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MINIREVIEW

Laboratory Diagnosis of Neonatal Herpes Simplex Virus Infections

William J. Muller,[a,b] **Xiaotian Zheng**[a,b]

[a]Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
[b]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA

ABSTRACT Herpes simplex virus (HSV) is a common and often benign infection in humans; although it less commonly affects newborns, infection in this age group can be devastating. Newborns often present with nonspecific clinical findings, making timely and accurate diagnosis of infection critical. A wide variety of tests are available for detecting herpes simplex virus infection, but only a subset are useful and validated in the newborn population. The current review summarizes available diagnostic testing for neonatal disease, including discussing limitations, unmet needs, and emerging data on molecular testing methods.

KEYWORDS diagnostics, herpes simplex virus, neonates

Herpes simplex viruses (HSV-1 and HSV-2) are common causes of infection. HSV-1 infection occurs in approximately 54% of U.S. adults and HSV-2 in approximately 16% (1). Initial infection generally occurs at skin or mucosal surfaces and may be clinically inapparent or result in the characteristic grouped vesicles that give the virus its name (herpes is Greek for "creep" or "crawl" [2]). After initial replication at these sites of entry, virus spreads to neurons that supply the initial region of infection, where viral genomes can form an episome harboring latent viral DNA. Periodically, viral replication can reactivate from this latent episomal DNA, leading to spread of virus to epithelial surfaces, viral shedding with possible transmission, and recurrent skin or mucosal lesions (3).

HSV infection is generally fairly benign or at most bothersome in older children and adults. Although infection in the newborn period is relatively rare, it is a disease that can be life threatening, making neonatal HSV infection one of the most challenging and important diagnoses in pediatric medicine. A thorough evaluation and accurate clinical diagnostic testing are critical to making this diagnosis and guiding appropriate treatment (3).

EPIDEMIOLOGY OF NEONATAL HSV

Estimated rates of neonatal HSV infection in the United States range between 1 in 2,000 to 1 in 13,000 live births, although estimates from studies in other countries suggest a lower incidence (4). Although neonatal HSV is considered rare, U.S. estimates are consistent with rates of bacterial meningitis in febrile infants under 1 month of age admitted to the hospital for evaluation for sepsis (5) or with rates of early onset group B streptococcal (*Streptococcus agalactiae*) sepsis (6), two other important clinical presentations in the newborn. Although commonly thought to be caused primarily by HSV-2, due to the association of that virus with genital infection, rates of both genital infection and neonatal disease due to HSV-1 are increasing and may be comparable to HSV-2 in some populations (7). Clinical management of newborns with HSV infection is not influenced by virus type (8), though some evidence suggests that neurologic outcomes may be worse if infection involves the central nervous system and is due to

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Address correspondence to Xiaotian Zheng, XTZheng@luriechildrens.org.

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TABLE 1 Risk factors for acquisition of neonatal HSV infection[a]

Risk factor	Odds ratio
Isolation of HSV from maternal genital tract at delivery	346
Primary-episode maternal infection in third trimester	33.1
Use of invasive monitoring	6.8
Delivery before 38 weeks	4.4
Maternal age <21 yrs	4.1
Caesarian delivery	0.14

[a]Data from reference 12.

HSV-2 compared with that due to HSV-1 (9–11). Knowing which virus type is causing neonatal infection can therefore influence discussions with families about mechanisms of transmission and prognosis. There is no significant increase in difficulty or cost to provide HSV type information for laboratory diagnosis of neonatal infection.

Neonatal infection occurs the vast majority of the time upon exposure of the baby to infected maternal secretions at the time of delivery, with postnatal or *in utero* infection much less common. Maternal infection may be classified as primary episode, nonprimary first episode, or reactivation based on her serologic status at the time of delivery (12). Primary-episode infection is defined by evidence of HSV-1 or HSV-2 from genital secretions in the absence of HSV antibodies. Nonprimary first-episode infection involves evidence of HSV-1 or HSV-2 in genital secretions with antibodies present only against the opposite virus type (i.e., seropositive only for HSV-1 with HSV-2 isolated from genital secretions, or seropositive only for HSV-2 with HSV-1 isolated from genital secretions). Reactivation occurs when a mother is seropositive for the same HSV type isolated from secretions. Risk factors for transmission of HSV from mother to baby were defined by a landmark study involving prospective observation of almost 60,000 pregnancies (12); the highest risk for neonatal disease occurs when primary-episode maternal infection occurs in the third trimester (Table 1), with isolation of virus from the vaginal tract at delivery the factor associated with the highest risk of neonatal disease (12). Although this is commonly attributed to the inability of the mother to generate a protective antibody response to the infection that may be passed onto the infant prior to delivery, infants born to mothers with primary-episode genital infection late in pregnancy are also likely to be exposed to significantly larger amounts of virus during the birth process than those born to mothers with reactivation of genital infection (3). Additional risk factors for neonatal HSV not identified in the Brown et al. study (12) include maternal fever at delivery (13) and duration of membrane rupture of more than 4 h (14).

CLINICAL PRESENTATION AND MANAGEMENT OF NEONATAL INFECTION WITH HSV

Neonatal HSV comprises an overlapping set of three clinical syndromes: disease isolated to the skin-eyes-mucous membranes (also known as SEM disease), disease of the central nervous system (i.e., encephalitis, which may occur with or without skin and/or mucous membrane involvement), and disseminated disease (with or without encephalitis and/or skin/mucous membrane involvement) (15). These clinical syndromes have certain differences in their presentation, treatment, and prognosis and influence the set of diagnostic tests required for complete evaluation of possible neonatal HSV disease. Clinical presentation of neonatal HSV is nonspecific and may be subtle, and a high index of suspicion on the part of clinicians is important in making the diagnosis (16). Many infants may have symptoms of infection for several days before presenting to medical attention (11, 13).

Neonatal SEM disease comprises approximately 45% of neonatal HSV cases and confers the best prognosis. Patients with SEM disease often present around day of life 10 to 12 (11) and may have skin lesions that test positive for the virus or may only have swabs of the eye or nasopharynx from which HSV is detected. Due to the risk of spread to the central nervous system (CNS), SEM disease is treated for 14 days with the antiviral acyclovir given intravenously (16).

Central nervous system disease due to HSV in the neonate (i.e., neonatal HSV encephalitis) involves approximately 30% of cases. Patients often present around day of life 16 to 19 (11), and may present with lethargy or seizures. Testing of cerebrospinal fluid (CSF) by PCR confirms the diagnosis, though imaging changes may also provide support. Importantly, CSF characteristics such as white blood cell count should not influence the decision to test CSF for HSV by PCR, as pleocytosis may not be present, particularly early in presentation (13). Neonatal HSV encephalitis is treated for 21 days with intravenous (i.v.) acyclovir, with most experts recommending repeat testing of CSF prior to stopping treatment to confirm clearance of viral DNA (16). Mortality from HSV encephalitis in the newborn period has been dramatically reduced by high-dose acyclovir treatment; however, neurologic morbidity remains significant, even with subsequent suppressive oral treatment (discussed further below) (17).

Disseminated neonatal HSV occurs in approximately 25% of cases (16). Patients often present to medical attention around day of life 10 to 12 (11). Virus spreads from the site of initial inoculation through the bloodstream, where it may infect multiple organs, including the lungs, liver, kidneys, and brain. Of the three clinical syndromes associated with neonatal HSV, disseminated disease confers the highest risk for mortality, with death in up to one-third of patients even with appropriate antiviral treatment (16). As for encephalitis, i.v. treatment is continued for 21 days (16).

Because the different clinical syndromes of neonatal HSV have overlapping characteristics, the testing that is indicated for newborns undergoing evaluation for the disease does not differ based on suspicion of one or another syndrome. Swabs from the conjunctivae, mouth, nasopharynx, and anus should be sent for culture (if available) or PCR, as should swabs of any skin vesicles that are present. CSF and blood should be sent for HSV PCR, with blood also sent for measurement of alanine aminotransferase (ALT) (8). The clinical syndrome will be based on the results of these tests, with isolated SEM disease diagnosed if all testing is negative other than the surface swabs and/or vesicles, and CNS disease diagnosed if CSF PCR is positive (whether or not surface or vesicle samples are positive) but there is no evidence of visceral involvement (e.g., pneumonia or abnormal liver function) to suggest disseminated disease (8, 16). Although a positive blood PCR suggests viral dissemination, a recent study suggests this test may be positive in patients suspected of isolated SEM or CNS disease (18), and so this test alone should not be used for classification of the syndrome in a given patient (8).

Although resistance to acyclovir is rare in neonatal HSV infection, it has been described in patients whose mothers previously received suppressive acyclovir treatment (19). Phenotypic resistance testing of HSV isolates can be considered in patients who do not appear to have a clinical response to acyclovir.

DIAGNOSTIC TESTING

The importance of a reliable and prompt etiologic laboratory diagnosis of neonatal HSV is underscored by its nonspecific clinical presentation and the fact that early antiviral therapy can reduce severity and improve outcomes (20). Although HSV can be detected by various methodologies, selection of the appropriate assays specific in this setting enhances the quality of the results and avoids delaying reporting. For example, serology or specific antibody detection is very useful in the demonstration of maternal HSV infection status (to differentiate primary-episode, nonprimary first-episode, and reactivation infections) but are not helpful in testing neonatal samples. Since the highest risk of transmission occurs when mothers acquire infection late in pregnancy (12), negative serologic testing in the newborn does not rule out HSV disease. Similarly, since IgG in the newborn reflects passively transferred maternal antibody, positive testing in the newborn does not help in the diagnosis of HSV disease, given the high seroprevalence of HSV antibodies in the general population (16).

Detailed virologic methodology for HSV diagnosis has been reviewed comprehensively recently (21). The following assays are available for consideration.

Conventional virus culture. Viruses present in patient samples are propagated in mammalian cells. Conventional virus culture is performed in flasks or tubes that have a cell monolayer grown on the inner surface. Multiple commonly available cell types can be used, but their sensitivities for virus recovery are different. Rabbit kidney cells, guinea pig embryo cells, and mink lung cells are considered more sensitive than others, including human diploid fibroblast (such as MRC-5 and WI 38) and A549. Although they can be used, Hep-2 and Vero (African green monkey kidney) cells are less sensitive for testing samples with lower virus load (13, 22–25). After sample inoculation, virus culture is incubated and microscopically examined to identify cytopathic or cytopathogenic effect (CPE), cellular morphological changes indicating virus growth. During the 2-week incubation period, the majority (95%) of positive growth for HSV is observed during the first 5 days (21).

Virus grown in culture is identified by the detection of viral antigens. Currently, the most widely used method for HSV identification and typing in virus culture is immunofluorescence staining of the cells. These monoclonal antibody-based immunofluorescence staining reagents recognize HSV type-specific epitopes and are sensitive, specific, and standardized and are readily available from diagnostic companies for HSV typing.

"Shell vial" and other modified culture methods. In the 1980s, a technique involving preincubation centrifugation of culture, coupled with immunofluorescence staining of cells before CPE is observed (also known as the "shell vial" technique), was successfully developed. This method reduced HSV culture time to 1 to 2 days. A commercial assay employing genetically engineered cells for HSV specific detection, the enzyme-linked virus inducible system (ELVIS), is also available for use and allows detection of virus without needing a fluorescence microscope.

As a general rule, when collecting samples for virus culture, swabs made of a wooden shaft and calcium alginate should be avoided, because these materials affect virus infectivity in culture (21, 24, 25). Utilization of virus transport medium, maintenance of refrigerated temperature, and prompt transportation to the laboratory are all important to ensure the viability of virus and to enhance recovery. Studies have found that, compared to refrigeration, storing samples at room temperature significantly reduces recovery of the virus by culture. It is therefore important to store samples after collection and during transportation at 4°C or refrigerated. Storage in a deep freezer, below −70°C, is recommended if the sample cannot be processed within 2 to 3 days (21, 23–25). In addition, repeated freezing and thawing or storage in a frost-free freezer also affects virus for culture and should be avoided.

Virus culture-based assays are sensitive and very specific and are used as reference methods. The cost is moderate. However, report times vary from one to a few days. The virus cultures are manually performed and require expertise. Culture-based HSV detection is offered by public health laboratories.

HSV direct antigen detection. Currently, for direct detection of HSV in clinical specimens, a widely used method in a clinical virology laboratory is the direct fluorescent antibody assay (DFA). Clinical specimens containing cells, collected from lesions, can be placed on a microscopic slide directly or after cytocentrifugation before being fixed and stained with HSV-specific antibodies. The performance of the assay varies based on factors such as sample quality and skills of the testing personnel. The strengths of the assay include short testing time (hours) and relatively lower cost.

Molecular methods. Detection of HSV DNA by nucleic acid amplification methods has effectively enhanced laboratory diagnosis of HSV infection. These tests significantly improved assay sensitivity and shortened report time compared to those of other methods. Historically, most testing laboratories relied on laboratory developed tests; standardized commercial assays with FDA clearance status have only been available very recently. The principles of these commercial assays include real-time PCR and isothermal amplification. These assays are only cleared by the FDA for testing samples collected from cutaneous or mucocutaneous lesions and/or CSF (Table 2). All these

TABLE 2 FDA-cleared molecular assays for HSV[a]

Assay and manufacturer	Virus detected and HSV gene target	Principle	Instrument(s)	Intended use
Lyra Direct HSV 1 + 2/VZV, Quidel	HSV-1 and -2, VZV (amplification targets not specified by manufacturer)	Real-time PCR	Life Technologies QuantStudio Dx, Applied Biosystems 7500 Fast Dx, Cepheid SmartCycler II System	Cutaneous or mucocutaneous lesion samples
Solana HSV 1 + 2/VZV, Quidel	HSV-1 and -2, VZV (amplification targets not specified by manufacturer)	HDA	Solana instrument	Cutaneous or mucocutaneous lesion samples
AmpliVue HSV 1 + 2, Quidel	HSV-1 and -2 (amplification target not specified by manufacturer)	HDA	AmpliVue cassette (disposable and self-contained cartridge)	Cutaneous or mucocutaneous lesion specimens
illumigene HSV 1&2, Meridian Bioscience	HSV-1 and -2, HSV gB	LAMP	illumipro-10	Cutaneous or mucocutaneous lesion specimens
Simplexa HSV 1 & 2 Direct, DiaSorin	HSV-1 and -2, HSV DNA polymerase	Real-time PCR	LIAISON MDX	Genital lesion; cutaneous/mucocutaneous swab samples; CSF
MultiCode-RTx HSV 1&2, EraGen (currently under Luminex)	HSV-1 and -2 HSV gB	Real-time PCR	Roche LightCycler	Vaginal lesion
Aries HSV 1&2 assay, Luminex	HSV-1 and -2 (amplification target not specified by manufacturer)	Real-time PCR	Aries system	Cutaneous or mucocutaneous specimens
FilmArray ME panel, BioFire	HSV-1 and -2 plus 12 other pathogens (amplification target not specified by manufacturer)	Real-time PCR, multiplex	BioFire instrument	CSF for meningitis and/or encephalitis

[a]HSV, herpes simplex virus; VZV, varicella zoster virus; HSV gB, HSV surface glycoprotein B; ME, meningitis/encephalitis; HDA, helicase-dependent isothermal DNA amplification; LAMP, loop-mediated isothermal DNA amplification.

molecular assays provide HSV type identification. In addition, to avoid potential false-negative results caused by inhibiting factors occasionally present in clinical specimens, all of these assays include the use of a built-in internal control to monitor possible reduction of enzymatic amplification efficacy. These nucleic acid amplification-based assays are sensitive and specific, and most can be completed within 1 to 2 h. Compared to virus culture and antigen detection, these molecular methods require instrumentation, and their supply costs are also slightly higher.

Nucleic acid amplification methods have been demonstrated to be superior for HSV diagnosis and have become standard for certain sample types such as CSF. However, the following unmet needs are still associated specifically with neonatal HSV diseases.

To assist virologic diagnosis of neonatal infections, the American Academy of Pediatrics recommends HSV culture (if available) or PCR for surface samples (mucous membranes, including the eye, mouth, nasopharynx, and anus) and skin vesicles and HSV PCR assay for CSF and whole blood (8). Swab samples collected from mucous membranes, particularly the eye, are often helpful for diagnosis in patients who do not have characteristic skin lesions (which should also be unroofed and swabbed, if present), with one study finding $\geq$90% of cultures from skin lesions or eye swabs positive in patients with neonatal disease (11). However, data suggest considerable variations about sample collection and test ordering. A recent survey conducted among hospital medicine and emergency department (ED) physicians showed consistency of testing CSF for HSV by PCR but reduced HSV testing rates (only 70% or less) for surface samples, vesicle samples, or blood (26).

For diagnostic laboratories, implementation of PCR for testing skin and surface samples is challenging. Since a positive HSV culture result from a sample collected 12 to 24 h after delivery is evidence of virus replication (8), result interpretation seems to be straightforward. However, there are insufficient data about the performance and interpretation of nucleic acid amplification assays, such as PCR, for these samples in neonates. Moreover, currently, there are no molecular assays cleared by the FDA for testing mucosal (eye/nasopharynx/anus) samples. Except for a limited number of reference laboratories, it is difficult for hospital-based diagnostic laboratories to collect a sufficient number of cases to perform extensive validation of the performance characteristics of PCR. Recently, Dominguez et al. reported comparison of PCR with surface sample culture for the diagnosis of neonatal HSV disease (27). A total of 97 surface samples that had been tested using both shell vial and tube culture methods were retrospectively tested with PCR. Seven of these cases had clinical HSV disease. PCR detected HSV in 6 cases (85.7%), while culture was positive for 3 of them. Both methods were shown to have 100% specificity. Although more data need to be collected, results of this report are helpful and encouraging.

Testing HSV DNA on blood samples by PCR has recently been shown to be beneficial for laboratory diagnosis of neonatal infection. Cantey et al. reported on 6 years of data obtained from two medical centers (28). In 21 neonatal cases, HSV was detected in blood samples (plasma; J. B. Cantey, personal communication) from all patients, including 6 cases (29%) for which blood HSV PCR was the first or the only positive diagnostic test result. The authors reported an additional case documenting detection of HSV by PCR of blood in a neonate before the onset of clinical signs of the infection (29). Melvin et al. reported blood PCR results from a large group of neonatal HSV patients treated at Seattle Children's Hospital (18). Testing of plasma samples was positive in 83% of the 63 neonates diagnosed with HSV disease, and detection of the virus in plasma did not necessarily indicate disseminated disease: HSV PCR was also positive in 78% of patients with clinical SEM disease and 64% thought to have isolated CNS disease (18, 30). Interestingly, higher HSV DNA copy numbers in blood correlate with disseminated disease compared to CNS and SEM disease and are also associated with increased risk of mortality (18, 31). More clinical study and test standardization for testing blood samples are needed. While blood PCR is important in assisting diagnosis of neonatal HSV disease, results are not recommended to be used to guide duration of therapy (8). HSV DNA copy numbers in blood can remain elevated in some patients for many weeks

(18), but the clinical significance of this observation is uncertain, and there are no recommendations for longitudinal testing of blood in patients being treated for neonatal HSV. In contrast, PCR of spinal fluid near the end of a treatment course is indicated in patients with HSV encephalitis, as detection of HSV DNA in the CSF at that time is an indication for prolonging treatment (8, 16).

For diagnostic laboratories, a practical question regarding testing HSV in blood is the selection of sample type. Whole blood is recommended to be tested by the American Academy of Pediatrics (AAP) (8). Some reports have used plasma or serum samples (18, 28), which are also used to test for other viruses by PCR for the purpose of reducing potential inhibition of amplification and/or for sample stability during transportation and storage. There are no data regarding the level of HSV DNA in different blood cellular and noncellular compartments during neonatal infection. Therefore, more data on the relative advantages of whole blood versus plasma for detection (and potentially quantification) of HSV by PCR will be useful. Currently, there are no FDA-cleared HSV molecular assays for any blood sample types, so users need to establish assay parameters.

Although HSV PCR performed on CSF is the most sensitive test for virologic confirmation of CNS disease, a negative result does not rule out the possibility of CNS infection. This may be due to the nature of CNS viral infections (including HSV encephalitis) or early sample collection time during the disease course (4, 8, 13, 30, 32). Therefore, when clinical suspicion is high, the collection of multiple samples is recommended (32). Histologic findings and virus culture of brain biopsy tissue, if available, can also confirm the diagnosis of HSV encephalitis. Interestingly, although HSV DNA copy number is associated with mortality, CSF viral load is not associated with neurologic outcome (18).

In clinical laboratories, screening criteria for testing HSV on CSF samples have been well established and used to effectively improve test utilization (33–35). Suggested criteria include testing CSF samples collected only from patients who are immunocompromised, who have CSF white blood cell counts of >5 (or 10) cells/mm^3, who have elevated CSF protein levels, or who are older than 2 years of age. Therefore, these screening criteria do not apply when testing samples for neonatal HSV disease.

PROGNOSIS

Mortality from neonatal HSV disease was dramatically improved by the introduction of high-dose acyclovir treatment as standard care (15). Prior to the availability of effective antiviral treatment, mortality at 12 months after neonatal infection was more than 50%, with high-dose acyclovir reducing these rates to less than 30% for disseminated disease and less than 5% for encephalitis (15). Even with appropriate treatment, survivors of neonatal HSV disease remain at risk for recurrent skin lesions, recurrent CNS disease, and neurodevelopmental impairment (36). Accordingly, suppressive oral acyclovir treatment was commonly prescribed to survivors of neonatal HSV disease to decrease the chances of these complications. A recent multicenter study validated the use of suppressive treatment for decreasing the frequency of skin recurrences in patients with a history of SEM disease and for improving neurodevelopmental outcomes among patients with a history of CNS involvement (17). Nevertheless, a significant proportion ($\sim$30%) of survivors of neonatal HSV involving the CNS had abnormal neurologic outcomes in this study, even with suppressive treatment. Additional treatment options are needed that may minimize or mitigate neurologic injury in these patients.

PREVENTION

The protective effect of Caesarian delivery on the risk of neonatal HSV disease (Table 1) has led to national guidelines indicating Caesarean delivery for women with a history of genital infection who are in labor and either have active genital lesions or prodromal symptoms such as genital pain or burning (37). Although recurrent maternal disease

confers a lower risk of transmission to the newborn than primary infection (12), transmission may still occur in this setting, and the risk of operative delivery is considered to be balanced by the risk of serious disease in the newborn. Importantly, Caesarian delivery itself is not completely protective against HSV transmission, even when delivery occurs prior to the rupture of membranes (38). Caesarian delivery is not recommended in asymptomatic women, even when there is a history of genital HSV infection (37).

Guidance is available for managing asymptomatic infants with potential exposure to HSV at delivery due to the presence of visible genital lesions in the mother (39). In this situation, type-specific maternal serology is helpful in assessing the risk of transmission to the baby and in guiding the management of the infant, with testing and treatment based on the classification of maternal infection as primary episode, nonprimary first episode, or reactivation. Note that for infants born to mothers with a history of genital HSV but no active lesions, the risk of neonatal HSV is low (8), and many experts recommend close clinical observation with a low threshold for testing.

Suppressive antiviral treatment is commonly used in pregnant women with a history of genital HSV infection, but whether serologic screening of all pregnant women for prior HSV infection should be pursued remains controversial (40). Use of serology to routinely screen for genital HSV has been discouraged by the U.S. Preventive Services Task Force due to the potential for a high rate of false-positive results (41). Although there are insufficient data to support a benefit of suppressive acyclovir treatment of the mother in reducing transmission to newborns, this approach has been shown to reduce rates of Caesarian delivery (42). American College of Obstetricians and Gynecologists (ACOG) recommendations are to offer suppressive acyclovir treatment to women with active recurrent genital HSV at or beyond 36 weeks of gestation (37); although the drug is considered safe to use in all stages of pregnancy, guidelines from the Centers for Disease Control and Prevention note that there is insufficient evidence to routinely offer antiviral suppressive therapy late in gestation for HSV-2-seropositive women without any history of genital outbreaks (43). Notably, vertical transmission of HSV to newborns can occur even in the setting of suppressive acyclovir treatment of the mother, and at least one case of transmission of acyclovir-resistant HSV has been reported (19).

It is interesting to consider the observation that detection of HSV from the vaginal tract at the time of delivery confers a >300-fold increased risk of neonatal disease for the baby (12). This suggests there could be clinical benefit from rapid diagnostic testing of vaginal fluid in the delivery room. Such testing could be achievable with current technology, but has not to the authors' knowledge been developed or investigated.

A number of attempts at developing vaccines to prevent HSV infection or disease have been pursued, but those which have made it to clinical trials have proved disappointing (44–46). One challenge for the field is whether vaccines that prevent primary HSV infection or genital disease can be ensured to prevent vertical transmission of infection to newborns (47).

SUMMARY

Neonatal HSV infection can cause devastating disease, warranting prompt recognition and aggressive patient management. Due to the nonspecific nature of clinical symptoms, laboratory virological diagnosis contributes to earlier diagnosis and ensures targeted treatment. Current guidelines recommend virus culture and/or PCR assays on multiple samples, including surface samples (eye, mouth, nasopharynx, and anus), skin lesions, blood, and CSF samples. More studies are needed to establish or verify the application of nucleic acid amplification assays in detecting HSV from surface samples and to determine the optimal testing matrix (plasma versus whole blood) from blood samples.

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