



L a b o r a t o r y *News*

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Molecular Diagnostic Testing for Herpes Simplex Virus Infections Expands to Swab Samples from Skin and Mucosal Lesions

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Beginning May 25th 2010, Marshfield Labs will accept swab samples from genital, skin and mucosal lesions for PCR detection of Herpes Simplex Virus (HSV-1 and HSV-2).

We encourage providers to order PCR testing in place of viral culture when applicable because the PCR test offers substantially better sensitivity for these viruses. In-house testing of culture negative samples revealed that 20% were positive by the new PCR method; the positive results were confirmed by a reference laboratory.

Sample collection will remain unchanged and providers should send swabs in M4-RT multi-microbe medium kept at refrigerated temperature. The lab test code for swabs will be the same test code currently in place for CSF samples (HS12PCR).

In the Combined Medical Record, the test name HSV by PCR will be used for both CSF and swab samples. The sample source is listed in the *Lab by Date* view; however, providers should be aware that the *Lab by Panel* view does not show the sample source.

Tissue samples for HSV-1 and HSV-2 will continue to be sent to Mayo Medical Laboratories under test code MISC until Marshfield Labs has the opportunity to validate tissue samples using the PCR method.

Background

The family Herpesviridae includes several viruses that infect humans. In addition to HSV-1 and HSV-2, the family

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HOW TO ORDER THIS TEST

HERPES SIMPLEX VIRUS
(HSV)1/2 BY RAPID PCR
LAB TEST CODE: HS12PCR
COM: HSV by PCR
Centricity: Herpes Simplex I/II
by PCR
DOWNTIME: Write-in (Form II)

Specimen

Swabs: Vesicular fluid and cellular material from the base of a lesion (skin, eye, oral, genital). Specimens from early stage vesicular lesions rather than ulcerative or crusted lesions should be obtained. Place swabs in M4-RT multi-microbe medium and break off swabs at least 0.5 inch below top of tube. Specimens collected on wood-shafted, cotton or calcium alginate swabs are not acceptable.
CSF: 0.5 mL spinal fluid collected in a sterile vial.

Storage

Swabs: Refrigerate. Frozen or non-refrigerated specimens are unacceptable.
CSF: Refrigerate. Frozen CSF is acceptable - avoid freeze/thaw cycles.

Available

Set up Monday through Friday.
One day analytical time.

Qualitative interpretation reported as Negative, Positive, or Indeterminate for Herpes Simplex Virus.

CPT Code

87529

Please direct questions to the Molecular Pathology Laboratory or Dr. Uphoff at 800-222-5835.

also includes varicella zoster virus, Epstein-Barr virus, cytomegalovirus and human herpes viruses 6-8. HSV is a common viral pathogen that is found worldwide and is responsible for various infectious manifestations in neonates, children and adults. HSV-1 and HSV-2 can be found in and released from the lesions that the viruses cause, but they are also released between outbreaks from skin in the absence of an obvious lesion. Generally, a person can only acquire HSV-2 infection during sexual contact with someone who has a genital HSV-2 infection. HSV-1 can cause genital herpes, but it more commonly causes infections of the mouth and lips, so-called "fever blisters." HSV-1 infection of the genitals can be caused by oral-genital or genital-genital contact with a person who has an HSV-1 infection. Genital HSV-1 outbreaks recur less regularly than genital HSV-2 outbreaks. Following primary infection, HSV can reside sequestered within the sensory neurons and has the ability to reactivate causing recurrent clinical or sub-clinical infection. Viral shedding during both reactivation and latency is, therefore, of considerable significance in virus transmission. Thus, transmission can occur from an infected person who does not have a visible lesion and may not know that he or she is infected.

By 40 years of age, approximately 90 percent of adults are seropositive for antibodies to HSV serotype 1 and/or 2, emphasizing not only the ubiquity of the viruses but the inherent limitations of immunoserologic testing for diagnostic purposes. According to CDC estimates released in March 2010, about 1 in 6 Americans (16.2 percent) between the ages of 14 and 49 is infected with HSV-2.

Diagnostic Testing for Herpes Simplex Virus Infections

HSV-1 and HSV-2 can cause several infections including genital lesions; encephalitis; meningitis; oropharyngeal, cutaneous and ocular infections; and neonatal systemic or localized infections.

There are several clinical circumstances that emphasize the importance of identifying and differentiating between the specific serotypes responsible for the infectious process:

- It has recently been documented that approximately 30 percent of first episodes of genital herpes are caused by HSV-1. The initial presentation of these infections is identical to those caused by HSV-2 but recurrences are less frequent with HSV-1.
- HSV-2 infections are known to increase risk for HIV-1 infections and other sexually transmitted diseases.
- In CNS infections, HSV-1 is more commonly associated with encephalitis, while HSV-2 is more often associated with aseptic meningitis.

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- The selection and administration of antiviral treatments in certain clinical situations may be based on the specific serotype of HSV identified.
- With appropriate laboratory testing, the provider may be able to recommend specific and directed counseling for the patient.

Testing Options

Laboratory testing presently includes virologic, immunoserologic and molecular studies.

- Cell Culture:
 - Cell culture isolation of HSV is an appropriate test choice provided the patient presents with genital or other mucocutaneous lesions that are appropriate for sampling. The sensitivity of viral culture is less than PCR and decreases as soon as the lesions begin to heal.
- Rapid Assay by Direct Fluorescent Antibody (DFA):
 - Rapid assay uses the DFA methodology. Like cell culture, the optimal specimen is a cellular scraping of a lesion that is obtained within a few days of onset. This methodology detects specific HSV antigen(s) and differentiates HSV-1 from HSV-2. While the turn around time for this assay may be faster than PCR, the PCR test is considered more sensitive.
- Serologic Testing:
 - Serologic testing allows detection of Type 1 and/or Type 2 HSV antibodies. The presence of antibodies to either or both HSV serotypes indicates exposure but does not differentiate between recent and past infection. HSV infected individuals may not be seropositive in the early stage of primary disease. If clinically indicated, retesting of these patients in 4-6 weeks is recommended. Results will be reported as POSITIVE, NEGATIVE, or EQUIVOCAL. In those instances when a result of EQUIVOCAL is reported, a second specimen should be resubmitted in 4-6 weeks. NEGATIVE results do not rule out the diagnosis of HSV disease. The time required to seroconvert following primary infection varies and the serum may have been obtained before the occurrence of detectable antibodies.
- Type Specific HSV DNA Detection by PCR:
 - PCR is the test of choice for the diagnosis of HSV infection of the central nervous system using a cerebrospinal fluid sample. CSF testing by PCR for suspected HSV disseminated disease of the neonate is also recommended.
 - PCR testing for HSV is also recommended for swab collected secretions or tissue biopsy specimens from skin and mucosal sites.
 - Results will be reported as POSITIVE, NEGATIVE, or INDETERMINATE. Indeterminate will be used when results are inconclusive due to inhibition of the PCR reaction. Repeat testing with a new specimen is recommended.


The following information is presented to optimize the laboratory detection of HSV infections based on selected specimen types and in various clinical settings:

Encephalitis, Meningitis, and Neonatal Disease			
Specimen Type	Specific Assay	Where It is Most Useful	Test Code
Cerebrospinal fluid	Herpes Simplex Virus, DNA Detection by PCR (Type 1 and 2 differentiation).	Sensitive assay useful for detecting and differentiating HSV 1 and 2 DNA in CSF in disseminated disease. Generally performed in suspected HSV neonatal disease. A negative result does not exclude a diagnosis of HSV infection.	COM: HSV by PCR Lab Code: HS12PCR
Genital Track Infections			
Specimen Type	Specific Assay	Where It is Most Useful	Test Code
Cellular material from base of lesion	Herpes Simplex Virus, DNA Detection by PCR (Type 1 and 2 differentiation).	Sensitive assay useful for detecting and differentiating HSV 1 and 2 DNA in active lesions. PCR has been shown to be more sensitive than culture.	COM: HSV by PCR Lab Code: HS12PCR
Serum	IgG Type-specific HSV antibodies 1 and 2.	Detects type-specific IgG antibodies in patients with recent or past, symptomatic or asymptomatic, infection.	COM: HSV Type Spec Ab Lab Code: HSV12AB
Cellular material from base of lesion	Herpes Simplex Virus Culture.	Culture is useful for isolating HSV virus from active tissue lesions. Viruses isolated are typed and reported as HSV 1 or as HSV 2.	COM: Culture, Herpes Lab Code: HSVC
Cellular material from base of lesion	Herpes Simplex Virus Antigen.	Antigen detection identifies and differentiates HSV 1 and 2 in cellular material from an active tissue lesion.	COM: HSV Antigen, Lesion Lab Code: HSAG
Tissue biopsy	Herpes Simplex Virus, DNA Detection by PCR (Type 1 and 2 differentiation), assay performed by Mayo Medical Laboratories.	Sensitive assay useful for detecting HSV DNA in tissue specimens. Assay differentiates between HSV 1 and 2 DNA.	COM: HSV by PCR, Tissue Lab Code: MISC

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1. Seroprevalence of Herpes Simplex Virus Type 2 Among Persons Aged 14-49 Years, United States, 2005-2008. MMWR, April 23, 2010; 59(15);456-459.
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3. Weinstock H, Berman S, Cates W. Sexually transmitted diseases among American youth: Incidence and prevalence estimates, 2000. *Perspectives on Sexual and Reproductive Health* 2004; 36:6-10.
4. Xu F, Sternberg M, Kottiri B, McQuillan G, Lee F, Nahmias A, Berman S, Markowitz L. National trends in herpes simplex virus type 1 and type 2 in the United States: Data from the National Health and Nutrition Examination Survey (NHANES). *JAMA* 2006; Vol 296: 964-973. 


Changes to Cystic Fibrosis Carrier Detection Test in COM (Clinical Order Manager) and Portal Order Screens

Timothy S. Uphoff, PhD, DABMG, MLS(ASCP)^{CM}

As of May 2010, Marshfield Labs will have a new enhanced format for Cystic Fibrosis Carrier Detection Test (Cystic Fibrosis, DNA) in Clinical Order Manager (COM) and Portal Order screens. Required clinical information has been streamlined for ease of use. If the patient's information is submitted electronically, a duplicate paper request does not need to be sent along with the specimen.

The following items summarize the information that will be requested.

- Ethnicity:
Ethnicity should be indicated for all patients using a drop-down choice or users may free text other ethnicities or admixtures of more than one ethnicity.
- Reason for Testing:
The reason for testing should be indicated *either* in section A if the testing is for carrier screening, *or* section B if testing a patient with a suspected diagnosis of Cystic Fibrosis (CF) or a CF related disorder.
 - If for carrier screening, complete only section A.
To accurately incorporate a family history of CF in the interpretation please include:
 - The relationship between the patient and affected relative.
 - Whether the relative is affected or a carrier of CF.
 - The identity of the relative's mutations if known.
 - If the patient's partner is a known carrier of CF.
 - If the patient has a suspected diagnosis of CF or a CF related disorder, complete only section B.
 - All clinical symptoms should be indicated.
 - The results of sweat testing should be included.

If you have any questions, please feel free to contact the Molecular Pathology Laboratory or Dr. Uphoff at 800-222-5835. 

Relationship Between HbA1c and a Patient's Estimated Average Glucose

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In 2008, the American Diabetes Association (ADA) published a study that defined an *Algebraic Relationship Between HbA1c and a Patient's Estimated Average Glucose (eAG)*^[1]. The ADA also determined that the HbA1c could be reliably reported as eAG, which would be in the same units as a patient's daily glucose self-monitoring.

Diabetic persons are quite familiar with their glucose values and understand that concentrations are specific to the moment in time that blood is taken. However, that result does not give any indication of what their glucose concentrations are at other times of day or over time, as does the HbA1c test. Since eAG uses the same units (mg/dL) as glucose meters, the ADA hopes that using the eAG will help patients understand its utility and better monitor their long-term glucose management.

Conversion of the HbA1c to eAG follows from a simple regression equation:

$$eAG_{\text{mg/dL}} = 28.7 \cdot \text{HbA1c} - 46.7$$

Therefore, inherently, one has no more value than the other. The benefit of the eAG is that clinicians can use it as a tool to explain to patients what their HbA1c result means, relative to glucose. The following table shows the relationship between the two.

HbA1c (%)	eAG (mg/dL)
6	126
6.5	140
7	154
7.5	169
8	183
8.5	197
9	212
9.5	226
10	240

An eAG calculator is also available on the ADA website (<http://professional.diabetes.org/GlucoseCalculator.aspx>). In addition, this web page includes links to the original study^[1] and other useful information.

Because diabetics are more likely to monitor their glucose before meals (when their blood glucose levels are low), the eAG is not expected to match the average glucose concentration that a patient would get from self-monitoring. That is, the average of multiple glucose meter readings by a patient is likely to be lower than their eAG, which, like HbA1c, reflects their true average glucose, including postprandial levels.

Beginning May 2010, the eAG is included on laboratory reports as an adjunct to the HbA1c result.

Please direct questions to Lab Customer Service at 800-222-5835.

[1] Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, and Heine RJ for the A1C-Derived Average Glucose (ADAG) Study Group. "Translating the A1C assay into estimated average glucose values." *Diabetes Care*. 2008; 31:1473-1478. 