Diagnosis of Herpes Simplex Virus
Introduction

Herpes simplex virus (HSV) is a common human pathogen found worldwide which produces a wide range of diseases. HSV specifically infects skin, mucous membranes and neurons of the dorsal root ganglia where the virus establishes lifelong, latent infection.

There are currently eight known types of herpes viruses which affect humans, with two specific Herpes simplex viruses (HSV1 & HSV2) that are responsible for a range of common clinical conditions.

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<th>Virus</th>
<th>Condition</th>
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<td>Herpes Simplex Virus 1, HSV-1</td>
<td>Cold Sores / Oral Herpes</td>
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<td>Neonatal HSV</td>
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<td>Genital Herpes</td>
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<td>Herpetic Whitlow</td>
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<td>Herpes Simplex Virus 2, HSV-2</td>
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Genital herpes is the second most prevalent sexually transmitted infection in Australia with up to one in eight sexually active adults infected. About 20% of HSV-2 seropositive patients have overt recurrences of genital herpes and 60% have lesions but they are not recognised. The remaining 20% have true asymptomatic shedding. For this reason genital herpes infections are often not recognised or properly diagnosed. Therefore people with genital herpes do not always receive the appropriate treatment. Neonatal herpes varies in prevalence from 1 in 2500 live births in the USA to 1 in 13,000 births in Australia. Most neonatal HSV infections occur through delivery via an infected birth canal and most cases are due to asymptomatic viral shedding in the mother. HSV-2 causes 70% of cases of neonatal herpes, but as the number of genital HSV-1 infections increase this may result in a rise in cases of neonatal herpes cases due to HSV-1.

Cold sores (oro-facial) herpes are often recurrent and are usually caused by HSV-1 and acquired in early childhood. Over 70% of adults in Australia have evidence of infection with this virus. Lesions may be widespread in people with eczema and severe in those who are immunocompromised. In primary HSV infection of the oropharynx, the most common manifestation is gingivostomatitis and this may be accompanied by fever and submandibular lymphadenopathy.
HSV-1 is a major causative agent of keratitis and blindness in the world. Recurrent HSV keratitis results in the accumulation of scarred lesions and therefore ocular HSV infections require antiviral therapy to prevent scarring.

The Importance of Diagnosis

There is no cure for HSV infections. Once a primary infection of a herpes virus establishes latency, it will persist in the host's cells for life.

Antiviral medication is available to treat most herpes virus infections and has been shown to decrease symptomatic recurrences of genital herpes, including the frequency of viral shedding and the transmission of the virus to sexual partners.

All patients with suspected genital herpes simplex infection requiring therapy should have confirmation of infection. The Pharmaceutical benefits Scheme (PBS) requires doctors to provide proof of microbiological confirmation of the diagnosis, whenever they prescribe antiviral medication (viral culture, antigen detection or nucleic acid amplification by PCR). It is important to note that confirmation of the diagnosis should not delay appropriate therapy.

Methods of Diagnosis

The PBS have specified three methods of diagnosis, however, there are four diagnostic techniques available in Australia – viral isolation, direct antigen detection, PCR and type-specific serology.

Virus Isolation

Virus isolation by culture has traditionally been the laboratory “gold standard” and is most sensitive when lesions are fresh and moist. Taking a sample for culture requires breaking a fresh lesion (oral or genital) and collecting vesicle fluid and/or cells rubbed from the base of a lesion, using a dacron/ rayon-tipped plastic swab. This is then transported in an appropriate viral transport medium. Virus isolation as a method of diagnosing herpes infections requires optimal collection and transport conditions and some pathology laboratories no longer routinely use this method. However, reference laboratories still use this technique.

Culture results, when available, distinguish between HSV-1 and HSV-2 infection and swabs can be taken from mucocutaneous sites such as endocervical swabs, anogenital swabs, throat and conjunctival swabs. A negative test result does not exclude infection.
**Direct Antigen Detection**

Direct Antigen detection sample collection requires vesicle fluid and cells rubbed from the base of a lesion, using a dacron/rayon-tipped plastic swab. Tissue or material from the eye or CFS may also be submitted for rapid antigen detection if herpes encephalitis or ocular infection is suspected. This is then transported in an appropriate viral transport medium.

Direct Antigen detection is a rapid, type specific and sensitive method for detecting HSV. Procedure involves a direct immunofluorescence using fluorescein- labeled monoclonal antibodies specific for HSV antigens. It has high sensitivity, when vesicles are present, however, sensitivity will fall as lesions heal. A negative test result does not exclude infection.

**PCR**

Genomic detection by polymerase chain reaction (PCR), including Real time PCR is highly sensitive, fully automated and more rapid than viral culture or direct antigen detection. PCR allows simultaneous detection and differentiation of HSV-1 and HSV-2. It is more sensitive than other detection methods late in infection when the lesions have begun to crust. In Australia, most private pathology laboratories use either an in-house PCR or a commercial kit.

The most commonly used commercial kit is Roche LightCycler HSV 1/2 Detection Kit but the Artus HSV-1/2 PCR kit is also available. A negative test result does not exclude infection.

**Type Specific Serology**

Viral culture and PCR methods are accurate and reliable tests for diagnosing Herpes simplex infections when lesions are apparent. However, patients often present when lesions are healed or when lesions are absent. In addition, HSV may fail to grow in the laboratory or the specimen may be inadequate for antigen detection or PCR.

In this situation, a blood sample can be taken for detection of antibodies to HSV. HSV antibody testing can reveal acute infection or previous exposure. HSV type specific antibody testing is available using Western blot or enzyme immunoassay. Western blot is considered the gold standard in serological testing for HSV antibody but the test is technically difficult, expensive and is only available in a small number of laboratories.
There are now a number of commercially available ELISA tests for type-specific HSV infection. These tests are relatively simple to perform in the laboratory, and are now being produced by a number of companies. Their sensitivity varies dependent on the antigen preparation used in the test; most now use recombinant antigens. The reliability and reproducibility of these tests also varies, and may be dependent upon the experience of the particular laboratory. The most appropriate test for use in particular situations has yet to be determined. Some of the tests only detect HSV-2 infections; others are able to detect both HSV-2 and HSV-1 infections. Currently the HSV-1 tests are specific, but have unacceptably low sensitivity. HSV-2 specific tests appear to be more sensitive but one widely used test has been shown to have a relatively high rate of false positives.

Type Specific Serology may be useful where culture and PCR are repeatedly negative, to identify infected patients and to help them recognise symptoms and to reduce the chance of transmission to sexual partners. HSV type-specific IgM can be detected in acute infection, usually within a few days of clinical lesions, but may take up to 3 weeks to appear. Specific IgM response may also appear in severe clinical recurrences. Individual episodes of recurrent genital herpes cannot be reliably diagnosed by serological testing.

Other methods for the diagnosis of herpes simplex virus such as kits for the rapid detection of antibody to HSV-1 and HSV-2 have been developed but are not used routinely in Australia. A multiplex bead-based assay for type specific antibody detection has also been developed.

Further Reading

The AHMF produces a wide range of clinical guidelines which can be used as further resources, including - The Use of Type Specific Serology for the Diagnosis of Genital Herpes, Managing Genital Herpes, Reducing the Sexual Transmission of Genital Herpes, Managing Oral Herpes and Herpes Infections of the Eye. These and other clinical guidelines are available from the AHMF.
BIBLIOGRAPHY


Ashley RL. Sorting out the new HSV type-specific antibody tests. Sex Transm Infect 2001;77:232-237.


