Herpes Simplex Virus and Varicella-Zoster Virus

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ABSTRACT The most common specimens from immunocompromised patients that are analyzed for detection of herpes simplex virus (HSV) or varicella-zoster virus (VZV) are from skin lesions. Many types of assays are applicable to these samples, but some, such as virus isolation and direct immunofluorescent antibody testing, are useful only in the early phases of the lesions. In contrast, nucleic acid (NA) detection methods, which generally have superior sensitivity and specificity, can be applied to skin lesions at any stage of progression. NA methods are also the best choice, and sometimes the only choice, for detecting HSV or VZV in blood, cerebrospinal fluid, aqueous or vitreous humor, and from mucosal surfaces. NA methods provide the best performance when reliability and speed (within 24 hours) are considered together. They readily distinguish the type of HSV detected or the source of VZV detected (wild type or vaccine strain). Nucleic acid detection methods are constantly being improved with respect to speed and ease of performance. Broader applications are under study, such as the use of quantitative results of viral load for prognosis and to assess the efficacy of antiviral therapy.

HERPES SIMPLEX VIRUS

Description and Pathophysiology of Infection

Herpes simplex viruses (HSV) are enveloped large DNA viruses (approximately 152,250 to 154,750 base pairs, depending on HSV type, and 90 transcriptional units). HSVs are α-herpesviruses (family Herpesviridae) and are divided into three major clades. There are two HSV types (HSV 1 and 2) that are genetically distinct but that are colinear and share roughly 83% genomic homology (1). HSV from mucocutaneous lesions, or from asymptomatic shedding in the oral or genital secretions from an infected contact, enters the skin/mucosa of a new host through minor breaks or abrasions to infect the underlying epithelium. Infection at the dermal–epidermal junction produces characteristic skin lesions. These begin as macules and papules that culminate in vesicles that contain infectious virus (Fig. 1A). These form pustules that rupture after 2 days; the resulting ulcers and crusts form within 96 hours. Vesicles and pustules are most likely to contain infectious virus, whereas ulcers and crusts reliably contain HSV DNA. Local vesicle formation is the hallmark of HSV infection, but vesicles may not be appreciated on mucosal surfaces because they rupture shortly after forming (Fig. 1B). An essential characteristic of α-herpesviruses during primary infection is entry into a permanent latent relationship with sensory neurons, with the potential for subsequent reactivation to cause recurrent local infections (2).

The first infection with either type of HSV is considered a primary infection. A subsequent infection with the other HSV type is termed a nonprimary first-episode (or initial) infection with that new type (3). The clinical course of first-episode infections lasts 10 to 17 days, with fever, malaise, and headache sometimes present in the early phase. Nonprimary infections may be milder or...
truncated (4). HSV from the base of either primary or nonprimary first-episode lesions ascends in the sensory nerves that innervate the infected skin/mucosa to reach sensory ganglia, where it infects neurons and remains latent for life. In the first year after first-episode infection, approximately one-third of days are associated with reactivation of HSV in neurons (5). This HSV can travel retrograde in the sensory nerves to the area of skin/mucosa that was initially infected, and it either causes asymptomatic shedding (if at a mucosal surface) or may propagate in the skin/mucosa to cause new skin lesions similar to the initial infection, which is termed recurrent genital herpes or recurrent oral herpes infection (“cold sores; fever blisters”). Approximately 65% of reactivations are asymptomatic (6). Reactivation frequency declines with increasing interval after the first-episode infection. The outcome of reactivated latent HSV may be determined by the HSV-specific T-cell responses in the vicinity of the termini of axons where the reactivated HSV emerges (7). The clinical course of recurrent HSV is shorter than primary infection, lasting less than one week, and consists of fewer, smaller, and less painful lesions than the initial infection (4) (Fig. 1C). Systemic symptoms are rare with recurrences.

**FIGURE 1** (A) First-episode HSV in an HIV-infected patient. (B) Genital HSV in a renal transplant recipient. (C) Typical recurrent HSV. (D) Atypical chronic recurrent HSV in a bone marrow transplant recipient.
Features of HSV Infection in Immunocompromised Patients

Disease spectrum

When immunocompromise is moderate, many patients have disease typical of the immunocompetent host. More severely immunocompromised patients often have local lesions that are atypically large and deep in the dermis; these may also heal slowly or fail to heal (8) (Fig. 1D). These chronic skin lesions can develop an atypical verrucose appearance.

Disruption of the tegument (as a consequence of intubation, nasogastric tube, and drug-induced oral ulceration) may result in tracheitis, esophagitis, and oral mucositis (9). Additional manifestations that occur in both immunocompetent and immunocompromised patients include meningitis (10) (usually with first-episode infection), encephalitis (11), keratitis, and retinal necrosis (12).

Host factors and subgroups

The common denominator of host factors resulting in complications is depression of T-cell-mediated immune responses. These host factors include: a) acquired defects in HSV-specific adaptive immunity (certain cancers, chemotherapy, steroids, biological modifiers, radiation, transplant antirejection therapy); b) congenital immune deficiency, including mutations in interferon and inflammatory signaling pathways that are a factor in some cases of pediatric HSV complications (13, 14); c) newborns, who can develop disseminated HSV or HSV encephalitis because they are slow to develop adequate T-cell adaptive responses (15); d) pregnancy, wherein disseminated HSV may develop as a consequence of the physiologic pregnancy-related depression in T-cell responses (16).

Morbidity and mortality

Significant morbidity occurs when painful atypical severe and chronic mucocutaneous lesions are present (17). HSV tracheitis, esophagitis, and mucositis can complicate care for severely immunocompromised patients (9). Dissemination with hepatitis and multiple organ involvement occurs but is a very rare complication of HSV. HSV encephalitis is less rare. HSV retinitis may destroy vision. Prognostic factors include: level of HSV-specific T-cell immunity prior to immunocompromise, extent and duration of immunocompromise at the time of HSV infection, dissemination and organ involvement, and delay in antiviral treatment.

Therapeutic considerations

Treatment is often instituted when the diagnosis is considered on clinical grounds, prior to confirmation. The decision to treat by the oral or intravenous route will depend on the severity and extent of immunocompromise. Topical antiviral drugs are not utilized as sole therapy, but serve as an adjunct for treating HSV keratitis, and some experts suggest that the addition of topical therapy may accelerate healing of skin lesions in immunocompromised patients. Duration of therapy is determined by the severity of the disease and the response to therapy. Immunocompromised patients have a significant chance of developing drug-resistant HSV infections, so careful observation of the response to treatment is essential, often with the assistance of laboratory methods.

Epidemiology

HSV in both immunocompromised and immunocompetent individuals is spread by close contact (kissing, heterosexual and homosexual intercourse, and oral-genital contact) with an index case. Most commonly HSV is acquired from an asymptomatic shedder of HSV, rather than by exposure to infected lesions. This is because asymptomatic shedding of HSV is very common from the mouth or genitals of a previously infected person (point prevalence of 10% or greater) (18). Recurrence frequency is greater in immunocompromised patients.

Goals of laboratory testing

Screening and prevention. Serologic screening to determine prior infection with HSV can be useful when extensive immunosuppression is planned, since seropositive patients will benefit from antiviral prophylaxis to suppress recurrent outbreaks. HSV serology may be useful in discordant couples to direct the use of condoms and other protective measures during pregnancy when the male partner is seropositive and the female is seronegative (19).

Diagnosis and prognosis. The clinical diagnosis of genital herpes has both poor sensitivity and specificity in immunocompetent individuals (20), and laboratory confirmation of HSV is especially important when the manifestations are atypical in immunocompromised patients. In addition, infection of visceral organs (liver, lung, brain) may only be apparent by detecting HSV in blood, cerebrospinal fluids (CSF) and tissue biopsies, especially when this occurs in the absence of skin lesions. Accurate diagnosis of intraocular infection requires confirmation by identifying HSV in corneal swabs from keratitis or in aqueous or vitreous aspirates for necrotizing retinitis (21). Isolation of HSV is required for antiviral drug sensitivity testing. Typing of an isolate has prognostic value, since in the immunocompetent host,
HSV 1 in the genital area reactivates much less frequently than HSV 2 (22, 23). Congenital infection with HSV 1 is associated with a higher risk of systemic dissemination and death compared to HSV 2 (24).

Treatment and monitoring. An etiologic diagnosis is essential to optimize the use of antiviral therapy for a sick newborn, evaluating vaginal lesions during the peripartum period, and evaluation for encephalitis, and to determine the etiology of esophagitis, tracheitis, oral mucositis, anal ulceration, necrotizing retinitis, and disseminated infection in the absence of characteristic skin lesions in an immunocompromised patient. Any unexplained skin lesion or mucositis (even in patients receiving chemotherapy) should be tested for the presence of HSV. Sequential testing for HSV or HSV DNA in blood, CSF, or intraocular fluid can provide early clues as to the extent of the infection (25–27), and adequacy of antiviral therapy as determined by the failure to clear HSV due to antiviral drug resistance (25–27). This is also true of the persistence of HSV in skin lesions for extended periods (28, 29).

**Laboratory testing options, applications, interpretation**

**Serology**

HSV serology is used to identify patients previously infected with HSV. This will guide antiviral prophylaxis against recurrent HSV in severely immunocompromised patients and also could be used to direct prophylaxis in discordant couples (30). Differentiating HSV 1 and 2 past infections by serology may be challenging but can be reliably accomplished by Western blot in research laboratories (31). Among the FDA-approved HSV serology tests, HerpeSelect immunoblot and the enzyme-linked immunosorbent assay (ELISA) kits manufactured by Focus Diagnostics have sensitivity and specificity ≥90% (32, 33). The kits use the antigenic differences in glycoprotein G (gG) between HSV 1 and HSV 2 to discriminate between antibodies against the two viruses. gG-based multiplexed flow immunoassays have sensitivities and specificities similar to the gG-based ELISA and immunoblot, and they have the added advantage of a fully automated platform (34, 35). A rapid point-of-care test using gG2 without requiring special instrumentation is available. This has ≥90% sensitivity and specificity for the diagnosis of past HSV 2 infection (36). Other ELISA kits for detection of HSV 1 or HSV 2 IgG that use whole HSV antigen have high sensitivity but only 60 to 70% specificity (37).

Serology is infrequently used to diagnose acute HSV infection. Occasionally, when a single serum sample is available, recent infection can be diagnosed by detecting anti-HSV IgM and the absence of IgG anti-gG antibodies, which generally appear >1 month after primary infection (38). Potential problems when attempting the diagnosis of acute or recent HSV infection using IgM include: 1) a sensitivity of only 70%, 2) persistence for 2 to 6 months after the acute infection; 3) occasional detection during reactivations (39), and 4) problematic detection in immunocompromised patients who may have a limited immune response or may have received intravenous immune globulin. In addition, since IgM antibodies derive from naive B cells and produce antibodies that have not been refined through affinity maturation, these antibodies often lack specificity and may generate false-positive results.

The presence of anti-HSV antibodies in CSF or aqueous fluid was used before the advent of the polymerase chain reaction to diagnose HSV infection in the CNS and eye, respectively. Note that failure to detect HSV DNA does not necessarily rule out HSV etiology, particularly when the patient has already begun antiviral therapy. In such instances when CNS infection is a strong consideration, an alternative approach has been to determine serum-to-CSF HSV-specific IgG ratios. Specific antibody in CSF is normally at lower titer than in serum, but during an infection in the CNS, specific antibody is made at the infected site. Thus, the ratio of antibody in CNS to the titer in serum will be much higher than in uninfected patients. This is demonstrated by measuring antibody in CSF and serum obtained at the same time. This altered ratio can persist for up to 2 months (40). This approach might not work in patients who are not able to make antibody. It requires suitable controls to demonstrate that the antibodies in CSF are specific for HSV and to rule out contamination of the CSF sample with blood. Quantifying albumin, total IgG, or specific antibodies to a second virus (e.g., VZV) are suitable controls. However, while such methods have been used successfully, PCR is the preferred method of diagnosing HSV encephalitis or retinal necrosis (see below) (40, 41) (Table 1).

**Culture**

Prior to the introduction of PCR, conventional tube culture techniques were the gold standard for HSV diagnosis. They may require as long as 7 days (usually 2 to 5 days) to provide a result, which contrasts with a turnaround time of one day for shell vial cultures and potentially only a few hours for some PCR methods (42). In addition, tube tissue cultures use cytopathic effect as a readout, which requires a skilled technologist.
### Table 1: Laboratory methods for the diagnosis of HSV infection

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Sample processing</th>
<th>Turnaround time</th>
<th>Results and clinical utility</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td><strong>Nonmolecular methods</strong></td>
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<tr>
<td>Serology</td>
<td>Antibody detection</td>
<td>Can use either serum or plasma</td>
<td>1–5 days</td>
<td>HSV-IgG indicates prior infection</td>
<td>Identifies patients who are at risk for reactivation of HSV and might benefit from prophylactic antivirals</td>
<td>May be falsely negative in a severely immunocompromised patient</td>
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<td><strong>Virus cultures</strong></td>
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<tr>
<td>Tube culture</td>
<td>Viral growth</td>
<td>Specimen taken to the laboratory as soon as possible; avoid exposure to heat and organic solvents</td>
<td>1–5 days</td>
<td>Characteristic cytopathic effect; can confirm with fluorescein-conjugated monoclonal antibodies; type-specific antibodies will provide additional information</td>
<td>Can establish HSV as the causative virus Can determine the type of HSV The viral isolate can be tested for susceptibility to antiviral agents</td>
<td>Care required in obtaining and transporting specimen Processing time is longer than for other methods Sensitivity will vary with the type of skin lesion and for noncutaneous specimens Not as sensitive as molecular methods</td>
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<tr>
<td>Shell vial assay</td>
<td>Viral growth detected with immunocytochemistry; fluorescent microscopy</td>
<td>Care needed for specimen collection as above</td>
<td>16–24 hours</td>
<td>Infectious foci detected with fluorescein-conjugated monoclonal antibodies; can determine type at the same time</td>
<td>Specific for HSV and determining HSV type More sensitive and more rapid than conventional tube cultures</td>
<td>Care required in obtaining and transporting specimen Not as sensitive as molecular methods</td>
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<td><strong>Antigen detection</strong></td>
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<tr>
<td>Direct fluorescent antibody assay (DFA)</td>
<td>Fluorescent microscopy</td>
<td>Scraping of skin lesion; care must be taken in collecting sample; must collect lesion cells but avoid contaminating the sample with blood</td>
<td>3–6 hours</td>
<td>Detection of specific fluorescence establishes etiologic diagnosis</td>
<td>Rapid diagnosis Relatively inexpensive</td>
<td>Limited to use for skin lesions Sensitivity varies with quality of the sample submitted and with skill of the microscopist Generally less sensitive than culture or nucleic acid methods Cannot be used on healing skin lesions</td>
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<td><strong>Molecular methods: nucleic acid detection</strong></td>
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<tr>
<td>PCR</td>
<td>DNA detection</td>
<td>Tissue, swabs, aspirates, scabs, and body fluids Mucocutaneous lesions</td>
<td>1–2 days</td>
<td>Viral genomic copies per PCR input or Qualitative</td>
<td>Highly sensitive and specific Verify response to therapy</td>
<td>May be negative when treatment is initiated very early prior to testing Less sensitive than PCR</td>
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<td>Isothermal amplification</td>
<td>DNA detection</td>
<td>Mucocutaneous lesions</td>
<td>0.5–2 days</td>
<td>Qualitative</td>
<td>Rapid procedure More sensitive than culture Moderate complexity</td>
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</table>
The sensitivity of conventional tube tissue culture for HSV is only 60 to 80% compared with PCR (43). Shell vial cultures are combined with a genetically engineered virus-inducible cell system that produces color when infected with HSV1 or HSV2. This has a turnaround time of <24 h and lower technical complexity. Specificity approaches 100%, but sensitivity is ≤98% compared with conventional tube tissue cultures (43, 44). Similar sensitivity and specificity is attained with shell viral cultures that contain a monolayer of susceptible cells grown onto a thin cover slip, placed into a flat-bottom tube with medium, and inoculated with the clinical sample. Within 24 hours, the cover slip can be removed and virus detected using monoclonal fluorescence-tagged anti-HSV antibodies. Multiple studies using HSV culture-based methods demonstrated an average sensitivity of 70% among 14 studies (range was 25% and 89%) compared with the sensitivity achieved with PCR (43). The result of any culture-based method is greatly influenced by the duration and character of the lesions. Samples from vesicles are most likely to be culture positive, whereas samples of ulcerated lesions are less often positive, and scabs from crusted lesions contain no viable virus. In addition, swabbing the lesions with alcohol or other antiseptics may inactivate the virus. On the other hand, a viral isolate is required for culture-based testing of sensitivity to antiviral drugs, or to provide material for extensive genotypic analysis for antiviral resistance analysis and research. Until recently, culture-based methods for viral detection were more economical than molecular methods such as PCR, but automation and other efficiencies of PCR have narrowed the cost differential between viral culture and PCR for many viruses. HSV may be the last exception, because it grows rapidly and the cytopathic effect is readily identified. Although some molecular tests may provide same-day results, their cost is still much higher than that of shell vial culture (Table 1).

**Antigen-based assays**

Direct immunofluorescence assays (DFA) are available for HSV detection. Collecting a suitable specimen for antigen-based direct detection assays is crucial to achieving a reliable result. Samples from skin lesions must be collected aggressively enough to ensure the collection of infected cells, but not so aggressive that the sample is contaminated with blood. A large proportion of samples are inadequate for DFA analysis due to low number of cells (45). Cytospin has been proposed to mitigate this shortcoming (46). Overall, DFA is substantially less sensitive than PCR or viral culture with sensitivity as low as 50% compared with PCR and 70% compared with viral culture (47). Beyond the requirement for an adequate sample, successful performance of DFA requires an experienced microscopist capable of distinguishing between HSV-specific fluorescent labeling and background staining. False-negative results can also occur if the sample is contaminated with patient blood, a consequence of cold competition by host antibodies. Reliance on a single monoclonal detection antibody for DFA can be problematic, since the target epitope may be lost through random mutation. The detection antibody should be directed toward a major epitope of an abundantly produced viral protein in order to maximize sensitivity. For type-specific detection, the agent-specific antibodies must recognize an epitope that is not shared by HSV-1 and HSV-2. Direct antigen methods have the advantages of: 1) relative ease of performance; 2) providing a rapid result with same-day turnaround times routinely feasible, and 3) less cost than PCR. Some specimens are never suitable for this assay, such as blood, CSF, and aqueous or vitreous humors (Table 1).

**Morphological tests**

Giemsa-stained skin scrapings (“Tzanck smear”) and electron microscopy are discussed with the tests for varicella-zoster virus (below).

**Nucleic acid amplification-based assays**

HSV molecular assays, currently most prominently PCR methods, outperform both culture and direct antigen detection methods by a substantial margin, including primary infection, reactivation, or reinfecion. Methods include monoplex formats (48, 49), multiplex formats (multiple DNA targets in one assay) (50, 51), and real-time PCR (18, 51). Depending on the number of target DNAs included in the assay, multiplexing may result in diminished sensitivity due to limited availability of reagents during cycling. Nested PCR methods are not recommended for diagnostic uses; while they can increase sensitivity, specificity and accuracy may be compromised due to amplification of weakly analogous, unrelated sequences that can occur with high cycle numbers or by the increased potential for contamination. Loop-mediated isothermal amplification (LAMP) is an alternative method that has the advantage of not requiring a thermocycler or other expensive equipment. A LAMP method for HSV was described that was 100% specific compared with PCR, with sensitivity ranging from 78% to 96% on different clinical samples (52). The strengths of the method include high specificity, rapid performance, and moderate cost; limitations include sensitiv-
ity, the requirement to use multiple primers targeting 6 to 8 regions in the DNA, and the inability to perform follow-up procedures on the amplification products, e.g., cloning. Another nucleic acid amplification-based method that has been applied to the diagnosis of HSV infection is helicase-dependent amplification (HDA) (53). HDA is also an isothermal method that differs from LAMP in its reliance on the dissociation of the DNA strands and in its reliance on only a single primer pair, as with PCR. Evaluation of 307 clinical samples using PCR as a gold standard indicated that the HDA method was 98.3% specific and 100% sensitive. Strengths include high performance specifications and the ability to further evaluate the amplification products, e.g., for cloning or sequencing. Disadvantages include the relatively high cost of reagents (despite not requiring a thermocycler), that the technique is still primarily performed in research laboratories, and that high-throughput platforms are not yet available.

Analytical validation of HSV-specific molecular methods typically includes testing panels of quantified doses of purified HSV DNA to establish sensitivity, and testing of high-copy-number preparations of closely related herpesviruses and other pathogens to evaluate specificity. Clinical validation is done by applying the method to samples from cases of known etiology. PCR assays are expected to perform at 100% specificity and 90% or higher sensitivity. Real-time HSV PCR methods can reliably detect as few as 10 copies of HSV DNA in a sample (18). Although direct comparisons have not been published, multiplex real-time PCR is slightly less sensitive, detecting 10 to 100 copies reliably (51). Wald et al. showed that PCR was 4-fold more sensitive than virus culture when tested in more than 36,000 clinical specimens (18). The specificity of PCR is excellent, and carefully designed methods discriminate the DNA of closely related human herpesvirus species. A turn-around time of 1 to 2 days is readily achieved. Helicase-dependent amplification appears to have performance specifications comparable to PCR, and LAMP assay, while somewhat less sensitive than PCR, is considerably more reliable than culture.

In addition to excellent sensitivity and specificity, nucleic acid methods can detect HSV DNA in specimens that are generally unsuitable for antigen-based direct detection or culture-based assays, such as scabs from crusted lesions, scrapings from maculopapular lesions, CSF, blood, urine, urethral and vaginal swabs, eye fluids, saliva, and biopsy/autopsy specimens from suspected cases of disseminated disease. Although viral DNA is detectable in CSF samples in 96% of cases of HSV encephalitis, failure to detect does not necessarily rule out HSV etiology, particularly when samples are obtained in the first few days of the disease, in which case testing a later specimen will detect additional cases. False-negative results might occur when the cell and protein content of CSF are minimally elevated (27, 54) or when the patient has received antiviral therapy.

HSV PCR has been used on oral fluid for the early and reliable detection of Bell’s palsy (55). Similarly, testing of intraocular fluid has been successfully used to confirm HSV-associated uveitis (36).

Quantitative PCR has prognostic value and can be used for therapeutic monitoring in many viral infections. Among herpesviruses, this technique has been fully developed for cytomegalovirus and Epstein–Barr virus infections, because these viruses can be present in blood or CSF or tissues without causing disease. Although HSV can be shed asymptomatically at mucosal sites, HSV is not found in blood or CSF or internal organs in the absence of disease. Hence, quantitative methods are not important for establishing the etiologic role of HSV, although studies of HSV encephalitis showed that high HSV loads in the CSF may indicate a worse prognosis (57, 58). Persistence of high viral titer in ocular fluid, CSF, or blood may be an indicator of virus that is resistant to antiviral therapy.

A number of factors can affect the reliability of quantitative PCR. These include the efficiency of the nucleic acid extraction method used and the preparation and/or amplification of the standard curve template. More generally, clinical samples may include a number of recognized inhibitors of PCR, such as organic contaminants, metals, chelators, and humic acids (59). As such, quantitative PCR results should be considered within the context of an individual study, and then only in the context of a single assay using the same standard curve template (Table 1).

Therapy
First-line antiviral therapy is performed with nucleoside analogues (acyclovir, valacyclovir, famciclovir). Treatment is often instituted when the diagnosis is considered on clinical grounds, even before it is confirmed. Oral administration is utilized when immunocompromise is not severe and the illness is not considered life threatening. Otherwise initial therapy is provided by the intravenous route. Topical antiviral drugs are not utilized as sole therapy but serve as an adjunct for treating HSV keratitis, and some experts suggest that the addition of topical therapy may accelerate healing of skin lesions in immunocompromised patients. Resistance to nucleoside
analogue is a potential problem in patients who are slow to clear their infection, usually because of mutations in the HSV thymidine kinase (TK) that activates nucleoside analogues. Second-line drugs that do not require activation by TK include foscarnet, which is chosen as the alternative, and cidofovir, which is generally more toxic. Sequential testing for HSV after instituting therapy may be useful in deciding on the length of therapy and as an indicator of resistance to antiviral therapy.

New categories of potential HSV antivirals, such as lipid-conjugated nucleotide analogues (brincidofovir) and helicase–primase inhibitors (AI316 and BAY 57-1293) are under investigation (60).

**Antiviral sensitivity testing**

HSV antiviral sensitivity testing is usually performed with phenotypic assays that measure some aspect of HSV replication in tissue culture in the presence or absence of antiviral agents. Tissue culture-based methods may have different read-outs, including plaque reduction, EIA-based antigen reduction, and enzyme-linked virus inhibitor reporter assay (61, 62). These assays are lab-developed and are not available in FDA-approved kits. Raft cultures have also been used for phenotypic assays. That approach involves seeding collagen rafts with freshly isolated normal human keratinocytes at the air–liquid interface, leading to the development of a fully differentiated epithelium (63). HSV cytopathic effects are monitored in the presence and absence of antiviral agents. Recently, a method has been adapted to shell vial culture for the detection of a chemiluminescent signal rather than a cytopathic effect, which reduces assay turnaround time, is amenable to automation, and makes interpretation less subjective (64).

Genotypic assays are in development since HSV DNA mutations have been described encoding resistance to nucleoside analogues (65–67). These are located in well-defined regions of the HSV TK gene or, less commonly, in the UL30 gene that encodes the catalytic subunit of the viral DNA polymerase. Rapid methods are available to evaluate the target sequences in TK or polymerase for established sequence variations associated with resistant strains of HSV (68, 69). Foscarnet and cidofovir, unlike the other herpesvirus antivirals, have TK-independent mechanisms of action to inhibit the DNA polymerase, and resistance mutations are found only in the polymerase gene (70). However, because mutations that completely cover the full complement of resistance mutations are not fully described and are so numerous, genotypic assays are not available for clinical management.

Genotyping strategies generally depend on completely sequencing these two genes. There is no subset of nonsynonymous changes that would reliably identify antiviral resistance. Mutant HSV strains resistant to acyclovir emerge more frequently in immunocompromised patients; the highest rates observed are in hematopoietic stem cell transplant recipients (71).

**VARICELLA–ZOSTER VIRUS**

**Description of the Agent and Pathophysiology**

Varicella–zoster virus is an enveloped large DNA virus (about 125,000 base pairs [range 124,472 to 125,459]; at least 71 open reading frames; 68 unique genes). VZV is an α-herpesvirus (family Herpessviridae) consisting of a single serotype divided into nine clades that correlate with the geographic origin of the isolate (72, 73). VZV infection of a naive individual results in varicella. This primary infection occurs through the inhalation of infectious droplets or through contact with fomites from skin lesions (74, 75). VZV infects predominantly T cells (76, 77) trafficking through VZV-infected oropharyngeal lymphoid tissue, and the resulting viremia causes infection of many organs (generally asymptomatically) and seeds the skin (77). Approximately 14 to 17 days after initial infection (range 10 to 21 days) an exanthem appears, beginning as macules, which progress within hours to papules and then vesicles (72). Crops of new lesions appear for 4 to 5 days before crusting ensues. Mild systemic symptoms may be present initially. The disease is often more severe in adults, including significant organ involvement (78, 79).

VZV in vesicles is thought to access the termini of sensory neurons at the dermal/epidermal junction and to ascend in the sensory nerves to the sensory ganglia. VZV remains latent in sensory neurons for life (80, 81). Alternatively, or in addition, VZV may reach sensory neurons as a result of the viremia that occurs during varicella. Latent VZV can reactivate later in life. This may be inapparent (subclinical) and its frequency is unknown (82–84). If the level of VZV-specific T-cell-mediated immunity (VZV-CMI) is depressed (as commonly occurs in immunocompromised patients) when reactivation occurs, VZV may propagate and cause an infection in the ganglion (ganglionitis). This causes the typical neuropathic pain (designated the prodrome) that precedes the rash of herpes zoster (HZ). This pain occurs in the distribution of the skin innervated by the sensory nerve, which is called a dermatome. VZV ganglionitis is followed by VZV descending in the sensory nerve to the skin innervated by that nerve. This results in a charac-
teristic vesicular cutaneous eruption that reproduces the stages of varicella, but only in the affected dermatome (72). Usually this is accompanied by nociceptive pain because of the damaged skin. The skin lesions heal in 10 to 17 days, but the pain may linger because of the inflamed or damaged neurons in the affected ganglion. Systemic symptoms are usually not present or are mild.

Vesicle formation is the hallmark of both types of VZV infection. Vesicles are on multiple areas of the body during varicella (typically most prevalent on the face and trunk), whereas they are distinctly unilateral and dermatomal with HZ.

Features of VZV Infection in Immunocompromised Patients

Varicella disease spectrum

When immunocompromise is moderate, varicella may have a typical course. When immunocompromise is greater, varicella may take longer to crust; there may be a greater number of lesions; and there may significant visceral disease, especially pneumonia, encephalitis, and hepatitis (72). Atypical features include abdominal pain as an early symptom before skin lesions appear, and disseminated disease without rash (or before rash) (72, 85, 86).

HZ disease spectrum

When immunocompromise is moderate, HZ may have a typical course. When immunocompromise is greater, HZ is often more extensive and more painful; there may be more contiguous dermatomes involved. Rarely, bilateral disease occurs or multiple separated dermatomes are affected. Meningoencephalitis may accompany HZ. A common complication is disseminated VZV infection, which may involve a varicella-form rash, and more importantly may produce visceral disease, especially pneumonitis, encephalitis, and hepatitis (85, 87, 88). Occasionally this occurs without apparent skin lesions. The skin lesions in chronic HZ have a nonvesicular verrucous appearance (89). Reactivation of VZV can produce progressive outer-retinal necrosis in immunocompromised patients (90).

Host factors and subgroups

For both varicella and HZ, the complications are those resulting from deficits in T-cell-mediated immune responses. Untreated varicella in children with leukemia typically has an extended course of illness (14 days or longer) with large lesions that require a prolonged period to resolve (86, 91). Leukemia patients are also more susceptible to varicella pneumonia, which is sometimes fatal, and to a variety of other complications, including hepatitis, thrombocytopenia, myelodysplasia, nephritis, and esophagitis. Children who develop varicella following organ transplantation, chemotherapy, high-dose steroids, radiation therapy, biological modifiers and transplant antirejection therapy are at elevated risk for progressive VZV disease. They are also more susceptible to secondary bacterial infections, most notably Group A streptococcal infections. Children with congenital immunodeficiency disorders, such as combined immunodeficiency, T-cell disorders, NK cell deficiency, and mutations in interferon and inflammatory signaling pathways, often develop severe, sometimes fatal, complications from varicella (72, 92, 93). Although maternal immunoglobulin confers some protection during the first several months of life, newborns and infants are substantially more prone to developing severe varicella because their immune system has not completely matured (94). This is especially problematic when a pregnant woman develops varicella shortly before or after delivery, thus denying the infant the benefit of transplacental maternal antibody (95). Similarly, fetuses in utero may develop severe congenital infection when the mother develops varicella (96). Pregnant women may develop complications from varicella as a consequence of pregnancy-related immunosuppression (97), although a large cohort study concluded that VZV pneumonia in this group is relatively rare (98). HZ is generally not complicated in pregnant women.

Varicella is commonly more severe in adults, although the underlying host deficits are not understood (79). One study determined that, while adults accounted for only 10% of varicella cases, a quarter of varicella-related hospitalizations and 69% of varicella deaths occurred in adults (99). HZ incidence is greatly elevated among persons over the age of 60, with a well-documented association with the waning of T-cell immunity (100, 101). Increased susceptibility to HZ is associated with all diseases and clinical therapies that are attended by suppression of T-cell-mediated immune responses (100, 102).

Morbidity and mortality

Morbidity is increased when the manifestations of varicella or herpes zoster are prolonged and disseminated infection results in multiorgan visceral disease (lung, liver, brain). This can be fatal. Local pain of HZ may be more severe in immunocompromised patients (107). VZV retinitis may destroy vision. Prognostic factors include: 1) the level of VZV-specific T-cell-mediated immunity prior to immunocompromise, 2) the extent and
duration of immunocompromise at the time of VZV infection, 3) dissemination and organ involvement and 4) delay in antiviral treatment.

**Epidemiology**

**Varicella**

Prior to the implementation of universal varicella immunization in 1996, annual cases of varicella in the U.S. equaled the U.S. annual birth cohort (4 million), with roughly 11,000 VZV-attributable hospitalizations and 150 deaths each year (104). High rates of single- and two-dose varicella vaccine coverage have reduced the incidence of varicella in the U.S. by at least 10-fold, with proportional reductions in varicella-related hospitalizations and deaths (105). This has also resulted in a similar decrease in varicella in immunocompromised patients.

One aspect of varicella epidemiology that distinguishes it from other childhood vaccine-preventable diseases is that a VZV-naive person may also be infected by exposure to a patient with HZ; however, varicella is less readily acquired from an HZ contact than from a varicella contact (106, 107).

**Herpes zoster**

In both immunocompetent and immunocompromised patients, HZ results from reactivation of latent VZV in sensory ganglia and thus only occurs in individuals who previously had varicella. The incidence of HZ increases with age, very likely because of the decline in VZV-CMI that characterizes aging (72). The increase in frequency and severity of HZ that occurs with immunocompromise is also closely correlated with defective VZV-CMI (102, 108).

**Goals of Laboratory Testing**

Routine serological screening of persons with an elevated risk of occupational VZV exposure (e.g., daycare workers, healthcare professionals) who have negative or uncertain histories of varicella is recommended, and persons identified as susceptible should receive the varicella vaccine (104). VZV serology to identify VZV-susceptible individuals as part of outbreak control is also recommended (104). Serologic screening to determine prior infection with VZV (varicella) can be useful when immunosuppression is planned. The HZ vaccine, which substantially prevents HZ in immunocompetent individuals, provides partial protection to many types of immunocompromised patients who receive the vaccine in advance of their immunosuppression (109). Some immunocompromised patients can safely receive the HZ vaccine while mildly immunocompromised (e.g., HIV infection after good response to antiretroviral therapy) (110). In addition, seropositive patients will benefit from prophylactic antiviral therapy through periods of severe immunosuppression (111). After exposure to VZV, some VZV-susceptible immunocompromised patients may be considered for passive immunization with VZV hyperimmune globulin (104). This decision may require testing to determine VZV immune status.

Varicella and HZ can often be diagnosed reliably on clinical grounds; however, the sharp decline in varicella incidence, coupled with substantially modified breakthrough varicella disease that can occur in previously vaccinated individuals, have complicated diagnosis and reduced the level of experience among physicians for diagnosing varicella. As such, laboratory confirmation of varicella has become more important. In addition, PCR protocols capable of discriminating vaccine varicella from wild-type virus are now commercially available, but confirmation of varicella vaccine adverse events is currently available in only several laboratories (112, 113). Detection of VZV is important when the manifestations of varicella or HZ are atypical in immunocompromised patients; it is especially important when visceral disease, especially brain, liver, and the retina, is involved in the absence of pathognomonic skin lesions (114).

Evaluating VZV or VZV DNA in blood after starting therapy can give early clues to the adequacy of antiviral therapy and the possibility of drug resistance (84). This is also true of the persistence of VZV in skin lesions for extended periods.

**Laboratory Testing Options, Applications, Interpretation**

**Serology**

VZV serological techniques are primarily useful for classifying patients as varicella-experienced or varicella-susceptible. Certain assays can be used to provide more descriptive data; for example, detection of VZV-specific IgM antibody provides evidence for recent VZV exposure (primary infection, reactivation or reexposure) (115), and IgG avidity can be used to determine whether recent primary infection (within 3 months) has occurred (116).

The large number of serological assays available for detecting antibodies to VZV vary widely in performance specifications and uses. In general, those that are reasonably sensitive suffer from lack of specificity, and vice versa. Sensitivity varied from 40% to 98%, with associated sensitivity declining from 80% to 40%. The least reliable method was 40% sensitive and 97% specific,
and the most reliable was 76% sensitive and 78% specific. Many commercial VZV IgG methods can reliably detect seroconversion after wild-type VZV infection, but false-negative results may be 20% or more (D.S. Schmid et al., unpublished observation). None reliably detect seroconversion after varicella vaccination (Schmid, unpublished observation). The majority of these methods use various preparations from VZV-infected cell lysates.

Only three methods have been demonstrated to detect seroconversion to varicella vaccine with adequate reliability: fluorescent antibody to membrane antigen (117, 118), glycoprotein ELISA (gpELISA) (116, 119), and time-resolved fluorescence immunoassay (120, 121). These methods are highly sensitive and specific (>90% sensitivity, 80 to 90% specificity on well-defined sera). gpELISA uses a standard ELISA format and is the simplest method to perform; time-resolved fluorescence immunoassay is similar in setup but requires a specialized plate reader capable of quantifying the fluorescence signal; and fluorescent antibody to membrane antigen, while also sufficiently reliable, is cumbersome to perform and requires an experienced fluorescent microscopist for accurate interpretation. A turnaround time of 1 to 2 days is technically feasible for all three techniques. The costs of the methods are comparable.

In some immunocompetent patients when VZV was suspected as the cause of a neurologic syndrome but the PCR assay of CSF was negative, an alternative was to measure the ratio of VZV-specific IgG in CSF and serum (as described above for HSV) (122, 123).

A high-throughput VZV-neutralizing antibody test is currently under development for the quantification of neutralizing antibodies to VZV (D.S. Schmid, unpublished observation). This method has been successfully used as an in vitro correlate of protection for other vaccine-preventable viruses, such as measles and influenza (Table 2).

**Culture**

VZV culture is substantially less sensitive than other available direct-detection methods, such as PCR, even more so than for HSV. Moreover, isolation of VZV infection by standard cell culture methods can take 7 to 14 days. This can be effectively shortened to as little as 16 hours postinoculation using shell vial culture coupled with detection antibodies directed against VZV early antigens by DFA (124). Thus, standard cell culture methods are not often utilized, unless further characterization of a clinical isolate is desired. Because of the lability of VZV, successful isolation in culture is critically dependent on rapidly stabilizing the collected specimen in transport medium and inoculating it into a susceptible cell monolayer as expeditiously as possible (preferably within an hour or two). As a direct consequence of this requirement, virus culture frequently leads to a false-negative result. Head-to-head comparison of VZV culture with PCR revealed that as many as 50 to 75% of VZV PCR-positive samples may elude detection by viral culture (125–126). Additional steps (fluorescent antibody staining) are required to make the method specific for VZV. Finally, successful VZV culture is dependent on the type of sample collected. Freshly unroofed vesicles are the best specimens; saliva, macular, papular and ulcerated lesions are less reliable, and crusted lesions contain no infectious virus. In addition, care in the collection of the sample is vital; lesions should not be wiped with alcohol prior to collection, polyester swabs are preferred as a collection device, and the sample must be immediately placed into cold transport medium. Samples must not be frozen prior to culture, since VZV is extremely sensitive to freezing. Culture may also be falsely negative if patients have already begun antiviral therapy. Culture is primarily desirable when a viable isolate is needed for more extensive in vitro studies (Table 2).

**Morphological tests**

The “Tzanck smear,” which examines Giemsa-stained cells collected from skin lesions for the presence of multinucleated giant (i.e., syncytial) cells, has the disadvantage of indicating only that an alphaherpesvirus (VZV, HSV-1, or HSV-2) is causative. Similarly, electron microscopy is no longer used because of expense, equipment requirements, and turnaround time (127). Both methods lack sensitivity and the Tzanck smear also has relatively poor specificity; both have been rendered obsolete by currently available tests.

**Antigen-based assays**

The antigen-based direct detection method of choice is DFA, which uses agent-specific fluorophore-labeled monoclonal antibodies to detect VZV-encoded surface glycoproteins on infected cells collected from lesions (128). The method is critically dependent on the collection of an adequate specimen, which must be obtained by swabbing an unroofed vesicular lesion vigorously enough to ensure the inclusion of cells but not so aggressively that blood contamination occurs. Contaminating blood can include antibodies (directed at VZV or other related herpesviruses such as HSV) that can serve as cold competitors to the detection antibody and cause a false-negative result. Failure to collect a sufficient number of
**TABLE 2** Laboratory methods for the diagnosis of VZV infection

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Sample processing</th>
<th>Turnaround time</th>
<th>Results and clinical utility</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td><strong>Nonmolecular methods</strong></td>
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<tr>
<td>Serology</td>
<td>Antibody detection (IgG, IgM) ELISA or immunoblot Fluorescent antibody to membrane antigen</td>
<td>Can use either serum or plasma</td>
<td>6 hours</td>
<td>VZV-IgG indicates prior infection VZV-IgM implies acute or recent infection or reactivation</td>
<td>Identifies patients who can be infected after exposure to VZV</td>
<td>May be falsely negative in a severely immune-compromised patient Many commercial assays have low sensitivity and specificity</td>
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<tr>
<td><strong>Virus cultures</strong></td>
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<tr>
<td>Tube culture</td>
<td>Viral growth Light microscopy</td>
<td>Specimen taken to the laboratory as soon as possible; avoid exposure to high heat and organic solvents</td>
<td>7–14 days</td>
<td>Characteristic cytopathic effect. Can distinguish from HSV with virus-specific monoclonal antibody</td>
<td>Can establish VZV as the causative virus The viral isolate can be tested for susceptibility to antiviral agents</td>
<td>Care required in obtaining and transporting specimen Processing time is very long Sensitivity will vary with the type of skin lesion and for noncutaneous specimens Not as sensitive as molecular methods</td>
</tr>
<tr>
<td>Shell vial assay</td>
<td>Viral growth detected with immunocytochemistry; fluorescent microscopy</td>
<td>Care needed for specimen collection as above</td>
<td>16–24 hours</td>
<td>Infectious foci detected with fluorescein-conjugated monoclonal antibody</td>
<td>Specific for VZV More sensitive and more rapid than conventional tube cultures</td>
<td>Care required in obtaining and transporting specimen Not as sensitive as molecular methods</td>
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<tr>
<td><strong>Antigen detection</strong></td>
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<tr>
<td>Direct fluorescent antibody assay (DFA)</td>
<td>Scoring of skin lesion; care must be taken in collecting sample; must collect lesion cells but avoid contaminating the sample with blood</td>
<td>3–6 hours</td>
<td>Detection of specific fluorescence establishes etiologic diagnosis</td>
<td>Rapid diagnosis Relatively inexpensive If monoclonal antibodies are used to detect antigen, must use two antibodies targeting different epitopes to minimize false-negative results</td>
<td>Limited to use for skin lesions Sensitivity varies with quality of the sample submitted and with skill of the microscopist Generally less sensitive than culture or nucleic acid methods Cannot be used on healing skin lesions</td>
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<tr>
<td>Method</td>
<td>DNA detection</td>
<td>Time</td>
<td>Qualitative or quantitative method</td>
<td>Highly sensitive and specific</td>
<td>Monitor response to therapy</td>
<td>Surrogate marker for antiviral drug resistance</td>
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<tr>
<td>LightCycler (FRET realtime PCR)</td>
<td>Ideal samples are swabs from vesicular lesions, scabs, saliva, CSF. Swabs and scabs (no transport medium) are stable indefinitely at ambient temperature; saliva and CSF should be frozen if not tested immediately</td>
<td>3 hours (1 hour for PCR amplification and detection)</td>
<td>Methods developed at CDC discriminate vaccine VZV from wild type virus in real time based on melt curve (FRET detection probe binds to site that includes a vaccine-associated single nucleotide polymorphism). Methods can be made quantitative by interpolating to a standard curve.</td>
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<tr>
<td>ABI cycler (7000, 7700, 7900) (TaqMan realtime PCR)</td>
<td>Same as for LightCycler</td>
<td>3 hours</td>
<td>Qualitative or quantitative method Several groups have developed methods capable of discriminating vaccine from wild type in real time. Methods can be made quantitative by interpolating to a standard curve.</td>
<td>Highly sensitive and specific</td>
<td>Monitor response to therapy</td>
<td>Rapid turnaround time Surrogate marker for antiviral drug resistance</td>
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<tr>
<td>Loop-mediated isothermal amplification (LAMP)</td>
<td>Same as for LightCycler</td>
<td>2–3 hours</td>
<td>Qualitative or quantitative method For VZV identification, not for discrimination of vaccine from wild type</td>
<td>Highly sensitive and specific</td>
<td>Not susceptible to inhibitory agents that can interfere with PCR</td>
<td>Generally less sensitive than PCR</td>
</tr>
</tbody>
</table>
infected cells in the sample is a common cause of a false-negative result. False-positive results can occur when the microscopist is insufficiently experienced at distinguishing background fluorescence from specific signal. Slide preparation for an adequately collected sample is straightforward. Once prepared, slides are stable and can be readily transported. The method has the advantage of being very rapid, providing a result in less than an hour; however, it is considerably less sensitive than VZV PCR, failing to detect nearly half of samples that were positive for VZV DNA in one study (129). Reliance on a single monoclonal detection antibody can be problematic, since two different groups have described the loss of a major immunoglobulin epitope through random mutation (130, 131). The assay is expandable to include detection antibodies for HSV1 and HSV2, but it is not suitable for specimens with limited or no cell content, such as blood, CSF, or vitreous humor (Table 2).

**Nucleic acid-based assays**

The most sensitive method for confirming VZV infection is PCR. The various methods reported in the literature are numerous. They include standard single-agent methods (132, 133), real-time PCR (134, 135), multiplex methods (134, 136, 137), quantitative PCR (137, 138), and nested PCR (138, 139). PCR methods detect up to twice as many infections as do the less sensitive non-PCR methods. PCR detection methods that amplify the target sequence and then characterize the amplicon based on molecular size and/or DNA sequencing have the feature of high sensitivity and specificity in common with more recently developed technologies. The use of restriction endonucleases or amplicon sequencing provides a means for detecting specific polymorphisms that distinguish vaccine strain from wild-type VZV, a crucial feature for documenting vaccine adverse events. These methods are being gradually replaced by real-time PCR assays, which are less cumbersome, more rapid, and have the further advantage of being single-tube methods, minimizing the risk of amplicon contamination. Multiplex PCR methods may be useful in clinical circumstances where a human alphaherpesvirus infection is suspected but where the specific etiology is unclear. Generally, these methods function comparably to single-agent PCR protocols when there are no more than three etiological agents targeted by the test, but with additional targeted agents, sensitivity may be reduced due to the depletion of reagents in the master mix. Nested PCR protocols may lead to enhanced sensitivity but may be subject to contamination problems arising from manipulating product from the first round of amplification.

Real-time methods have now been developed that can distinguish vaccine VZV from wild-type virus using Förster Resonance Energy Transfer (FRET)-based PCR (135, 140, 141) or TaqMan chemistry (142, 143). FRET PCR employs fluorescent anchor and detection probes; the anchor probe fluor is excited by the real-time cycler, emitting a different wavelength that in turn excites a short detection probe. The detection probe binds to a position that includes a vaccine-associated single nucleotide polymorphism and is excited only when both the anchor and detection probes are bound to the DNA template. The detection probe is designed to bind at temperatures approximately 10°C different, permitting discrimination of vaccine and wild-type on the basis of probe annealing temperature. TaqMan PCR also utilizes two probes, but in this instance each probe binds preferentially to either the vaccine target or the wild-type target. Both probes are coupled to a quencher, which prevents the emission of fluorescent signal until probe hydrolysis during replication dislodges the quencher molecule. These techniques reduce the time required to confirm the involvement of vaccine strain Oka VZV in clinical disease from two days (using either restriction fragment length polymorphism or DNA sequencing) to several hours.

PCR is applicable to a much broader range of samples than viral isolation or antigen detection. These include swabs of unroofed skin lesions (vesicles, papules, and macules), swabs from crusted lesions, CSF, saliva, peripheral blood, urine, throat swabs, biopsy samples, autopsy samples, and samples from patients receiving antiviral therapy. That said, there are qualitative differences in various specimens for VZV testing. Vesicle swabs and scabs are the most reliable specimens, followed by saliva. CSF can be a useful specimen for confirming VZV infection in encephalitis, meningitis, and a variety of neuropathies and vasculopathies (123), but viral DNA is frequently undetectable even in cases with high suspicion of VZV etiology (144, 145). As such, failure to detect VZV DNA in a CSF specimen does not rule out VZV involvement. In this situation, detection of high titers of VZV antibody in CSF, as described in the section on HSV serology, may provide the diagnosis. PCR is somewhat more expensive than other methods, such as culture and DFA, but the increase in reliability has led to nearly universal adoption by diagnostic testing firms, public health laboratories, and laboratories in many medical centers.

VZV PCR performed on oral fluid has been used for the early and reliable detection of Bell’s palsy in patients (55). Similarly, testing of intraocular fluid has been
successfully used to confirm VZV-associated uveitis (56) (Table 2).

**Quantitative DNAemia**

Among varicella cases, VZV DNA may be detected in peripheral blood as early as 14 days prior to rash onset and up to 8 days following the outbreak of rash illness (84). Quantitative PCR methods can measure VZV in circulation during disease, with dramatic reductions in detectable genome copies apparent shortly after disease onset in the immunocompetent host. Much of the viral DNA is not cell-associated and, as such, detectable in either serum or plasma (146). Cell-associated VZV in whole blood appears to be primarily associated with infected T lymphocytes (77). Measuring VZV DNAemia has demonstrable value for cases of varicella in immunocompromised children, where it has been used to establish etiology in atypical cases (hepatitis without rash, neurologic syndromes without rash, and in cases of modified breakthrough illness among vaccinated persons). Its use has also served as an indicator of underlying immunodeficiency (147).

Similarly, VZV DNA may be detected in the peripheral blood from HZ patients for a month or longer following the eruption of rash (84). While results from various studies are inconsistent, VZV DNA has been detected in blood samples from patients with postherpetic neuralgia, suggesting that continued replication of the virus in ganglia could explain the pain, and has been detected in saliva in patients with zoster sine herpette (discussed in reference 84). If VZV DNA is present in blood during HZ prodrome, its detection could potentially offer a means for earlier diagnosis and therapy. Quantification of VZV DNA in blood also appears to correlate with disease severity (146, 148), and persistence of VZV in blood or other fluids/lesions may be an indicator of treatment failure and resistance to antiviral therapy (Table 2).

**Therapy**

First-line antiviral therapy is with nucleoside analogues (acyclovir, valacyclovir, famciclovir). Oral administration is utilized when immunocompromise is not severe and the illness is not considered life-threatening. Otherwise, initial therapy is provided by the intravenous route. Topical antivirals play little role in treating herpes zoster. Resistance to nucleoside analogs is a potential problem in patients who are slow to clear their infection, usually because of mutations in the enzyme that activates these drugs. Second-line drugs that do not require activation include foscarnet, which is chosen as the next alternative, and cidofovir, which is generally more toxic. The most promising new antiviral for VZV is FV-100, a bicyclic pyrimidine nucleoside analogue, which was shown to inhibit VZV at subnanomolar levels (149). FV-100 is intended for single daily oral administration. Sequential testing for VZV after instituting therapy may be useful in deciding on the length of therapy and as an indicator of resistance to antiviral therapy.

**Antiviral Sensitivity Testing**

There are two general approaches to evaluating antiviral sensitivity or resistance for varicella-zoster virus. The first is simple tissue culture of VZV in the presence and absence of the antiviral agent. Conventional tissue culture and raft culture have been used with this approach, and the adaptation to an objective chemiluminescent test signal should be readily formatted for evaluating VZV, thereby reducing assay turnaround time and saving labor through automation (63, 64). This approach to antiviral sensitivity testing is essential for the evaluation of new antivirals, particularly those that may have entirely different modes of action than existing herpesvirus antiviral agents. That said, all but one of the currently licensed herpesviral antivirals are nucleoside analogues that operate as DNA chain terminators interfering with replication of many herpesviruses. Similar to HSV, the genomic markers for antiviral resistance are located in various sites within the viral thymidine kinase gene or, less commonly, in the ORF28 gene that encodes the catalytic subunit of VZV DNA polymerase. This is a natural consequence of the pharmacology of current herpesvirus antivirals, which are nearly all base analogue DNA chain terminators and thus inhibitors of replication. Since VZV grows very slowly in tissue culture, assessments of antiviral resistance in culture are regarded as relatively impractical in a clinical setting requiring a fast turnaround (126). Rapid methods have been developed that evaluate the target sequences in TK or polymerase associated with VZV resistance to antivirals (70, 150). A large number of amino acid sequence variations have been identified for the VZV TK and DNA polymerase gene products. Genotypic analyses are complicated by differences among wild-type VZV clades in the types of resistance mutations they carry. For example, in the study by Sauerbrei and coworkers (150), none of the acyclovir-resistant Clade 1, 3 or 5 isolates evaluated carried known mutations in the TK gene. Together, these three clades comprise roughly 85% of the strains circulating in North America and Europe (151, D.S. Schmid, unpublished observation).
ACKNOWLEDGMENT
The views included in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES
Herpes Simplex Virus and Varicella-Zoster Virus


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