LAB NEWS

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Clinical Virology Laboratory Newsletter

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Update in Herpes Simplex Virus PCR

Herpes simplex virus (HSV) is associated with a variety neurologic syndromes including: 1) a severe sporadic, temporal lobe encephalitis; 2) encephalitis in neonates, either alone or in combination with disseminated disease; 3) a self-limited meningitis in 36% of women and 13% of men with primary genital herpes[1]; 4) a benign recurrent lymphocytic meningitis usually associated with unrecognized genital herpes[2]. In addition, some cases of Bell's palsy, myelitis and radiculitis are associated with HSV. The majority of encephalitis cases outside the neonatal period are associated with HSV type 1, whereas most neonatal infections and meningitis cases are associated with HSV type 2.

Recently, it has been reported that one-fifth of cases of HSV encephalitis (HSVE) may be mild or atypical (i.e. no CSF pleocytosis or focal findings at presentation). Atypical HSVE is most frequently found in immunocompromised hosts or in asymmetric HSV infection involving predominantly the nondominant temporal lobe[3]. In addition, HSV has occasionally been found in CSF as a co-infection and without symptoms[4].

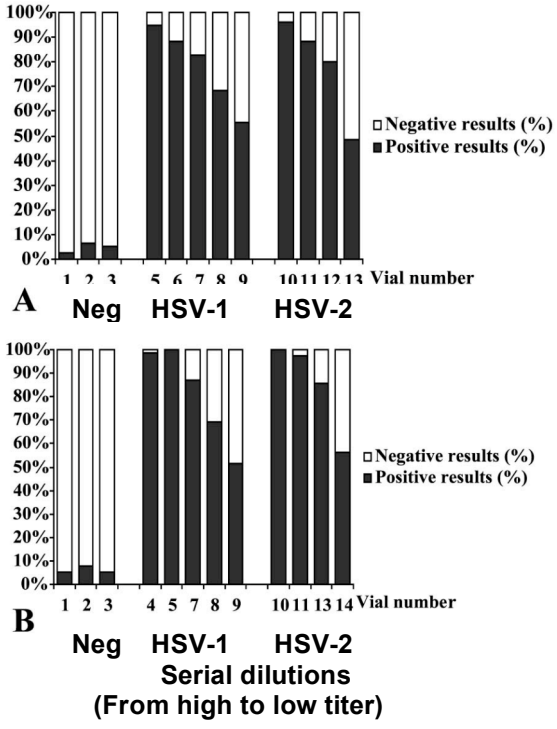
Role of molecular testing. Detection of HSV DNA in CSF by molecular amplification methods, such as the polymerase chain reaction (PCR), has greatly facilitated diagnostic testing and has essentially eliminated the need for brain biopsy. However, PCR protocols and quality control vary tremendously from lab to lab.

Variations in quality. In a recent study of 27 coded samples tested by 66 labs, 30 false positive HSV PCR results were reported, as well as 30-50% false negative results for samples containing low titers of 0.2-7 copies/μL.

Method used in the Virology Laboratory. In March 2005, real-time TaqMan PCR targeting the polymerase (pol) gene replaced the prior standard PCR[5,6]. The sensitivity of the assay is < 0.25-1 copy/μL CSF for HSV-1 and 0.5-5 copies/μL CSF for HSV-2. *Multiple assays were tested before the current assay was selected.*

Typing is by a separate real-time multiplex assay targeting the gB gene[7]. Since the typing assay is slightly less sensitive than the type-common assay, it is only used once a positive is identified. For some low positive samples, typing may not be successful.

Blinded Multi-center Study of HSV PCR



J Clin Virol 28:175-185, 2003)

Sample requirements. 0.5-1.0 ml CSF should be submitted to the Virology Laboratory. Note: Delays in transport and improper storage can adversely affect results.

Test availability and time to result. HSV PCR is performed Monday through Saturday. Samples *received in the Virology Laboratory* by 7:30-8 AM will be tested the same day, with results available by 5 PM.

Caution in interpretation of results.
Clinical correlation is essential in the interpretation of all laboratory tests.
HSV encephalitis**:** While the assay performed in Virology is highly sensitive, a negative CSF result cannot absolutely exclude HSVE. Published reports have found that false negatives can occur due to testing too early or too late, improper sample transport, or low volumes of CSF tested. HSVE is frequently fatal untreated. Therefore, if MRI shows compatible temporal lobe findings and no alternative diagnosis is established, continued treatment with acyclovir should be strongly considered. A second spinal tap with repeat CSF PCR or a brain biopsy may be indicated. (NOTE: A head CT obtained early in the course may be falsely negative.)

Recurrent meningitis: CSF samples for PCR should be collected at the onset of symptoms. Samples collected late in the course or between episodes of meningitis will usually be negative. In contrast with HSV encephalitis, HSV meningitis in the healthy host is a self-limited disease. However, antiviral therapy may shorten the course.

Positive results in the absence of disease: A true-positive CSF HSV PCR can be obtained without neurologic disease (e.g. with HSV reactivation from trigeminal ganglion in a patient with ophthalmic zoster)[8]. In addition, false positive results can occur with PCR due to cross-contamination, even in the best of laboratories[9]. With real-time PCR, the cycle threshold (C_T) when a sample becomes positive is an indicator of relative viral load. In our laboratory, when very low positive results are obtained, the test is repeated with doubling of the input CSF to determine if it is a repeatable result. At the same time, the Virology Laboratory director calls the physician to discuss the case and obtain clinical information. Some of these low positives do not repeat and are considered contaminants. If the result is still in question, excess CSF from other labs will be retrieved and the test repeated again. To our knowledge, we have not issued a false positive report.

We encourage clinicians to notify the laboratory whenever there are questions about a test result.

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