

Herpes Simplex Testing

Background Information

The herpes family contains Herpes Simplex virus (HSV) types 1 and 2, Varicella-zoster virus, Cytomegalovirus, Epstein-Barr virus, and human Herpes viruses 6 through 8.

HSV types 1 and 2 produce infections that are expressed in various clinical manifestations ranging from mild stomatitis to disseminated and fatal disease. The more common clinical conditions include gingivostomatitis, keratitis, encephalitis, vesicular skin eruptions, aseptic meningitis, neonatal Herpes, genital tract infections, and disseminated primary infection. Infections with HSV types 1 and 2 can differ significantly in their clinical manifestations and severity. HSV type 1 is closely associated with infections of the mouth and lips, although genital infections can be common in some populations. HSV type 2 is the cause of the majority (approximately 90%) of urogenital infections and is most often found in adults.

Diagnostic Testing and Interpretation

I. Culture

Cells or fluid from a fresh lesion are collected and placed into appropriate viral transport media. A portion of the specimen collected is then used to inoculate an appropriate cell culture suspension which is then incubated for several days. If the Herpes virus is in the specimen, typical cytopathic effect (syncytial formation) will be produced and type specific fluorescent antibody stains are used to identify the type (1 or 2) of Herpes virus present.

Viral culture has been recognized in the past as the gold standard in diagnosing Herpes virus infections. However, false negative results can occur that are often attributable to sampling error or the deterioration of the specimen in transport.

II. Viral Antigen Detection

Cells collected from a fresh sore are scraped and placed onto a microscope slide. Type specific antibody labeled with a fluorescent dye is then applied to the slide. The type specific antibody links to surface antigens on the virus and fluorescence is produced. This fluorescence is then detectable using a specific microscope that shows the antigen-antibody coupling to the virus as well as the morphologic feature of the infected cells. This test is used in conjunction with viral culture or, quite often, in place of culture to improve test turn-around-time.

III. Viral Antibody Testing

Two types of antibodies (IgG and IgM) can be detected in serological testing. A positive result with IgM antibodies indicates recent infection (usually within 21 days) whereas a positive result with IgG antibodies is usually associated with past exposure to the virus. Paired serum specimens, collected several weeks apart, can demonstrate a significant rise in IgM antibody level which indicates a recent infection if IgM methods are not available.

It is important to note that the presence of HSV1 and/or 2 antibodies may indicate past, primary, or reactivated infection, but cannot reliably distinguish between them. Furthermore, the continued presence or level of antibody detected cannot be used to determine the success or failure of therapy. In addition, a negative result does not necessarily rule out a primary or reactivated infection since specimens collected too early or too late in the course of the disease may have undetectable levels of antibody. This point is particularly important when comparing serologic methods to direct testing methods that use polymerase chain reaction technology.

Cross-reactivity with other viruses in the Herpes family occurs frequently. Epstein-Barr virus infections can produce false-positive results in HSV1 and 2 enzyme immunoassays. A heterotypic rise in anti-HSV antibody levels may also be observed in a primary or reactivated Varicella-zoster virus infection. Since rheumatoid factor (RF) binds to IgG in immunocomplexes, false-positive results may arise in specimens with RF and specific IgG. False-negatives may arise due to specific IgG competing with specific IgM. In pregnant women, the serologic test should never be used as the sole criterion for the diagnosis of current HSV infection.

Results obtained with antibody testing should serve as an aid to diagnose HSV infection and should not be interpreted as diagnostic independent of other testing technology and clinical impression.

IV. Polymerase Chain Reaction (TEM-PCR)

Herpes diagnostic testing using PCR is considered to be definitive; particularly in genital and dermal specimens. Viral nucleic acid is extracted from the specimen and specific primers directed to the target DNA produce a specific amplicon. Amplification of the amplicon in a very carefully controlled thermocycling step produces a large volume of PCR products. Following the addition of a fluorescent dye (SAPE), the amplicon of the Herpes virus is detected on either a flow cytometer or microarray platform. The specificity and sensitivity of the assay is very high; as few as 80 and 150 plaque forming units can be detected for HSV 1 and HSV2 respectively.

It is important to note that false negative test results can occur when patients are being actively treated with Acyclovir. HSV (DNA) rapidly disappears from the mucosal surfaces of patients receiving 400 mg twice daily thus reducing the recovery of virus in culture attempts by 100% and the detection with PCR by 80%.