleoside or nucleotide reverse transcriptase inhibitors (NRTI-NtRTIs) | | | |
| Abacavir (ABC) | Ziagen (GSK) | Converted to triphosphate analogue of dGTP by cellular kinases, competitive inhibitor of RT, viral DNA chain terminator; administered orally | Hypersensitivity reaction associated with HLA-B*5701 |
| Didanosine (ddI) | Videx (BMS) | Converted to dideoxy triphosphate analogue of dATP by cellular kinases<br>Activity and administration similar to ABC | Pancreatitis, peripheral neuropathy, nausea, diarrhea |
| Emtricitabine (FTC) | Emtriva (Gilead) | Converted to triphosphate analogue of dCTP by cellular kinases<br>Activity and administration similar to ABC | Minimal toxicity, skin hyperpigmentation, posttreatment exacerbation of hepatitis B coinfection |
| Lamivudine (3TC) | Epivir (GSK) | Converted to triphosphate analogue of dCTP by cellular kinases<br>Activity and administration similar to ABC | Minimal toxicity, posttreatment exacerbation of hepatitis B coinfection |
| Stavudine (d4T) | Zerit (BMS) | Converted to triphosphate analogue of dTTP by cellular kinases<br>Activity and administration similar to ABC | Peripheral neuropathy, lipodystrophy; motor weakness |
| Tenofovir alafenamide (TAF) | Vemlidy (Gilead) | Diester hydrolysis required for conversion to tenofovir, monophosphate analogue requires diphosphorylation by cellular kinases<br>Activity and administration similar to ABC | Asthenia, headache, GI symptoms, cough, posttreatment exacerbation of hepatitis B coinfection |
| Tenofovir disoproxil fumarate (TDF) | Viread (Gilead) | Same as TAF | Asthenia, headache, GI symptoms, cough, decrease in bone mineral density, lipodystrophy, posttreatment exacerbation of hepatitis B coinfection |
| Zidovudine (AZT or ZDV) | Retrovir (GSK) | Converted to triphosphate analogue of dTTP by cellular kinases<br>Activity and administration similar to ABC | Bone marrow suppression, GI symptoms, headache, insomnia |
| Nucleoside or nucleotide reverse transcriptase inhibitors (NRTI-NtRTI) combined formulations | | | |
| Abacavir (ABC) + lamivudine (3TC) | Epzicom (GSK) | See individual NRTIs above | See individual NRTIs above |
| Abacavir (ABC) + zidovudine (AZT) + lamivudine (3TC) | Trizivir (GSK) | See individual NRTIs above | See individual NRTIs above |
| Emtricitabine (FTC) + tenofovir (TDF) + efavirenz (EFV) | Atripla (Gilead and BMS) | See individual NTRIs-NtRTIs above | See individual NTRIs-NtRTIs above |
| Tenofovir (TDF) + emtricitabine (FTC) | Truvada (Gilead) | See individual NTRIs-NtRTIs above | See individual NTRIs-NtRTIs above |
| Nonnucleoside reverse transcriptase inhibitors (NNRTIs) | | | |
| Efavirenz (EFV) | Sustiva (BMS) | Noncompetitive inhibitor binds to HIV-1 RT close to catalytic site, disrupts normal polymerization function<br>Administered orally | Skin rash (Stevens-Johnson syndrome), psychiatric symptoms, CNS symptoms (e.g., dizziness, insomnia, confusion), elevated transaminases, teratogenic |
| Etravirine (ETR) | Intelence (Tibotec) | Activity and administration similar to EFV | Skin rash (Stevens-Johnson syndrome), GI symptoms |
| Nevirapine (NVP) | Viramune (BI) | Activity and administration similar to EFV | Severe hepatotoxicity, skin rashes (Stevens-Johnson syndrome) |
| Rilpivirine (RPV) | Edurant (Tibotec) | Activity and administration similar to EFV | Rash, depression, headache, insomnia, hepatotoxicity |

*(Continued on next page)*

**TABLE 1** Antiviral agents for HIV therapy[a] *(Continued)*

| Antiviral agent (abbreviation) | Trade name (pharmaceutical company[b]) | Mechanism of action/ route of administration | Major adverse effects[c] |
|---|---|---|---|
| Protease inhibitors | | | |
| Atazanavir (ATV) | Reyataz (BMS) | Peptidomimetic protease. Binds competitively to active site of HIV protease, prevents cleavage of viral polyprotein precursors, produces immature, noninfectious viral particles<br>Administered orally | Indirect hyperbilirubinemia, prolonged PR interval, hyperglycemia; fat redistribution; increased bleeding episodes with hemophilia, nephrolithiasis |
| Darunavir (DRV) | Prezista (Tibotec) | Nonpeptidic protease<br>Inhibits protease dimerization<br>Prevents cleavage of viral polyprotein<br>Administered orally | Skin rash (Stevens-Johnson syndrome), hepatotoxicity, hyperglycemia, fat redistribution, GI symptoms, elevated transaminase, increased bleeding episodes with hemophilia, nephrolithiasis |
| Fosamprenavir (FPV) | Lexiva (GSK) | Converted to amprenavir by cellular phosphatases<br>Activity and administration similar to ATV | Skin rash, GI symptoms, headache, hyperlipidemia, fat redistribution, elevated transaminases, hyperglycemia, increased bleeding episodes with hemophilia |
| Indinavir (IDV) | Crixivan (Merck) | Activity and administration similar to ATV | Nephrolithiasis/urolithiasis, GI symptoms, indirect hyperbilirubinemia, hyperlipidemia, hemolytic anemia, headache, hyperglycemia, fat redistribution, increased bleeding episodes with hemophilia |
| Lopinavir (LPV) + ritonavir (RTV) | Kaletra (Abbott) | Activity and administration similar to ATV | GI symptoms, asthenia, hyperlipidemia, elevated transaminase, hyperglycemia, hyperlipidemia, fat redistribution, elevated transaminases, increased bleeding episodes with hemophilia |
| Nelfinavir (NFV) | Viracept (Pfizer) | Activity and administration similar to ATV | Diarrhea, hyperlipidemia, hyperglycemia, fat redistribution, elevated transaminases, increased bleeding episodes with hemophilia |
| Ritonavir (RTV) | Norvir (Abbott) | Activity and administration similar to ATV | Severe GI symptoms, circumoral paresthesias, hyperlipidemia, hepatitis, asthenia, taste disturbance, hyperglycemia, fat redistribution, increased bleeding episodes with hemophilia |
| Saquinavir (SQV) | Invirase (Roche) | Activity and administration similar to ATV | GI symptoms, hyperlipidemia, elevated transaminase, headache, hyperglycemia, hyperlipidemia, fat redistribution, increased bleeding episodes with hemophilia |
| Tipranavir (TPV) | Aptivus (BI) | Nonpeptidic protease<br>Activity and administration similar to DRV | Hepatotoxicity, hyperglycemia, sulfa allergy skin rash, hyperlipidemia, fat redistribution, increased bleeding episodes with hemophilia, rare intracranial hemorrhage |
| Entry inhibitors | | | |
| Enfuvirtide (T20) | Fuzeon (Roche) | Binds to first heptad repeat in gp41, prevents conformational changes required for fusion of viral and cellular membranes<br>Administered by injection | Local injection site reactions, pneumonia, hypersensitivity reactions |
| Maraviroc (MVC) | Selzentry (Pfizer) | CCR5 coreceptor antagonist<br>Allosteric binding to CCR5 alters conformation, prevents gp120 binding<br>Administered orally | Upper respiratory infections, cough, pyrexia, rash, dizziness |

*(Continued on next page)*

**TABLE 1** Antiviral agents for HIV therapy[a] *(Continued)*

| Antiviral agent (abbreviation) | Trade name (pharmaceutical company[b]) | Mechanism of action/ route of administration | Major adverse effects[c] |
|---|---|---|---|
| Integrase strand transfer inhibitors | | | |
| Dolutegravir (DTG) | Tivicay (Viiv/GSK) | Prevents formation of covalent bond between unintegrated HIV DNA and host DNA, preventing formation of provirus<br>Administered orally | Headache, insomnia, fatigue, elevated AST/ALT, elevated CPK |
| Elvitegravir (EVG) + cobicistat (COBI) + TDF + FTC | Stribild (Gilead) | EVG: prevents formation of covalent bond between unintegrated HIV DNA and host DNA, preventing formation of provirus<br>Requires pharmacologic boosting<br>Administered orally<br>Cobicistat: pharmacokinetic enhancer, inhibits CYP3A4.<br>TDF and FTC: see protease inhibitors above | Coformulation EG-COBI-TDF-FTC: GI symptoms, renal impairment, decreased bone density |
| Raltegravir (RAL) | Isentress (Merck) | Prevents formation of covalent bond between unintegrated HIV DNA and host DNA, preventing formation of provirus.<br>Administered orally | Headache, GI symptoms, asthenia, fatigue, pyrexia, CPK elevation |

[a]Note: all NRTI/NtRTIs carry the warning of lactic acidosis and severe hepatomegaly with steatosis.
[b]Pharmaceutical companies: Abbott Laboratories, North Chicago, IL; BI, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT; BMS, Bristol-Meyers Squibb, Princeton, NJ; Gilead Sciences, Foster City, CA; GSK, GlaxoSmithKline, Research Triangle Park, NC; Merck & Co., Whitehouse Station, NJ; Pfizer, New York, NY; Roche Pharmaceuticals, Nutley, NJ; Tibotec Therapeutics, Division of Ortho Biotech Products, L.P., Raritan, NJ.
[c]Abbreviations: GI, gastrointestinal (symptoms include nausea, vomiting, and diarrhea); AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatine phosphokinase.

## Abacavir

### Pharmacology

The oral bioavailability of abacavir (ABC) is 83%. The plasma half-life is 1.5 h, and the intracellular half-life is 12 to 26 h. ABC can be administered with or without food. It is metabolized by alcohol dehydrogenase and glucuronyltransferase, and 82% of the metabolites are excreted by the kidneys. Placental passage has been demonstrated in animal studies (7). ABC penetration of the central nervous system (CNS) is adequate to inhibit HIV replication (9). ABC is recommended for therapy in combination with dolutegravir (DTG) and lamivudine (3TC). The guidelines recommend using caution when prescribing ABC in patients with high risk for cardiovascular disease, because studies have shown both a lack of association as well as increased risk of cardiovascular disease (10–12). ABC is contraindicated in patients who are positive for the HLA-B*5701 major histocompatibility complex class I allele, which is associated with a hypersensitivity reaction to the drug (5, 13). Combination formulations of two and three NRTIs and/or NtRTIs containing ABC are commercially available (Table 1).

### Drug Interactions

ABC decreases the level of methadone. Ethanol increases the concentration of ABC in plasma through common metabolic pathways (5).

## Didanosine

### Pharmacology

The oral bioavailability of didanosine (ddI) is 30 to 40%. The serum half-life is 1.5 h, and the intracellular half-life is >20 h. It should be administered without food. One-half of the drug is excreted by the kidney. There is low penetration of the CNS, but ddI has been shown to cross the human placenta (7). ddI is no longer recommended for use in treatment-naive patients (7).

### Drug Interactions

Administration of ddI with either d4T or tenofovir disoproxil fumarate (TDF) can increase the rate and severity of toxicities associated with each individual drug. Ganciclovir (GCV), valganciclovir (val-GCV), ribavirin (RBV), and allopurinol also increase ddI exposure, leading to increased ddI toxicity (5, 14, 15).

## Emtricitabine

### Pharmacology

The oral bioavailability of emtricitabine (FTC) is 93%. The plasma half-life is 10 h, and the intracellular half-life is >20 h. FTC can be administered with or without food. It is excreted mostly unchanged (86%) by the kidneys, and the remainder is eliminated in the feces. It has intermediate penetration of cells of the CNS (16) and has been shown to cross the placenta (7). FTC is recommended as a preferred drug in combination with tenofovir (TDF) in NNRTI-based, PI-based, or INSTI-based regimens for treatment-naive patients. Coadministration with 3TC is not recommended, because both drugs have similar resistance patterns and there is no therapeutic advantage for the combination (5).

### Drug Interactions

No significant interactions with other antiretroviral agents have been reported (5, 17).

## Lamivudine

### Pharmacology

The oral bioavailability of lamivudine (3TC) is 86%. The serum half-life is 5 to 7 h, and the intracellular half-life

is 18 to 22 h. The drug can be administered with or without food, and 71% is excreted by the kidney. 3TC crosses the human placenta (7) and has intermediate penetration of the CNS (16). 3TC is recommended in alternative dual-NRTI regimens with tenofovir (TDF or tenofovir alafenamide [TAF]), combined with either an NNRTI, PI, or INSTI for treatment-naive patients (5). Coadministration of 3TC with FTC is not recommended (see " Emtricitabine" above).

**Drug Interactions**

3TC is actively excreted by the kidney by the organic cationic transport system; therefore, possible interactions should be considered with other drugs that use the same pathway, such as trimethoprim-sulfamethoxazole (5).

### Stavudine

**Pharmacology**

The oral bioavailability of stavudine (d4T) is 86%. The serum half-life is 1.0 h, and the intracellular half-life is 7.5 h. d4T can be administered with or without food. Half of the drug is excreted by the kidneys. Placental passage occurs in animals, and d4T has intermediate penetrance of the CNS (7, 18). d4T is no longer recommended for use in treatment-naive patients because of toxicity (5). It has been replaced by ABC or zidovudine (ZDV) in first-line pediatric regimens (19).

**Drug Interactions**

d4T combined with ddI can increase the rate and severity of toxicities associated with each individual drug. ZDV and RBV inhibit the phosphorylation of d4T (20, 21).

### Tenofovir Disoproxil Fumarate and Tenofovir Alafenamide

**Pharmacology**

The oral bioavailability of the prodrug tenofovir disoproxil fumarate (TDF) metabolized to tenofovir is 25% without food and 39% with a high-fat meal, although the drug is administered without regard to meals. The serum half-life is 17 h, and the intracellular half-life is >60 h. The drug is excreted mostly unchanged (70 to 80%) by the kidneys. TDF has been shown to cross the placenta in animal studies, but it has low penetrance of the CNS (7, 16). It is less likely than other NRTIs-NtRTIs to be associated with mitochondrial toxicity; however, renal dysfunction and decreased bone mineral density have been reported with TDF use. TDF is recommended in initial regimens with dual NRTI-NtRTI combinations including FTC with elvitegravir (EVG) and cobicistat (COBI) (5, 22).

Tenofovir alafenamide (TAF) is another prodrug of tenofovir, which appears to be equally effective as an antiretroviral agent as TDF, but at a much lower dose (23). Consequently, TAF is associated with lower bone density loss and nephrotoxicity compared to TDF (22). TAF is approved in combined formulations such as EVG-COBI-FTC or darunavir (DRV)-ritonavir (RTV)/FTC for initial antiretroviral therapy (5).

**Drug Interactions**

TDF increases the concentration of ddI in plasma, leading to increased toxicity (14). There may be increased toxicity associated with coadministration of GCV, val-GCV, acyclovir (ACV), or cidofovir (CDV) (5).

TAF is a substrate for P-glycoprotein. Drugs that decrease TAF through this pathway include anticonvulsants, antimycobacterials, and St. John's wort (5).

### Zidovudine

**Pharmacology**

The oral bioavailability of zidovudine (ZDV) is 60%, with a serum half-life of 1.1 h and intracellular half-life of 7 h. ZDV can be administered without regard to meals. It is metabolized to the glucuronide form, which is excreted by the kidneys.

ZDV crosses the blood-brain barrier to achieve effective concentrations in the CNS (16) and also crosses the placenta. ZDV with 3TC is an alternative dual-NRTI backbone for combination regimens in pregnant women (7). It can be given intravenously to pregnant women during labor to prevent maternal-fetal transmission if the mother has ≥400 copies/ml of HIV or if the HIV viral load is unknown near the time of delivery. Intrapartum ZDV is no longer recommended for HIV-infected mothers who achieve virologic control on cART. ZDV can be administered orally to the child at birth either alone or in combination with nevirapine (NVP) and/or 3TC (7, 24). For adults and adolescents, ZDV can be given with 3TC as a dual-NRTI backbone with NNRTI-based and PI-based regimens. However, this is no longer considered a preferred or alternative regimen, because it requires twice-daily dosing and has greater associated toxicity than TDF-FTC or ABC-3TC (5).

**Drug Interactions**

ZDV inhibits the phosphorylation of d4T by thymidine kinase (24). RBV inhibits phosphorylation of ZDV (21). GCV and alpha-interferon may enhance the hematologic toxicity associated with ZDV (25, 26).

### NRTI/NtRTI Combination Formulations

There are multiple fixed-dose combinations involving NRTIs and NtRTIs, which are available as commercial formulations for convenience of administration: ABC-3TC-ZDV (Trizivir), ABC-3TC (Epzicom), FTC-TDF (Truvada), 3TC-ZDV (Combivir), FTC-TDF-efavirenz (EFV) (Atripla), FTC-rilpivirine (RPV)-TDF (Complera), FTC-EVG-COBI-TDF (Stribild), FTC/RPV/TAF (Odefsey), FTC/TAF (Descovy), and EVG-COBI-FTC-TAF (Genvoya). Clinical trials have shown the triple combination ABC-3TC-ZDV to be equivalent to PI-based regimens but inferior to NNRTI-based regimens (5). Therefore, ABC-3TC-ZDV is no longer recommended for initial therapy. The dual combinations are used as NRTI-NtRTI backbones in combination with an NNRTI, PI, or INSTI in triple- or quadruple-drug therapy. The triple coformulation FTC-TAF (or TAF)-EFV is a preferred initial regimen, while FTC-RPV-TAF and FTC-RPV-TDF (or TAF) are considered alternative regimens (7).

## Nonnucleoside Reverse Transcriptase Inhibitors

Drugs in the NNRTI class do not require intracellular anabolism for activation. There is no common structure; however, they bind noncompetitively to the HIV-1 RT close to the catalytic site. Disruption of DNA polymerization activity leads to premature DNA chain termination. The HIV-2 RT is resistant to this class of drugs (1).

There are currently four available NNRTIs: NVP, EFV, etravirine, and RPV. All are metabolized by the cytochrome P450 (CYP450) system, which also metabolizes the PIs (see below) and other drugs used to treat conditions associated with HIV infection. The common pathway can lead to serious interactions, which either induce or inhibit individual drug metabolism.

In the past NNRTIs were preferred for first-line therapeutic regimens with two NRTIs and/or NtRTIs for the

following reasons: (i) there is a low incidence of gastrointestinal symptoms; (ii) NNRTIs have a long half-life that tolerates missed doses; and (iii) use of NNRTIs saves PIs for future regimens. The disadvantages of the NNRTIs are (i) the relatively low number of mutations required to confer cross-resistance to many of the drugs in this class and (ii) side effects related to the CNS (7). As a result, NNRTIs are now components of recommended alternative regimens, with INSTIs replacing them in the preferred initial regimens.

### Efavirenz

#### Pharmacology

The oral bioavailability of efavirenz (EFV) is <1%. The serum half-life is 52 to 76 h. The drug should be administered without food. EFV is 99.5% protein bound in the plasma, mainly to albumin. CNS penetration is intermediate (16), but EFV has been shown to cross the placenta in animals (7). EFV is metabolized by CYP3A4 and CYP2B6 and is an inducer and inhibitor of CYP3A4. Glucuronidated metabolites are excreted in the urine (14 to 34%) and eliminated in the feces (16 to 61%). EFV-FTC-TDF (or TAF) is recommended as an alternative regimen except in pregnant women, because teratogenic effects have been observed in cynomolgus monkeys during the first trimester of pregnancy (7).

#### Drug Interactions

Dose modifications may be necessary for potential drug interactions between EFV and the following: indinavir (IDV), lopinavir-RTV (LPV-r), fosamprenavir (FPV), nelfinavir (NFV), saquinavir (SQV), clarithromycin, rifabutin, rifampin, simvastatin, lovastatin, methadone, itraconazole, anticonvulsants, and oral contraceptives (5). Contraindicated drugs are rifapentine, cisapride, midazolam, triazolam, ergot derivatives, St. John's wort, voriconazole, HCV PIs, and in treatment-experienced patients, atazanavir (ATV).

### Etravirine

#### Pharmacology

The oral bioavailability of etravirine (ETR) is unknown. The serum half-life is 41 h ± 21 h. Drug levels are reduced under fasting conditions; therefore, ETR should be taken with meals. ETR is 99.9% protein bound in plasma, mainly to albumin. It is not known whether ETR penetrates the CNS or crosses the placenta. ETR is metabolized by CYP3A4, CYP2C9, and CYP2C19. It induces CYP3A4 and inhibits CYP2C9 and CYP2C19. It is also an inducer of P-glycoprotein (5). ETR is eliminated in the feces (93.7%) and excreted in the urine (1.2%) (27). It has not been studied in large trials of treatment-naive patients and therefore is not recommended for treatment in this population. ETR is reported to be active against HIV-1 strains that are resistant to other NNRTIs, including HIV-1 group O (28); therefore, it is currently used in regimens for treatment-experienced patients who have failed therapy (1, 27, 29).

#### Drug Interactions

Dose modifications may be required for the following: LPV-r, SQV, antiarrhythmics, dexamethasone, erectile dysfunction drugs, warfarin, lipid-lowering drugs, diazepam, and antifungal agents. ETR should not be coadministered with the following drugs: EFV, NVP, ATV, FPV, tipranavir (TPV), hormonal contraceptives, St. John's wort, clarithromycin, antimycobacterials (if coadministered with RTV-boosted PI), and phenobarbital (5, 27).

### Nevirapine

#### Pharmacology

The oral bioavailability of nevirapine (NVP) is >90%, and the serum half-life is 25 to 30 h. NVP is 60% protein bound. Penetration into the CNS is high; the concentration in the cerebrospinal fluid is 45% of the concentration in plasma (16). NVP can be administered with or without food. It is both a substrate and an inducer of CYP3A4 and CYP2B6 (5). Glucuronidated metabolites are excreted in the urine (80%) and feces (10%). NVP is known to cross the human placenta (7). It has been used in resource-limited regions as a single oral agent in an intrapartum/newborn prophylaxis regimen to prevent mother-to-child transmission (7, 30). It is also under study as part of three-drug regimens to prevent perinatal transmission (7, 31). However, NVP has been associated with serious hepatic events and has a low barrier to resistance, and therefore, it is no longer considered a preferred or alternative agent for initial therapy. In certain circumstances NVP may be considered in women with $CD4^+$ T cell counts of ≤250 cells/$mm^3$ or in males with counts of ≤400 cells/$mm^3$ in the absence of moderate to severe hepatic impairment (Child-Pugh class B or C) (5).

#### Drug Interactions

NVP reduces the concentrations in plasma of IDV, SQV, oral contraceptives, fluconazole, ketoconazole, clarithromycin, and methadone (21). Coadministration of ATV, ETR, rifampin, rifapentine, St. John's wort, or HCV PIs with NVP is contraindicated (5).

### Rilpivirine

#### Pharmacology

The oral bioavailability of rilpivirine (RPV) is unknown, and the serum half-life is 50 h. It is not known whether RPV penetrates the CNS or crosses the placenta. RPV should be administered with food. It is a CYP3A4 substrate (5). RPV in combination with TDF-FTC or ABC-3TC is an alternative regimen for treatment-naive patients. However, RPV use is not recommended in patients with a pretreatment HIV viral load of >100,000 copies/ml, because it has been associated with virologic failure in these patients. In addition, patients with $CD4^+$ T cell counts of <200 cells/$mm^3$ are more likely to experience virologic failure when treated with an RPV-based regimen (5). RPV is metabolized by CYP3A4 and eliminated in urine and feces.

#### Drug Interactions

Drugs that are contraindicated are antimycobacterials, anticonvulsants, proton pump inhibitors, HCV PIs, dexamethasone, and St. John's wort (5).

## Protease Inhibitors

PIs, like the NNRTIs, require no intracellular anabolism for antiviral activity. The target is the HIV-encoded protease, which is required for posttranslational processing of the precursor gag polyprotein (32). Most PIs are peptidomimetic, because they contain the peptide bond normally cleaved by the protease (1). TPV and DRV are nonpeptidic molecules that are reported to inhibit protease dimerization as well as normal enzymatic activity (33). The relative activity of PIs against the HIV-1 versus HIV-2 protease varies among the drugs and is dependent on the amino acid sequences of the target binding sites (34).

PIs are commonly used in cART regimens in combination with NRTI and/or NtRTIs for maximum antiretroviral

activity and to minimize the development of resistance. PI-based regimens introduced initially led to treatment failure related to their limited bioavailability, frequent dosing, and toxicity. There are several characteristics of these drugs that lead to these treatment-related problems. They are highly bound to plasma protein, mainly alpha-1 acid glycoprotein (AAG) (35). The low concentration of unbound drug is responsible for the therapeutic activity as well as toxicity. PIs are substrates for P-glycoprotein and multidrug resistance-associated protein. These are efflux transporters, which enhance elimination of the drugs from cells in the intestine, liver, and kidneys and reduce intracellular drug concentrations (36). All of the PIs are metabolized in the intestine and liver by enzymes of the CYP450 system (37), mainly by CYP3A4, CYP2C9, and CYP2C19. An individual PI can induce and/or inhibit specific CYP450 isoenzymes, which can enhance or reduce its own metabolism or that of other PIs. As noted above, the CYP450 system metabolizes the NNRTIs and numerous other drugs that may be used for conditions associated with HIV infection. Thus, the choice of treatment regimens is complicated by multiple potential drug-drug interactions, which may enhance toxicity and/or require dose modifications of coadministered drugs (5).

Although most PIs are inhibitors of CYP3A4, RTV is the most inhibitory. For this reason, RTV is used in boosting regimens to improve the pharmacokinetic profile of a second PI (38). Subtherapeutic concentrations of RTV increase the systemic exposure of a second PI by reducing the rate of metabolism and increasing the half-life (37), which lowers dosing requirements and food effects for the second drug. An example is LPV, which alone has very little bioavailability and a very short half-life but in combination with RTV is used therapeutically in alternative regimens for treatment-naive patients and in salvage therapy (5, 38, 39). The effect of RTV on the pharmacokinetics of other PIs varies as a result of differences in interaction with components of the CYP450 system that determine bioavailability. Specific recommendations are described below for each drug.

### Atazanavir

**Pharmacology**

Atazanavir (ATV) is an azapeptide PI that differs structurally from other peptidomimetic PIs. The bioavailability is undetermined, and the serum half-life is 7 h. The bioavailability, however, is increased by administration with food. ATV is 86% protein bound and penetrates the CNS (40). It is metabolized in the liver by CYP3A4, and it is also an inhibitor of this enzyme. The metabolites are eliminated in the feces (79%) and urine (13%). ATV crosses the placenta at minimal levels. It is an inhibitor, inducer, and substrate for P-glycoprotein (7).

ATV has the advantage of once-daily dosing as well as a high genetic barrier to resistance. ATV boosted with RTV or COBI is a recommended alternative PI in regimens with TDF (or TAF)-FTC (5, 41).

**Drug Interactions**

Drugs that may require dose modifications or cautious use with ATV include antifungal agents, antiarrhythmics, clarithromycin, colchicine, oral contraceptives, anticonvulsants, rifabutin, erectile dysfunction agents, H2 receptor antagonists, antacids, and buffered medications. Drugs that are contraindicated for coadministration with ATV include IDV, NVP, ETR, EFV (in treatment-experienced patients), HCV PIs, antihistamines, bepridil, simvastatin, lovastatin, antimycobacterials, cisapride, proton pump inhibitors, neurologic agents, ergot derivatives, St. John's wort, and irinotecan (5).

### Cobicistat

**Pharmacology**

Cobicistat (COBI) is a structural analogue of RTV, but it has no direct antiviral activity for HIV or HCV. Like RTV it serves as a pharmacoenhancer for other antiviral agents. It is a component of several fixed-dose antiretroviral regimens: ATV-COBI, DRV-COBI, EVG-COBI-FTC-TDF (or TAF) (42, 43). It is not interchangeable with RTV for boosting FPV, SQV, or TPV. COBI should be given with food. COBI is 97 to 98% protein bound. The half-life is 3 to 4 h (higher with ATZ than DRV), and it is excreted in feces and urine. COBI is a substrate and a very strong inhibitor of CYP3A4 as well as an inhibitor of CYP2D6 and P-glycoprotein.

**Drug Interactions**

As an analogue of an HIV PI, COBI has a similar profile of drug interactions. It should not be administered with any PIs coformulated with RTV (FPV, SQV, TPV, LPV). Other contraindicated drugs include EFV, ETV, NVP, antiarrhythmics, macrolide/ketolide antibiotics, antifungals, antimycobacterials, HCV antivirals, neurologic agents, erectile dysfunction drugs, and anticonvulsants.

### Darunavir

**Pharmacology**

The bioavailability of darunavir (DRV) is 37% alone and 82% when boosted with RTV, and the serum half-life is 15 h when boosted. It should be administered with food. The plasma protein binding is 95%, mainly to AAG. DRV is metabolized in the liver by CYP3A4, for which it is an inhibitor, and it is an inducer of CYP2C9 and P-glycoprotein. It is eliminated in the feces (79.5%) and the urine (13.9%). DRV boosted with RTV is a preferred PI in regimens with two NRTIs or NtRTIs for treatment-naive patients and pregnant women (7).

**Drug Interactions**

Drugs that may require dose modifications are the antidepressants paroxetine and sertraline, erectile dysfunction drugs, antifungals, atorvastatin, and rosuvastatin. Drugs that are contraindicated are EFV, ETR, NVP, HCV PIs, neurologic agents, lovastatin, simvastatin, antimycobacterials, ergot derivatives, St. John's wort, cisapride, anticonvulsants, and fluticasone.

### Fosamprenavir

**Pharmacology**

Fosamprenavir (FPV) is a prodrug with no antiviral activity which must be converted to amprenavir (APV) by cellular phosphatases (44). The bioavailability of APV is undetermined, and the serum half-life is 7.7 h. It can be administered with or without food. The plasma protein binding is 90%. APV is metabolized in the liver by CYP3A4, for which it is an inhibitor and inducer (45). It is eliminated in the feces (75%) and urine (14%). It is not known whether APV crosses the placenta (7). FPV boosted with RTV has high penetrance in the CNS (16). However, it is not recommended for treatment-naive patients (5).

**Drug Interactions**

Drugs that may require dose modifications or cautious use with FPV include erectile dysfunction drugs, antifungals,

EFV, NVP, LPV/r, SQV, RTV, rifabutin atorvastatin, and methadone. Drugs that are contraindicated for coadministration with FPV include ETR, DLV, HCV protease and NS5A inhibitors, simvastatin, lovastatin, antimycobacterials, cardiac agents, cisapride, neurologic agents, antihistamines, ergot derivatives, St. John's wort, and oral contraceptives (5, 21).

### Indinavir

**Pharmacology**

The bioavailability of indinavir (IDV) is 65%, and the serum half-life is 1.5 to 2.0 h. IDV should be administered with low-caloric, low-fat food. It is 60% plasma protein bound, mainly to AAG (46). IDV is a substrate and an inhibitor of CYP3A4. The majority of the drug (83%) is eliminated as metabolites in the feces. There is minimal passage of IDV across the placenta (7), but RTV-boosted IDV penetrates the CNS (16, 47).

RTV-boosted or -unboosted IDV is not recommended as a component of PI-based regimens for treatment-naive patients, because of inconvenient dosing (unboosted) and the adverse complication of nephrolithiasis (RTV boosted) (5).

**Drug Interactions**

Coadministered drugs that may require dose modifications or cautious use include DLV, ddI, EFV, NFV, NVP, RTV, SQV, antiarrhymics, antifungal agents, anticonvulsants, calcium channel blockers, atorvastatin, methadone, colchicine, and vitamin C, especially in grapefruit juice. Drugs that are contraindicated for coadministration with IDV include ATV, TPV, amiodarone, simvastatin, lovastatin, antimycobacterials, ergot derivatives, neurologic agents, cisapride, erectile dysfunction drugs, and St. John's wort (5, 21).

### Lopinavir-Ritonavir

**Pharmacology**

LPV is administered only in combination with low-dose RTV (LPV-r), and the combined formulation (Kaletra) is commercially available. The bioavailability of LPV-r is undetermined, and the half-life is 5 to 6 h. The oral tablet formulation can be taken with or without food; the oral solution should be taken with food of moderate fat content. The plasma protein binding is 99%, mainly to AAG. LPV-r is an inhibitor and a substrate of CYP3A4 and to a lesser extent CYP2D6. It is eliminated mainly in the feces (82.6%) and urine (10.4%) as metabolites. LPV crosses the placenta (7). LPV-r has high penetration of the CNS (16) and is a component of recommended alternative PI-based regimens with two NRTIs/NtRTIs for treatment-naive patients (5).

**Drug Interactions**

Drugs that may require dose modifications when coadministered with LPV-r include erectile dysfunction drugs, rosuvastatin, atorvastatin, calcium channel blockers, and methadone. Drugs that are contraindicated for coadministration include DRV, FPV, TPV, simvastatin, lovastatin, oral contraceptives, neurologic agents, anticonvulsants, antiarrhythmics, antimycobacterials, antihistamines, cisapride, cardiac agents, HCV antivirals, ergot derivatives, fluticasone, and St. John's wort (5, 21).

### Nelfinavir

**Pharmacology**

The bioavailability of nelfinavir (NFV) is 20 to 80%, and the serum half-life is 3.5 to 5 h. NFV shows the greatest accumulation in cells of all the PIs; however, the protein binding is >98% (35). It should be administered with food. NFV is both an inhibitor and inducer of CYP3A4 (45). The majority of the drug (87%) is eliminated in the feces. There is minimal placental passage (7) and low penetration of the CNS (16). NFV is not recommended in PI-based regimens with two NRTIs and/or NtRTIs for treatment-naive patients because of lower antiretroviral efficacy (5). Boosting with RTV does not affect exposure.

**Drug Interactions**

Drugs that require dose modifications or cautious use include rifabutin, atorvastatin, anticonvulsants, methadone, and erectile dysfunction agents. Drugs that are contraindicated for coadministration with NFV include TPV, antiarrhythmics, simvastatin, lovastatin, antimycobacterials, cisapride, neurologic agents, antihistamines, ergot derivatives, St. John's wort, proton pump inhibitors, and oral contraceptives (5, 21).

### Ritonavir

**Pharmacology**

The oral bioavailability of ritonavir (RTV) is undetermined, and the serum half-life is 3 to 5 h. RTV should be administered with food. It is 98% plasma protein bound and is metabolized by CYP3A. The major metabolite is isopropylthiazole, which has the same antiviral activity as the parent drug. RTV is eliminated in the feces (86.4%) and urine (11.3%) (5). Passage across the placenta is minimal (7).

The main role of RTV in current HIV therapeutics is to enhance the pharmacokinetics of a second PI (38), because RTV is a very strong inhibitor of CYP3A4. Low-dose RTV is a pharmacoenhancer of IDV, FPV, SQV, LPV, ATV, TPV, and DRV. RTV alone in PI-based regimens is not recommended because of gastrointestinal intolerance (5). RTV-boosted PIs are recommended in combination with two NRTIs and/or NtRTIs in PI-based regimens for treatment-naive and treatment-experienced patients (5, 38).

**Drug Interactions**

As a very strong inhibitor of CYP3A4, RTV has numerous potential drug interactions requiring close monitoring (5, 21). Coadministered drugs that may require dose modifications or cautious use include antifungals, clarithromycin, atorvastatin, pravastatin, rosuvastatin, anticonvulsants, methadone, erectile dysfunction drugs, atovaquone, quinine, antidepressants, and theophylline. Drugs that are contraindicated for coadministration with RTV include ETR, antiarrhythmics, simvastatin, lovastatin, antimycobacterials, cisapride, neurologic agents, ergot derivatives, oral contraceptives, and St. John's wort.

### Saquinavir

**Pharmacology**

The oral bioavailability of saquinavir (SQV) is approximately 4%. The serum half-life is 1 to 2 h. SQV is both a substrate and inhibitor of CYP3A4 and P-glycoprotein. It should be administered with food. SQV is 97% bound to plasma proteins and is eliminated mainly in the feces (81%) (5). There is minimal passage of SQV across the placenta (7) and very low penetrance of the CNS (16). SQV RTV-boosted and unboosted regimens with two NRTIs and/or NtRTIs are not recommended for treatment-naive patients (5).

**Drug Interactions**

Coadministered drugs or foods that require dose modifications or cautious use include antifungal agents,

antiarrhythmics, atorvastatin, rosuvastatin, anticonvulsants, methadone, erectile dysfunction agents, proton pump inhibitors, and grapefruit juice. Drugs that are contraindicated for coadministration with SQV include TPV, DRV, antihistamines, fluticasone, simvastatin, lovastatin, antimycobacterials, cisapride, neurologic agents, oral contraceptives, ergot derivatives, HCV antivirals, St. John's wort, garlic supplements, and dexamethasone (5, 21).

### Tipranavir

#### Pharmacology

Tipranavir (TPV) is a nonpeptidic PI (48). The oral bioavailability is undetermined, and the half-life is 6 h. It can be administered with or without food. TPV is >99.9% protein bound in plasma to both albumin and AAG. It is metabolized mainly through CYP3A4, and it is also a CYP3A4 and CYP2C19 inducer. TPV is eliminated in the feces (82.3%) and urine (4.4%). It is not known whether TPV crosses the placenta (7), and penetration of the CNS is low (16). TPV requires coadministration with RTV to reach effective levels in plasma (37, 49). TPV is not recommended for use in PI-based regimens for treatment-naive patients. The current indicated use is in patients who are highly treatment experienced or who are infected with virus strains resistant to multiple PIs.

#### Drug Interactions

Coadministration of TPV with the following drugs may require dose modification: colchicine, rosuvastatin, methadone, antifungals, and anticonvulsants. Coadministration of the following drugs is contraindicated: ATV, ETR, FPV, LPN, NFV, SQV, cardiac agents, antimycobacterials, lovastatin, simvastatin, neurologic agents, ergot derivatives, cisapride, antihistamines, HCV antivirals, oral contraceptives, erectile dysfunction agents, St. John's wort, and fluticasone (5, 37, 49).

## Entry Inhibitors

Antiretroviral agents that target the entry of HIV into the host cell have been developed. Enfuvirtide (T20), a fusion inhibitor, was the first of these drugs to be approved. It is a linear synthetic peptide of 36 L-amino acids that binds to the first heptad repeat in the gp41 subunit of the HIV-1 envelope glycoprotein. The sequence of the peptide was derived from that of HIV-$1_{LAI}$, a subtype B strain (50). The binding prevents conformational changes that are required for fusion between the virus envelope and the cell membrane (51). Entry is inhibited, thereby preventing infection of the target cell.

Maraviroc (MVC), a CCR5 antagonist, is a second drug that targets viral entry. The use of this drug is dependent on the prior determination of the viral tropism, because only virus strains utilizing the CCR5 coreceptor (R5) are susceptible. The rationale for this antiviral target is that coreceptor tropism of primary HIV-1 infection is most commonly CCR5, and the switch to CXCR4 or dual tropism occurs much later in the course of infection. Allosteric binding of MVC to the CCR5 coreceptor results in a conformational change, which inhibits HIV-1 gp120 binding and viral entry into the target cell (52).

### Enfuvirtide

#### Pharmacology

The bioavailability of enfuvirtide (T20) by subcutaneous injection is 84% (51), and the serum half-life is 3.8 h. T20 is 92% protein bound in plasma. It is assumed that the metabolism of the drug produces the constituent amino acids, which enter the amino acid pool in the body and are recycled. It is not active against HIV-2, but there are recent data suggesting that it is active against HIV-1 non-B subtypes and possibly group O as well (53). Limited data indicate that T20 does not cross the placenta (7) and that it does not penetrate the CNS (54). T20 is not recommended for use in NNRTI- or PI-based regimens in treatment-naive patients, because of its low barrier to resistance, and it requires injection for delivery. T20 is currently used in salvage therapy regimens for treatment-experienced patients who have not responded to their current antiretroviral therapy (7, 55).

#### Drug Interactions

There is no evidence that T20 induces or inhibits any of the CYP450 isoenzymes; therefore, it is unlikely to interact with any of the drugs that are metabolized by the CYP450 system. No significant interactions with other antiretroviral drugs have been identified (51).

### Maraviroc

#### Pharmacology

Maraviroc (MVC) prevents HIV-1 binding of CCR5 (R5) strains to the CCR5 coreceptor but has no activity against CXCR4 (X4) strains. The bioavailability is 33%, and the serum half-life is 14 to 18 h. It is 76% protein bound in the plasma to both albumin and AAG. It can be administered with or without food. MVC is a substrate for CYP3A4 and P-glycoprotein and is eliminated in the feces (76%) and urine (20%). It is not known whether MVC crosses the placenta (7). Although MVC may be used in combination with two NRTIs and/or NtRTIs in treatment-naive patients known to have R5-tropic virus, it is not considered a preferred or alternative agent because of its twice-daily dosing schedule and need for tropism testing (7, 56).

#### Drug Interactions

Coadministration of MVC with the following drugs may require dose modification: antifungals, anticonvulsants, rifabutin, EFV, EVG boosted with COBI (EVG/c), raltegravir (RAL), ETR, and all PIs except TPV. Coadministration with antimycobacterials, HCV antivirals, and St. John's wort is contraindicated.

## Integrase Strand Transfer Inhibitors

INSTIs, a class of antiretroviral drugs, target the HIV-1 integrase enzyme that mediates transfer of the reverse-transcribed HIV-1 DNA into the host chromosome. The activity of this enzyme includes 3′ processing of the reverse transcribed DNA to generate hydroxyls at the 3′ ends of both strands followed by strand transfer that joins viral and host DNA. The approved integrase inhibitors are recommended with two NRTI/NtRTIs for first-line therapy regimens for treatment-naive patients (1, 7).

### Raltegravir

#### Pharmacology

Raltegravir (RAL) is active against HIV-1 group O isolates (28) as well as HIV-1 group M and HIV-2 (57). Its bioavailability has not been established, and its serum half-life is 7 to 12 h. It is 83% protein bound in plasma. RAL crosses the placenta (7). It can be administered with or without food. It is eliminated in the feces (51%) and urine (32%).

Clearance is by UDP-glucuronosyltransferase glucuronidation. It is not metabolized by the CYP450 enzymes. RAL with FTC-TDF or TAF is now an INSTI-based recommended regimen for treatment-naive patients (5, 58, 59).

**Drug Interactions**

Because RAL is not an inducer, inhibitor, or substrate of CYP450 enzymes, it does not affect the pharmacokinetics of most of the drugs that interact with the other classes of antiretroviral agents (5, 57, 59). Coadministration of the following drugs may require dose modification: antacids, antimycobacterials, anticonvulsants, ETR, and TPV boosted with RTV (TPV/r).

### Elvitegravir

**Pharmacology**

Currently, elvitegravir (EVG) is approved only in coformulation with other antivirals plus the pharmacoenhancer COBI (EVG-COBI-TDF or TAF-FTC; Stribild). It achieves therapeutic concentrations only when combined with COBI. EVG has a serum half-life of 13 h. It is 99% protein bound in plasma (60). EVG should be taken with food. Cerebrospinal fluid and placental penetration levels are unknown. The combination pill EVG-COBI-TDF-FTC is a recommended option for treatment-naive patients (5).

EVG is a CYP3A4 substrate and CYP2C9 inducer. COBI was developed for use with EVG, because COBI has no anti-HIV activity, but like RTV, it is a strong inhibitor of CYP3A4. The result is higher concentrations of EVG at lower doses (61). COBI also interacts with intestinal transport proteins to increase absorption of other anti-HIV drugs, including ATZ/c and DRV/c (42, 43, 59).

**Drug Interactions**

EVG is primarily metabolized by the CYP450 pathway and therefore interacts with other drugs that utilize this pathway. Because EVG is available only as a coformulated tablet, data regarding interactions of EVG alone are lacking. EVG-COBI-TDF-FTC administration should be separated from antacid administration by more than 2 hours. Coadministration of NVP, RPV, ATV boosted with COBI (ATV/c) (or with RTV [ATV/r]), DRV/c (or r), FPV/r, LPV/r, SQV/r, TPV/r, antimycobacterials, anticonvulsants, antidepressants, antifungal agents, neurologic agents, HCV antivirals, ergot derivatives, lovastatin, simvastatin, and St. John's wort is contraindicated (5).

### Dolutegravir

**Pharmacology**

DTG is approved for use in both HIV treatment-naive and treatment-experienced patients. It has been shown to have little cross-resistance with the other INSTIs, RAL, and EVG (62). DTG has a 14-hour half-life and can therefore be administered once a day in select patients. Twice-daily dosing is recommended in patients with known or suspected INSTI resistance and when coadministered with EFV, FPV/r, TPV/r, or rifampin (63). DTG can be administered with or without food (64). It is a P-glycoprotein substrate and is eliminated in the feces (53%) and urine (31%). DTG does not inhibit CYP450 enzymes, and therefore, like RAL, it does not interact with drugs that are metabolized by these enzymes.

**Drug Interactions**

Coadministration with the following drugs may require dose modification: anticonvulsants, EFV, FPV/r, TPV/r, and mycobacterials. The following drugs should not be coadministered with DTG: carbamazepine, phenytoin, NVP, phenobarbital, and St. John's wort.

## AGENTS AGAINST HEPATITIS C VIRUS

Increased understanding of the genome and virology of hepatitis C virus (HCV) has led to advances in the efficacy and tolerability of HCV treatment. Multiple direct-acting antivirals (DAAs) which interfere with specific steps in HCV replication have been developed. This has led to combination treatment regimens that are interferon free, pangenotypic, and administered in single daily doses. The four classes of DAAs defined according to their mechanism of action and therapeutic target are the nonstructural proteins 3/4A (NS3/4A) PIs, NS5B nucleoside polymerase inhibitors, NS5B nonnucleoside polymerase inhibitors, and NS5A inhibitors (65). DAAs are available in multiple fixed-dose combinations (summarized in Table 2). While RBV is still used in combination with DAAs, interferon-based regimens are no longer used because of their poor tolerability. Given the rapidly changing landscape of HCV treatment, please visit www.hcvguidelines.org for the most current information (66).

### NS3/4A Protease Inhibitors

NS3/4A PIs inhibit a serine protease involved in posttranslational processing of HCV by blocking the NS3 catalytic site or the NS3/NS4A interaction. In addition, NS3/NS4A PIs prevent blockage of TIR domain-containing adaptor protein-inducing interferon beta (TRIF)-mediated Toll-like receptor and Cardif-mediated retinoic acid inducible gene 1 signaling, which results in induction of interferons and promotion of viral elimination (67). The first-generation PIs telaprevir and boceprevir have been replaced by more potent and better-tolerated antivirals. Grazoprevir, paritaprevir, simeprevir, voxilaprevir, and glecaprevir are PIs available in the United States.

### Grazoprevir

**Pharmacology**

Grazoprevir is a pangenotypic PI that is only available in combination with the NS5A inhibitor elbasvir (68). Its absorption is not affected by meals and is 27% bioavailable. It has a predominantly hepatic distribution and is highly protein bound. It is metabolized hepatically, has a half-life of 31 hours, and is predominantly excreted in the feces. It can be used in patients with any degree of renal impairment, including those on dialysis, without the need for dose modifications. It is contraindicated in patients with Child-Pugh class B or C cirrhosis.

**Drug Interactions**

Grazoprevir is metabolized by CYP3A enzymes and should not be given with moderate and strong inducers or strong inhibitors of this system (69). It is also a substrate of OATP1B1/3 and should not be coadministered with drugs that inhibit this enzyme. Coadministration is contraindicated with rifampin, phenytoin, carbamazepine, St. John's wort, cyclosporine, and some antiretroviral agents such as PIs and EFV. Coadministration is not recommended with nafcillin, ketoconazole, etravirine, COBI, or modafinil.

### Paritaprevir

**Pharmacology**

Paritaprevir is coadministered with low-dose RTV for a pharmacologic boosting effect, and these drugs are available as a fixed-dose combination with ombitasvir, which is an

**TABLE 2** Antiviral agents for HCV therapy[a]

| Antiviral agents and approved fixed combinations | Trade name (pharmaceutical company[b]) | Mechanism of action/ route of administration | Major adverse effects |
|---|---|---|---|
| Ribavirin | Copegus (Genentech)<br>Rebetol (Merck) | Mechanism not established<br>Administered orally only in combination with another DAA | Anemia[c], myocardial infarction, teratogenic, hypersensitivity, impairment of pulmonary function, GI symptoms[d] |
| Sofosbuvir | Sovaldi (Gilead) | A nucleotide analogue inhibitor of HCV NS5B polymerase<br>Inhibits viral RNA synthesis<br>Administered orally | Fatigue, insomnia, headache, GI symptoms, bradycardia with amiodorone |
| Simeprevir | Olysio (Janssen) | Binds to NS34A protease active site, preventing viral replication<br>Administered orally | Photosensitivity, rash, bradycardia with amiodorone |
| Sofosbuvir + ledipasvir | Harvoni (Gilead) | NS5A inhibitor (ledipasvir) and nucleotide analogue inhibitor of HCV NS5B polymerase (sofosbuvir)<br>Administered orally | Fatigue, insomnia, headache, GI symptoms |
| Ombitasvir + paritaprevir + ritonavir + dasabuvir | Viekira Pak (AbbVie) | NS5A inhibitor (ombitasvir), NS3/4A protease inhibitor (paritaprevir), and NS5B RNA polymerase inhibitor (dasabuvir)<br>Ritonavir is an HIV protease inhibitor with no anti-HCV activity | Fatigue, rash, GI symptoms, insomnia, pruritus |
| Ombitasvir + paritaprevir + ritonavir | Technivie (AbbVie) | Same combination of antiviral agents as above, without dasabuvir | Same as above |
| Grazoprevir + elbasvir | Zepatier (Merck) | NS5A inhibitor (elbasvir), NS3/4A protease inhibitor (elbasvir) | Fatigue, headache, GI symptoms |
| Daclatasvir | Daklinza (Bristol-Meyers Squibb) | NS5A inhibitor for use with sofosbuvir | Fatigue, headache |
| Velpatasvir + sofosbuvir | Epclusa (Gilead) | NS5A inhibitor (velpatasvir), NS5B inhibitor (sofosbuvir) | Fatigue, headache |
| Voxilaprevir + velpatasvir + voxilaprevir | Vosevi (Gilead) | Protease inhibitor (voxilaprevir), NS5A inhibitor (velpatasvir), NS5B inhibitor (sofosbuvir) | Headache, fatigue, GI symptoms |
| Glecaprevir + pibrentasvir | Mavyret (Gilead) | Protease inhibitor (glecaprevir), NS5A inhibitor (pibrentasvir) | Headache, fatigue |

[a]Note: all DAA HCV antiviral agents carry the warning that HBV reactivation has been reported in coinfected patients being treated for HCV but not HBV and can lead to fulminant hepatitis.
[b]Pharmaceutical companies: AbbVie, North Chicago, IL; Bristol-Meyers Squibb, Princeton, NJ; Genentech, San Francisco, CA; Gilead, Foster City, CA; Janssen Pharmaceutica, Beerse, Belgium; Merck & Co., Inc., Whitehouse Station, NJ; Roche Pharmaceuticals, Nutley, NJ.
[c]Ribavirin can cause anemia via hemolysis or decreased red cell production.
[d]GI (gastrointestinal) symptoms include nausea, vomiting, and diarrhea.

NS5A inhibitor (70). Ombitasvir, paritaprevir, and RTV are typically given with the nonnucleoside NS5B inhibitor dasabuvir. Paritaprevir is well absorbed when administered with food, has a 53% bioavailability, and is very highly protein bound. Its half-life is 5.5 hours, and it is primarily excreted in the feces. Renal insufficiency is not expected to clinically affect levels of paritaprevir, and no dose adjustment is warranted by mild hepatic impairment. Its use is contraindicated in moderate to severe (Child-Pugh classes B and C) hepatic impairment.

**Drug Interactions**

Paritaprevir is metabolized by CYP3A4 and to a lesser extent by CYP3A5 (71). Common drugs that should not be coadministered are anticonvulsants, rifampin, St. John's wort, certain oral contraceptives, and salmeterol. Close monitoring with statins, cyclosporine, tacrolimus, and antiarrhythmics is warranted.

### Simeprevir

**Pharmacology**

Simeprevir was the first available second-generation PI, and it has been used in combination with sofosbuvir with or without RBV for chronic genotype 1 infection (72). It has a bioavailability of 62% with food and is very highly protein bound. Its half-life is 41 hours, and it is primarily excreted in the feces. No dose adjustment is necessary with renal insufficiency, and its use is not recommended in patients with moderate or severe (Child-Pugh classes B and C) hepatic impairment.

**Drug Interactions**

Simeprevir undergoes oxidative metabolism by CYP3A4 and possibly CYP2C8 and CYP2C19 (72). Significant inducers or inhibitors of these enzymes will lead to alterations in simeprevir concentrations. Simeprevir in turn can affect the levels of other drugs by inhibiting OATP1B1/3.

Coadministration with RTV, HIV PIs, EFV, NVP, statins, St. John's wort, and cyclosporine among many others is not recommended. Simeprevir can be safely administered with tacrolimus or sirolimus.

### Voxilaprevir

**Pharmacology**

Voxilaprevir is a pangenotypic inhibitor of the NS3/4A protease that has been studied in combination with sofosbuvir and velpatasvir for previously treated patients without sustained virologic response (73). It is well absorbed and highly protein bound and is primarily excreted in the feces. No dosage adjustment is required for mild or moderate renal impairment, and use is not recommended in patients with moderate or severe hepatic impairment.

**Drug Interactions**

Voxilaprevir is metabolized through CYP3A4. Coadministration with phenytoin, phenobarbital, oxcarbazepine, rifabutin, rifapentine, ATV, LPV, TPV, EFV, and cyclosporine is not recommended (74).

### Glecaprevir

**Pharmacology**

Glecaprevir is a pangenotypic inhibitor of the NS3/4A protease that has been formulated in combination with the NS5A inhibitor pibrentasvir for previously treated patients without sustained virologic response (75). It is highly protein bound and is primarily excreted in feces. No dosage adjustment is required with mild, moderate, or severe renal impairment, including those on dialysis. It is not recommended in patients with moderate or severe hepatic impairment.

**Drug Interactions**

Glecaprevir is metabolized by CYP3A. Coadministration with digoxin, carbamazepine, rifampin, ethinyl estradiol-containing medications such as combined oral contraceptives, St. John's wort, ATV, DRV, LPV, RTV, HMG-CoA reductase inhibitors, and cyclosporine is not recommended (75).

## NS5A Inhibitors

The NS5A protein plays a role in hepatitis C viral replication and assembly (76, 77). However, the precise molecular mechanisms by which it accomplishes these functions remain poorly characterized. While NS5A inhibitors are effective across all genotypes, they have a low barrier to resistance and variable toxicity profiles. They have been shown to have very high SVR rates among patients with genotype 1 infection when given in combination with other direct-acting antivirals with or without RBV (78, 79). Available NS5A inhibitors are ledipasvir, ombitasvir, elbasvir, velpatasvir, and pibrentasvir, each available in fixed-dose combinations with other direct-acting antivirals, and daclatasvir.

### Ledipasvir

**Pharmacology**

Ledipasvir is coformulated with sofosbuvir and is administered with or without RBV depending on the patient population (78). It is well absorbed and is highly protein bound. Its half-life is 47 h, and it is primarily excreted in the feces. While ledipasvir needs no dose adjustment for severe renal insufficiency, its coformulated drug sofosbuvir accumulates with severe renal impairment. No dose adjustment is needed for mild or moderate renal insufficiency or in the setting of moderate or severe (Child Pugh class B or C) hepatic impairment.

**Drug Interactions**

Ledipasvir undergoes slow oxidative metabolism via an unknown mechanism and is a substrate of the P-glycoprotein drug transporter (78). Coadministration is not recommended with rifampin, St. John's wort, carbamazepine, phenytoin, phenobarbital, or TPV. Increased gastric pH levels may decrease its absorption. Acid-suppressing agents can be coadministered if needed, but low doses should be used.

### Ombitasvir

**Pharmacology**

Ombitasvir is only available as a fixed-dose coformulation with the PIs paritaprevir and RTV, which is typically given with the NS5B inhibitor dasabuvir (70). These drugs are administered with or without RBV depending on the patient population, for the treatment of chronic genotype 1 infection (80). Ombitasvir-paritaprevir-RTV coadministered with RBV but without dasabuvir is used for genotype 4 infections (81). Ombitasvir is well absorbed when administered with food and has a bioavailability of 48%. Its half-life is 21 to 25 h, and it is primarily excreted in the feces. Renal impairment is not expected to significantly alter its levels, but it has not been studied in patients with severe renal insufficiency. Its use is contraindicated for patients with moderate to severe (Child Pugh classes B and C) hepatic impairment.

**Drug Interactions**

Ombitasvir is metabolized by amide hydrolysis and oxidative metabolism (70). Because it is coformulated with paritaprevir and RTV, which are metabolized by CYP3A4 and CYP3A5, the fixed-dose combination has considerable drug interactions and should not be administered with anticonvulsants, rifampin, St. John's wort, certain oral contraceptives, or salmeterol.

### Elbasvir

**Pharmacology**

Elbasvir is only available as a fixed-dose combination with the PI grazoprevir (69). It is administered with or without RBV depending on certain patient characteristics (82). Prior to administration of this drug, patients with genotype 1a infection should be tested for the presence of NS5A resistance-associated substitutions. Elbasvir has a bioavailability of 32% and is highly protein bound. Its half-life is 24 h, and it is primarily excreted in the feces. It can be used in patients with any degree of renal impairment without the need for dose modifications. It is contraindicated in patients with Child-Pugh class B or C cirrhosis.

**Drug Interactions**

Elbasvir undergoes partial oxidative metabolism via CYP3A, just like its coformulated drug grazoprevir (69). Coadministration is contraindicated with rifampin, phenytoin, carbamazepine, St. John's wort, cyclosporine, and some antiretroviral agents such as PIs and EFV. Coadministration is not recommended with nafcillin, ketoconazole, etravirine, COBI, or modafinil.

### Velpatasvir

**Pharmacology**

Velpatasvir is a pangenotypic NS5A inhibitor that is only available as a fixed-dose combination with the

NS5B inhibitor sofosbuvir (83). It is highly protein bound and has a half-life of 15 h. It is primarily excreted in the feces. No dose adjustment is necessary for mild or moderate renal insufficiency or Child Pugh class B or C hepatic impairment. Preliminary studies suggest that severe renal impairment does not affect levels of velpatasvir. However, its coformulated drug sofosbuvir accumulates with renal impairment.

**Drug Interactions**

Velpatasvir is a substrate of the P-glycoprotein drug transporter and should not be coadministered with rifampin, rifabutin, rifapentine, St. John's wort, carbamazepine, phenytoin, phenobarbital, oxcarbazepine, or TPV (83). Increased gastric pH levels may decrease its absorption. If acid-suppressing agents need to be used, only low doses of proton pump inhibitors should be given, and velpatasvir should be administered without food.

### Pibrentasvir

**Pharmacology**

Pibrentasvir is an NS5A inhibitor that is formulated in combination with glecaprevir for previously treated patients without sustained virologic response (75). It is highly protein bound and is primarily excreted in the feces. No dosage adjustment is required with mild, moderate, or severe renal impairment, including those on dialysis. It is not recommended in patients with moderate or severe hepatic impairment.

**Drug Interactions**

Pibrentasvir is not extensively metabolized and is excreted unchanged in the feces (75). Since it is coformulated with glecaprevir, which is metabolized by CYP3A, coadministration with digoxin, carbamazepine, rifampin, ethinyl estradiol-containing medications such as combined oral contraceptives, St. John's wort, ATV, DRV, LPV, RTV, HMG-CoA reductase inhibitors, and cyclosporine is not recommended.

### Daclatasvir

**Pharmacology**

Daclatasvir is typically used in combination with sofosbuvir (79). It is 67% bioavailable and is highly protein bound. Its half-life is 12 to 15 h, and it is primarily excreted in the feces. No dosage adjustments are required for renal or hepatic impairment.

**Drug Interactions**

Daclatasvir is primarily metabolized by CYP3A4 and should not be given with strong inducers or inhibitors of this enzyme (84). Coadministration is not recommended with rifampin, phenytoin, carbamazepine, or St. John's wort. Daclatasvir is also an inhibitor of P-glycoprotein transporter and organic anion transporting polypeptide 1B1 and 1B3. Dose adjustments of digoxin may be warranted when it is coadministered with daclatasvir.

## NS5B RNA-Dependent RNA Polymerase Inhibitors

NS5B is an RNA-dependent RNA polymerase that is also involved in posttranslational processing. Its structure is highly conserved across all hepatitis C virus genotypes, giving NS5B inhibitors activity against all six genotypes (67). There are two kinds of polymerase inhibitors: nucleoside/nucleotide analogues such as sofosbuvir and nonnucleoside analogues such as dasabuvir. Nucleoside/nucleotide analogues are activated within the hepatocyte through phosphorylation which competes with nucleotides, resulting in chain termination during RNA replication. As a class, nucleoside polymerase inhibitors have moderate to high efficacy against all six genotypes and a very high barrier to resistance. In contrast, nonnucleoside polymerase inhibitors are less potent and more genotype specific, with all drugs from this class having been optimized for genotype 1.

### Sofosbuvir

**Pharmacology**

Sofosbuvir is the first NS5B nucleoside polymerase inhibitor to have been developed, and it is used in various combinations with other direct-acting antivirals against hepatitis C (85, 86). It can be taken without regard to food and has a half-life of 0.4 h. It is primarily excreted in the urine. No dose adjustment is necessary for patients with a glomerular filtration rate greater than 30 ml/minute. Sofosbuvir exposure is increased in patients with severe renal impairment, including patients on dialysis. It can be used without regard to hepatic impairment.

**Drug Interactions**

Sofosbuvir is a substrate of the P-glycoprotein drug transporter, so inducers of these enzymes may decrease sofosbuvir levels (85). Coadministration is not recommended with rifampin, rifabutin, rifapentine, St. John's wort, carbamazepine, phenytoin, phenobarbital, oxcarbazepine, or TPV. Coadministering sofosbuvir and amiodarone is also not recommended, because of reports of symptomatic bradycardia and fatal cardiac arrest.

### Dasabuvir

**Pharmacology**

Dasabuvir is packaged with ombitasvir-paritaprevir-RTV (70, 71). It is 70% bioavailable and very highly protein bound. Its half-life is 6 hours, and it is excreted primarily in the feces. Because of its lower potency and higher threshold for resistance, it is used as an adjunct to more potent direct-acting antivirals.

**Drug Interactions**

Dasabuvir is metabolized by CYP2C8 and CYP3A. It should not be coadministered with anticonvulsants, rifampin, St. John's wort, ethinyl estradiol-containing products, or salmeterol.

### Ribavirin

**Pharmacology**

The bioavailability of ribavirin (RBV) is reported to be 52% (87) and is increased by a high-fat meal; therefore, RBV should be administered with food. The half-life in plasma is 120 to 170 h, and the drug may persist in other body compartments for up to 6 months. The pathway for elimination has not been determined. RBV appears not to be a substrate for the CYP450 isoenzymes. It is used as a standard therapy, always in combination with other antiviral agents, for the treatment of HCV. RBV monotherapy is not effective against HCV infection.

RBV has been used as a monotherapy to treat other RNA viruses, including respiratory syncytial virus, Lassa fever virus, influenza virus, parainfluenza virus, and hantavirus; however, there are no conclusive data demonstrating RBV treatment efficacy (88–95). An aerosolized formulation of RBV (Virazole; Valeant Pharmaceuticals, Costa

Mesa, CA) has been approved for the treatment of hospitalized infants and young children with severe respiratory syncytial virus lower respiratory tract infections.

**Drug Interactions**

Coadministration of ddI or d4T with RBV is contraindicated. ZDV plus RBV is linked to higher rates of anemia (5, 96).

## AGENTS AGAINST HEPATITIS B VIRUS

Because a large percentage of patients are coinfected with HIV, agents with activity against HBV are categorized according to whether they have activity against both viruses or only HBV. Of the drugs that are specifically approved for HBV, only telbivudine (LdT) is active against HBV, while 3TC, TDF, TAF, adefovir (ADV), and entecavir (ETV) are active against both HBV and HIV (Table 3). Though neither ADV nor ETV is currently recommended for the treatment of HIV, use of these agents should be avoided in HIV/HBV-coinfected patients who are not on a suppressive antiretroviral regimen in order to prevent the development of HIV resistance. FTC is approved only for HIV, but it has been shown to have activity against HBV (97, 98). The common target for antiviral drugs active against both viruses is the RT function of the HIV and HBV replication enzymes (99–101).

Chronic HBV infection plays an important role in the morbidity and mortality of HIV-infected patients (102). The strategy for selecting antiviral therapy regimens for coinfected patients is based on the need to treat one or both viruses. If only HIV requires treatment, drugs with activity against both HIV and HBV, such as 3TC or TDF, should be withheld. If only HBV needs to be treated, drugs without HIV activity, such as LdT, can be used. However, it is recommended that all patients with HIV and HBV coinfection be started on cART regardless of CD4 count, because this may slow the progression of liver disease (98). cART regimens with dual NRTIs and/or NtRTIs, such as TDF with 3TC or FTC, that suppress replication of both viruses are preferred (98, 102). 3TC monotherapy rapidly selects for HBV resistance (103); therefore, combination therapy with one NRTI (3TC or FTC) and one NtRTI (TDF or ADV) is required. Of note, there are eight HBV genotypes (A to H), each with certain geographic predilections (104). There is evidence that the genotype impacts interferon responsiveness. In particular, in the treatment of hepatitis B e antigen-positive chronic hepatitis B, greater rates of hepatitis B e antigen seroconversion have been observed for genotype A than for genotype D and for genotype B than for genotype C (105). A correlation between genotype and treatment response to other anti-HBV therapies has not been established.

### Nucleoside/Nucleotide Analogues

#### Adefovir Dipivoxil

**Pharmacology**

Adefovir (ADV)-dipivoxil is a diester prodrug that is converted to the active drug ADV. ADV-dipivoxil is administered without regard to food, and the bioavailability is 59%. The half-life of ADV is 7.5 h, and it is excreted by the kidneys. There are no data for placental passage of the drug.

ADV was originally developed as an antiretroviral drug; however, the high dose required for HIV therapy is associated with nephrotoxicity (101). A much lower dosage is effective against HBV (99, 102). ADV is effective for treatment of chronic HBV infection. The rate of viral load decline is slower, but development of drug resistance is delayed compared to that seen with other NRTIs and NtRTIs (106) that are active against HBV. The primary

**TABLE 3** Antiviral agents for HBV therapy[a]

| Antiviral agent (abbreviation) | Trade name (pharmaceutical company[b]) | Mechanism of action/route of administration | Major adverse effects |
|---|---|---|---|
| Adefovir dipovoxil (ADV) | Hepsera (Gilead) | Prodrug converted to the nucleotide monophosphate analogue of adenosine<br>Inhibitor of HBV RT DNA polymerase, viral DNA chain terminator<br>Administered orally | Headache, asthenia, GI symptoms, nephrotoxicity |
| Entecavir (ETV) | Baraclude (BMS) | Guanosine analogue inhibitor of HBV RT DNA polymerase functions: priming, reverse transcription, positive-strand DNA synthesis<br>Administered orally | Headache, fatigue, dizziness |
| Lamivudine (3TC) | See HIV antivirals, Table 1 | Cytidine analogue inhibitor of HBV RT DNA polymerase | Minimal toxicity (see HIV antivirals) |
| Telbivudine (LdT) | Tyzeka (Novartis) | Thymidine analogue inhibitor of HBV RT DNA polymerase | Fatigue, increased creatine kinase, headache, myopathy, cough, GI symptoms |
| Tenofovir (TDF/TAF) | See HIV antivirals, Table 1 | Inhibitor of HBV DNA polymerase | Asthenia, headache, GI symptoms (see HIV antivirals) |

[a]Note: Except for interferon, all HBV antiviral agents are N(t)RTIs, some of which have anti-HIV activity (see Table 1), and all carry the warning of lactic acidosis and severe hepatomegaly with steatosis. All agents also carry a warning of severe acute exacerbations (flares) of HBV in patients who have discontinued anti-HBV therapy.

[b]Pharmaceutical companies: BMS, Bristol-Meyers Squibb, Princeton, NJ; Gilead Sciences, Foster City, CA; Novartis Pharmaceuticals Corporation, East Hanover, NJ.

role of ADV is in the treatment of 3TC-resistant HBV infection (107). However, TDF and entecavir have largely replaced ADV for this indication.

**Drug Interactions**

ADV is not a substrate, inhibitor, or inducer of any of the CYP450 isoenzymes. There is no interaction with 3TC, ETV, or TDF. It is possible that drugs that reduce renal function or compete for active tubular secretion could increase the concentration of ADV and/or the coadministered drug in serum.

### Emtricitabine

**Pharmacology**

See the discussion on HIV antiviral agents above and Table 1. Emtricitabine (FTC) is approved for antiviral therapy in HIV-infected patients. It has activity against HBV but is not licensed for HBV antiviral therapy. FTC and 3TC are biochemically similar and appear to be interchangeable for potential use in treatment of HIV-HBV-coinfected patients. However, they also share the same HBV resistance mutations; therefore, combined therapy with these two drugs is not recommended (101, 102). In addition, as with 3TC, severe acute exacerbations of HBV can occur once therapy is discontinued (98).

**Drug Interactions**

See the discussion on HIV antiviral agents above.

### Entecavir

**Pharmacology**

The bioavailability of entecavir (ETV) is approximately 100%, and the half-life is 24 h. ETV should be administered without food. It is excreted by the kidney (62 to 73%) mainly as unmetabolized drug. ETV maintains activity against 3TC-resistant HBV, but a higher dose is recommended for patients with 3TC-resistant HBV infection (102). ETV has shown low activity against HIV; however, there is evidence that resistance mutations are selected. For this reason, it is recommended that ETV be used in HIV-coinfected patients only if they are receiving effective antiretroviral therapy (99, 102, 108).

**Drug Interactions**

ETV is not a substrate, inhibitor, or inducer of any of the CYP450 isoenzymes. There is no interaction with 3TC, ADV, or TDF. It is possible that drugs that reduce renal function or compete for active tubular secretion could increase the concentration of ETV and/or the coadministered drug in serum.

### Lamivudine

**Pharmacology**

See the discussion on HIV antiviral agents above as well as Table 1. Lamivudine (3TC) was the first nucleoside analogue that was approved for chronic HBV infection. Because it has activity against both HIV and HBV, it has been effective in reducing loads of both viruses in plasma as part of cART regimens. However, HBV-specific drug resistance mutations are selected over long-term therapy at a higher rate (20% per year) in coinfected patients than in those that are HIV negative (90). Selection of HBV drug resistance mutations eventually decreases efficacy for treatment of chronic hepatitis. Discontinuation of 3TC in HBV-infected patients can produce severe flare-ups of hepatitis, which are usually self-limited but have been fatal in a few cases. Another common problem is the rebound viremia that occurs when therapy is terminated (98). This is thought to be derived from the viral covalently closed circular DNA (cccDNA), which is not affected by nucleoside or nucleotide therapy and remains in the infected hepatocytes (101). For coinfected patients, recent recommendations suggest using combination dual NTRI-NtRTI therapy that includes TDF to reduce the rate of selection of HBV 3TC-resistant strains (109).

**Drug Interactions**

See the discussion on HIV antiviral agents above.

### Telbivudine

**Pharmacology**

The bioavailability of telbivudine (LdT) is 68%, and it can be administered with or without food. The half-life is 40 to 50 h, and the drug is excreted mainly by the kidneys. LdT has a relatively low genetic barrier to resistance; therefore, it is not recommended as a first-line drug for treatment of chronic HBV (110). Hepatitis exacerbations have been reported upon discontinuation of LdT.

**Drug Interactions**

LdT does not alter the pharmacokinetics of other nucleoside or nucleotide analogues used in the treatment of HBV (e.g., 3TC, ADV, or TDF). Coadministration with PEG-IFN 2a may be associated with increased risk of peripheral neuropathy (99).

### Tenofovir Disoproxil Fumarate and Tenofovir Alafenamide

**Pharmacology**

See the discussion on HIV antiviral agents above as well as Table 1. Tenofovir disoproxil fumarate (TDF) is approved for treatment of both HBV- and HIV-infected patients. It does not show cross-resistance with HBV 3TC-resistant mutants, and it appears to have a lower potential for selection of resistance mutations. For this reason, TDF and FTC or TDF and 3TC are recommended as dual-nucleoside backbones in therapeutic regimens to reduce the possibility of selection of HBV drug-resistant strains in coinfected patients who are on antiretroviral therapy (98, 99, 102, 109). TAF is another prodrug of tenofovir, which appears to be effective as TDF in treating hepatitis B but at a much lower dose (111).

**Drug Interactions**

See the discussion on HIV antiviral drugs above.

## AGENTS AGAINST HERPESVIRUSES

Most of the antiviral compounds that are approved to treat the eight human herpesviruses are nucleoside or nucleotide analogues, which inhibit DNA replication. Several of these compounds require phosphorylation by a virus-encoded enzyme as well as cellular kinases for activation. The ultimate target of most of these drugs is the viral DNA polymerase, although other enzymatic steps in DNA synthesis also may be inhibited (1). In addition to the nucleoside and nucleotide analogues, the antiherpesvirus compounds include a pyrophosphate analogue (foscarnet [FOS]) that targets the viral DNA polymerase directly and an entry inhibitor (docosanol). The structure, mode of action, route of administration, and adverse effects of each drug are summarized in Table 4.

**TABLE 4** Antiviral agents for herpesviruses

| Antiviral agent (abbreviation) | Trade name (pharmaceutical company[a]) | Mechanism of action/route of administration | Major adverse effects | Antiviral activity |
|---|---|---|---|---|
| Acyclovir (ACV) | Zovirax (GSK) | Converted to guanosine monophosphate by viral kinase<br>Converted to triphosphate by cellular kinases<br>DNA chain terminator<br>Oral or intravenous formulations | Minimal toxicity<br>GI symptoms,[b] headache, nephrotoxicity<br>Precipitation in renal tubules if maximum solubility exceeded | HSV-1, HSV-2, VZV |
| Valacyclovir (Val-ACV) | Valtrex (GSK) | L-Valyl ester prodrug of ACV with increased bioavailability<br>Activity same as ACV | GI symptoms, headache, dizziness, abdominal pain, nephrotoxicity, thrombotic thrombocytopenia, hemolytic uremic syndrome (high dosage) | HSV-1, HSV-2, VZV, HCMV[c] |
| Cidofovir (CDV) | Vistide (Gilead) | Cytidine nucleotide analogue<br>Converted to di- and triphosphate by cellular kinases<br>DNA chain terminator (2 successive molecules required); intravenous administration with probenecid | CDV: renal toxicity, decreased intraocular pressure, neutropenia, fever<br>Probenecid: headache, GI symptoms, rash | HCMV,[d] HSV-1, HSV-2, VZV |
| Foscarnet (FOS) | Foscavir (AstraZeneca) | Pyrophosphate analogue<br>Noncompetitive inhibitor of DNA polymerase pyrophosphate binding site<br>Intravenous formulation only | Renal impairment, fever, nausea, anemia, diarrhea, vomiting, headache, seizures, altered serum electrolytes | HCMV, HSV-1, HSV-2, EBV |
| Ganciclovir (GCV) | Cytovene (Roche) | Guanosine analogue<br>Converted to monophosphate by HCMV UL97 kinase or HSV or VZV TK<br>DNA chain terminator<br>Oral and intravenous formulations | Fever, neutropenia, anemia, thrombocytopenia, impaired renal function, diarrhea | HCMV,[e] HSV-1, HSV-2 |
| Valganciclovir (Val-GCV) | Valcyte (Roche) | Oral prodrug of GCV with increased bioavailability<br>Activity same as GCV | Diarrhea, neutropenia, nausea, headache, and anemia | HCMV[e] |
| Letermovir | Prevymis (Merck) | Inhibitor of HCMV terminase complex | Nausea, diarrhea, vomiting, peripheral edema, cough, headache, fatigue, abdominal pain | HCMV |
| Penciclovir (PCV) | Denavir (Novartis) | Guanosine analogue<br>Mode of action similar to ACV<br>Limited DNA chain elongation<br>Topical formulation only | Headache and application site reaction no different from placebo | HSV-1[f] |
| Famciclovir | Famvir (Novartis) | Oral prodrug of PCV<br>Mode of action same as PCV | Headache, GI symptoms, anorexia | HSV-1, HSV-2, VZV |
| Trifluridine | Viroptic (Monarch) | Mode of action not established, may inhibit viral DNA synthesis<br>Ophthalmic aqueous solution for topical use | Burning on instillation and palpebral edema, punctate keratopathy, hypersensitivity reaction, stromal edema, keratitis sicca, hyperemia, increased ocular pressure | HSV-1[g] |
| Docosanol | Abreva (GSK) | Prevents HSV entry into cells by inhibition of fusion between HSV envelope and cell membrane<br>Nonprescription topical cream formulation | Headache and skin rash | Oral HSV |

[a]Pharmaceutical companies: AstraZeneca, Wilmington, DE; BMS, Gilead Sciences, Foster City, CA; GSK, GlaxoSmithKline, Research Triangle Park, NC; Monarch Pharmaceutical, Bristol, TN; Novartis, East Hanover, NJ; Roche Pharmaceuticals, Nutley, NJ.

[b]GI (gastrointestinal) symptoms include nausea, vomiting, and diarrhea.

[c]Valacyclovir is used in some transplant settings for HCMV prophylaxis.

[d]Cidofovir also has reported activity against human papillomavirus, polyomavirus, adenovirus, and poxvirus.

[e]Ganciclovir and valganciclovir also have *in vitro* activity against EBV, HHV-6, HHV-7, and HHV-8.

[f]Penciclovir is used to treat herpes labialis but also has activity against HSV2 and VZV.

[g]Trifluridine is used to treat herpes keratitis but also has activity against HSV2 and VZV.

## Acyclovir and Valacyclovir

### Pharmacology

The pharmacokinetics of acyclovir (ACV) after oral administration has been evaluated in healthy volunteers and in immunocompromised patients with herpes simplex virus (HSV) and varicella-zoster virus (VZV) infection. The plasma protein binding for valganciclovir (val-ACV) is 13.5 to 17.9% and for ACV is 22 to 33%. The bioavailability of ACV administered as val-ACV is 54%, while the bioavailability resulting from oral ACV is 12 to 20%. The ACV half-life is 2.5 to 3.3 h in patients with normal renal function but increases to 14 h in patients with end-stage renal disease (112). ACV may be administered with or without food. Valacyclovir (val-ACV), the L-valyl ester prodrug, is rapidly converted to ACV after oral administration (112). ACV is phosphorylated by the viral thymidine kinases of HSV-1, HSV-2, and VZV and by the UL97 kinase of human cytomegalovirus (HCMV) (113).

ACV is excreted by the kidney with inactive metabolites 9-[(carboxymethoxy) methyl] guanine and 8-hydroxy-9-[2-(hydroxyethoxy)methyl] guanine. A dosage adjustment is recommended for patients with reduced renal function (112).

### Spectrum of Activity

ACV and val-ACV are active against HSV-1, HSV-2, VZV, and Epstein-Barr virus (EBV) (4). Of note, ACV and val-ACV are active only against replicating virus. Therefore, their role in the treatment of EBV-associated disease processes, which are primarily driven by latent virus, is limited (114). In addition, both drugs have some activity against HCMV. Although ACV and val-ACV are not recommended for HCMV treatment, they have been used prophylactically to prevent HCMV disease in some patients following transplantation (115, 116).

### Drug Interactions

There are no clinically significant drug-drug interactions in patients with normal renal function.

## Cidofovir

### Pharmacology

Cidofovir (CDV) is a nucleotide analogue of deoxycytidine monophosphate, which does not require a virus-encoded enzyme for activation. After phosphorylation by cellular kinases, CDV diphosphate becomes the active nucleotide triphosphate, which inhibits the HCMV DNA polymerase. In HCMV, two successive CDV molecules must be incorporated for complete chain termination (117).

CDV must be administered with probenecid (118, 119). Approximately 90% of the CDV dose administered is recovered unchanged in the urine within 24 hours. The half-life is 2.4 to 3.2 h. When CDV is administered with probenecid, the renal clearance of CDV is reduced to a level consistent with creatinine clearance, suggesting that probenecid blocks active renal tubular secretion of CDV (118). *In vitro*, CDV is less than 6% bound to plasma or serum proteins.

### Spectrum of Activity

CDV is active against several herpesviruses, including HCMV, HSV, and VZV (4). CDV also has antiviral activity against poxviruses (120), adenovirus (121), polyomaviruses (122), and human papillomavirus (123, 124).

### Drug Interactions

No clinically significant interactions have been identified for CDV. However, the required administration of probenecid with CDV may produce drug-drug interactions resulting from the potential block of acidic drug transport in the kidney (118).

## Foscarnet

### Pharmacology

Pharmacokinetic data indicate that foscarnet (FOS) undergoes negligible metabolism, appears to be distributed widely by the circulation, and is eliminated via the renal route. The available data, however, indicate that the pharmacokinetics of the drug varies among patients and within the individual patient, particularly with regard to plasma FOS levels (125). The FOS terminal half-life determined by urinary excretion is 87.5 ± 41.8 h, possibly due to release of FOS from bone (126). Approximately 90% of FOS is excreted as unchanged drug in urine. Systemic clearance of FOS decreases and half-life increases with diminishing renal function, which may require FOS dosage modification (127).

### Spectrum of Activity

Although FOS is active against several herpesviruses, including HSV, HCMV, VZV, and EBV, it is most commonly used to treat drug-resistant HSV and HCMV.

### Drug Interactions

Because FOS is reported to decrease calcium concentrations in serum, caution is advised for patients receiving agents known to affect calcium levels in serum such as intravenous pentamidine. Renal impairment is a major adverse effect of FOS; therefore, the use of FOS in combination with other potentially nephrotoxic drugs such as aminoglycosides and amphotericin B (128) should be avoided.

## Ganciclovir and Valganciclovir

### Pharmacology

Ganciclovir (GCV) is an acyclic nucleoside analogue of 2′-deoxyguanosine, which requires phosphorylation by a viral kinase to become active. GCV monophosphate is subsequently phosphorylated to the di- and triphosphate forms by cellular kinases (1, 4).

val-GCV, the L-valyl ester prodrug of GCV, is rapidly converted to GCV after oral administration (129). Val-GCV should be administered with food. The bioavailability of val-GCV is 60.9% compared to 5.6% for the oral formulation of GCV. The half-life of GCV is 4 h in healthy volunteers and 6.5 h in transplant recipients (130, 131). GCV is only 1 to 2% protein bound. Renal excretion of unchanged drug by glomerular filtration and active tubular secretion is the major route of elimination (91%).

### Spectrum of Activity

GCV is active against HCMV as well as HSV-1, HSV-2, VZV, EBV, HHV-6, HHV-7, and HHV-8 (132–135).

### Drug Interactions

Coadministration of GCV with ddI results in significantly increased levels of ddI (133). Coadministration of GCV with ZDV requires dose modifications of both drugs because of their common adverse hematological effects of neutropenia and anemia. Dosage modifications may also be required with drugs that inhibit renal tubular secretion, such as probenecid. Imipenem-cilastatin should not be administered with GCV (133).

### Letermovir

**Pharmacology**

Letermovir prevents HCMV replication by inhibiting the terminase complex (pUL51, pUL56, pUL89), resulting in an inability to cleave concatemeric genomic viral DNA and package genomes into preformed virus capsids. It is orally bioavailable and has a half-life of 12 hours. It is primarily excreted in the feces. In a phase 3, double-blind trial of HCMV-seropositive hematopoietic stem cell transplant recipients, the efficacy of letermovir in preventing active HCMV infection was compared to placebo through week 24 after transplant (152). The trial found that 37.5% of patients on letermovir developed active CMV infection versus 60.6% of patients on placebo. Most cases of active HCMV infection were asymptomatic DNAemia. HCMV disease was rare. Adverse events were similar in the two groups, and myelotoxic and nephrotoxic events were similar. The results of this study led to FDA approval for letermovir in 2017.

**Spectrum of Activity**

Letermovir is active only against HCMV and does not have activity against other herpesviruses, including HSV and VZV.

**Drug Interactions**

Letermovir is highly protein bound and metabolized in the liver. It is a P-glycoprotein and CYP3A4 inhibitor and can increase serum concentrations of amlodipine, atorvastatin, cilostazol, cyclosporine, and ibrutinib, among others.

### Penciclovir and Famciclovir

**Pharmacology**

Famciclovir is the oral prodrug diacetyl 6-deoxy analogue of penciclovir (PCV) (136), which undergoes rapid conversion to the active compound, PCV. Famciclovir was developed to improve the bioavailability of the parent compound (137). PCV is available only as a 1% cream for the topical treatment of herpes labialis (138). The bioavailability of PCV is 77%, and the half-life is 2 h. It can be given with or without food. PCV is <20% protein bound and is eliminated in the urine (73%) and feces (27%) (139). Although PCV is structurally related to ACV, it has a higher affinity for the HSV thymidine kinases than ACV. However, ACV triphosphate has a higher affinity for the HSV DNA polymerase than does PCV triphosphate. As a result, the two compounds have similar anti-HSV potencies (140).

**Spectrum of Activity**

PCV and famciclovir are active against HSV-1, HSV-2, and VZV (141). Neither of these compounds is active against other human herpesviruses.

**Drug Interactions**

No clinically significant drug interactions have been identified for PCV.

### Trifluridine

**Pharmacology**

Trifluridine is a fluorinated pyrimidine nucleoside approved for the topical treatment of epithelial keratitis caused by HSV (142). It has activity against HSV-1, HSV-2, and vaccinia virus (143). Intraocular penetration of trifluridine occurs after topical instillation into the eye. Decreased corneal integrity or stromal or uveal inflammation may enhance the penetration of trifluridine into the aqueous humor. Systemic absorption following therapeutic dosing with trifluridine appears to be negligible (144).

**Drug Interactions**

There are no reported drug interactions by the topical route of administration.

### *n*-Docosanol

**Pharmacology**

*n*-Docosanol exhibits *in vitro* antiviral activity against several lipid-enveloped viruses including HSV-1, HSV-2, and respiratory syncytial virus (145). A topical preparation of *n*-docosanol is available without prescription as a 10% cream for the treatment of herpes labialis.

**Drug Interactions**

There are no reported drug interactions with topical administration.

### Other Drugs against Herpesviruses

There are several antiviral agents that are undergoing clinical trials or that are approved for conditions other than antiviral therapy. Maribavir is an antiviral agent in the benzimidazole drug class (146). Maribavir is not phosphorylated by the UL97 kinase but inhibits UL97 kinase activity directly. It has been found to be effective *in vitro* against GCV-resistant strains of HCMV, with taste disturbances as the only adverse side effect (147). Unfortunately, phase 3 clinical trials in stem cell and liver transplant recipients found maribavir to be inadequate for prevention of CMV disease (148, 149). However, new clinical trials have been launched to address speculation that the lack of demonstrable efficacy may be due to inadequate dosing (https://clinicaltrials.gov; NCT02927067 and NCT02931539).

Brincidofovir is an orally administered lipid conjugate of CDV (150). It has *in vitro* activity against all of the herpesviruses, including GCV-resistant CMV and ACV-resistant HSV, as well as polyomaviruses, poxviruses, and adenovirus (151). However, severe diarrhea and increased mortality led to the failure of a phase III trial (https://clinicaltrials.gov; NCT01769170). New formulations may lead to future trials to pursue the treatment of targeted patients.

Two helicase/primase inhibitors, pritelivir and amenamevir, have shown efficacy in phase II studies (153, 154). Pritelivir and amenamevir show *in vitro* activity against HSV-1 and HSV-2, while amenamevir also shows activity against VZV.

Two additional drugs that are approved for other medical conditions have been reported to have antiviral activity against HCMV, although no clinical trials have been conducted. These are leflunomide, which is approved for treatment of rheumatoid arthritis (155, 156), and artesunate, which is an antimalarial agent (157, 158).

## AGENTS AGAINST INFLUENZA VIRUSES

The two classes of antiviral agents for the treatment of influenza are M2 protein inhibitors and neuraminidase inhibitors (159–161). The structure, mode of action, route of administration, and adverse effects of each drug are summarized in Table 5. Recommendations for the use of these antivirals for influenza prevention and therapy are available from the Centers for Disease Control and Prevention website (http://www.cdc.gov/flu).

**TABLE 5** Antiviral agents for influenza virus

| Antiviral agent | Trade name (pharmaceutical company[a]) | Mechanism of action/ route of administration | Major adverse effects |
|---|---|---|---|
| Amantadine/ rimantadine | Symmetrel/Flumadine (Endo/Forrest) | Prevents release of nucleic acid by interfering with viral M2 protein<br>Administered orally | CNS symptoms,[b] GI symptoms[c] |
| Oseltamivir | Tamiflu (Genentech) (Gilead [licensor]) | Sialic acid analogue<br>Competitive inhibitor of neuraminidase affecting release of influenza virus particles from host cells<br>Administered orally | GI symptoms[c] (usually mild), transient neuropsychiatric symptoms[d] |
| Peramivir | Rapivab (Biocryst) | Same as oseltamivir<br>Administered intravenously | GI symptoms[c], leukopenia/ neutropenia |
| Zanamivir | Relenza (GSK) | Same as oseltamivir<br>Administered by oral inhalation | Respiratory function deterioration after inhalation |

[a]Pharmaceutical companies: Biocryst Pharmaceuticals, Durham, NC; Endo Pharmaceuticals, Inc., Chadds Ford, PA; Forrest Laboratories, Inc., St. Louis, MO; Licensor: Gilead Sciences, Inc., Foster City, CA; GSK, GlaxoSmithKline, Research Triangle Park, NC.
[b]CNS symptoms include confusion, anxiety, insomnia, difficulty concentrating, dizziness, hallucinations, and seizures.
[c]GI (gastrointestinal) symptoms include nausea, vomiting, and anorexia.
[d]Neuropsychiatric symptoms include self-injury and delirium.

## M2 Protein Inhibitors

The virus-encoded M2 protein facilitates the hydrogen ion-mediated dissociation of the matrix protein-ribonucleoprotein complex within the endosome and the release of the viral ribonucleoprotein into the cytoplasm of the host cell. The M2 inhibitors block the passage of $H^+$ ions through the M2 ion channel, which prevents uncoating of the virus (1, 162, 163).

### Amantadine and Rimantadine

The adamantanes differ in their metabolism and adverse effects, but they have similar antiviral activity against influenza A viruses. Neither drug has activity against influenza B viruses. Recent reports indicate that both the seasonal influenza virus, H3N2, and the current pandemic virus, H1N1, have a high incidence of resistance to both drugs (1, 164, 165); therefore, the adamantanes are no longer recommended for influenza prophylaxis and empiric therapy.

## Neuraminidase Inhibitors

The influenza virus neuraminidase is an envelope glycoprotein that cleaves the terminal sialic residues, releasing the virion from the infected cell. The virus-encoded neuraminidase allows the influenza virus to spread from cell to cell. Three neuraminidase inhibitors are approved for the treatment of influenza A and B viruses: oseltamivir, zanamivir, and peramivir (166, 167). Of these, oseltamivir is the most widely used. In 2007 to 2008, a high percentage of seasonal H1N1 influenza virus isolates were resistant to oseltamivir as the result of a single amino acid substitution, but they remained sensitive to zanamivir (164, 168). However, for the 2013–2014 season the CDC reported that 98.2% of the 2009 H1N1 pandemic virus strains were susceptible to oseltamivir and 100% were susceptible to zanamivir (https://www.cdc.gov/flu/about/qa/antiviralresistance.htm).

### Oseltamivir

#### Pharmacology

Oseltamivir phosphate is an ethyl ester prodrug that requires ester hydrolysis for conversion to the active form, oseltamivir carboxylate. After oral administration, oseltamivir phosphate is readily absorbed from the gastrointestinal tract and is extensively converted to oseltamivir carboxylate, predominantly by hepatic esterases (169). At least 75% of an oral dose reaches the systemic circulation as oseltamivir carboxylate. The binding of oseltamivir carboxylate to plasma protein is low. The plasma half-life is 6 to 10 h. There are fewer side effects if administered with food. Oseltamivir carboxylate is not further metabolized and is eliminated in the urine (170). The efficacy of oseltamivir in preventing naturally occurring influenza illness has been demonstrated in treatment and prophylaxis studies (171–173).

#### Drug Interactions

Studies of oseltamivir suggest that clinically significant drug interactions are unlikely, because neither the drug nor the metabolite oseltamivir carboxylate is a substrate for the CYP450 isoenzymes or for glucuronyltransferases. The potential exists for interaction with other agents such as probenecid that are excreted in the urine by the same pathways (170). Oseltamivir should not be administered in a time period 2 weeks before and 48 h after administration of live influenza vaccine.

### Peramivir

#### Pharmacology

Peramivir was approved in late 2014 for the treatment of uncomplicated influenza. Peramivir has poor oral bioavailability and is therefore only available as an intravenous formulation that is administered as a single dose. It is primarily eliminated by the kidneys (174).

#### Drug Interactions

There are no significant drug interactions (174).

### Zanamivir

Zanamivir treatment has been shown to reduce the severity and duration of naturally occurring, uncomplicated influenza illness in adults (175). Zanamivir is administered only to the respiratory tract by oral inhalation using a blister pack (176). The contents of each blister are inhaled using a specially designed breath-activated plastic device for the inhaling powder. This route rapidly provides high local concentrations at the site of delivery. Because of the respiratory

route of administration, zanamivir is contraindicated in patients with underlying airway disease such as asthma. As noted above, the H1N1 strains that have become resistant to oseltamivir remain sensitive to zanamivir.

### Pharmacology

The absolute oral bioavailability of zanamivir is low, averaging 2%. After intranasal or oral inhaled administration, a median of 10 to 20% of the dose is systemically absorbed, with maximum concentrations in serum generally reached within 1 to 2 hours. The remaining 70 to 80% is left in the oropharynx and is eliminated in the feces. The median serum half-life ranges between 2.5 and 5.5 hours, and the systemically absorbed drug is excreted unchanged in the urine. The low level of absorption of the drug after inhalation produces low concentrations in serum with only modest systemic zanamivir exposure (170).

### Drug Interactions

Zanamivir is not metabolized; therefore, there is a very low potential for drug-drug interaction (177).

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