



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

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## Routine Cerebrospinal Fluid Viral Cultures No Longer Performed

Timothy Uphoff, PhD, DABMG, MLS(ASCP)<sup>CM</sup> ; Thomas Novicki, PhD, DABMM; Thomas Fritsche, MD, PhD

As of September 5, 2012, Marshfield Labs will discontinue viral culture of cerebrospinal fluid (CSF). Polymerase chain reaction (PCR) amplified nucleic acid testing for enterovirus, herpes simplex virus (HSV) and varicella-zoster virus (VZV) are performed at Marshfield Labs and are superior tests for detection of these viral pathogens in CSF samples.

### BACKGROUND

Only a few viruses are cultivable from CSF and the sensitivity of CSF viral culture is often extremely poor (Gorgievski-Hrisoho, Schumacher, et al., 1998). Viral detection by culture can take up to 14 days. Viral nucleic acid detection by PCR is a much more rapid and sensitive method that has replaced routine viral cultures of CSF. A large retrospective study of more than twenty-two thousand CSF viral cultures showed 99.7% of isolates were enteroviruses, HSV, or VZV (Polage and Petti, 2006). The remaining 0.3% of viral pathogens detected consisted of cytomegalovirus (CMV) and adenovirus. This study also found that PCR detected 48% more enteroviral infections (246/949) than viral culture (124/949). The HSV positivity rate by viral culture was 0% among 1,290 samples tested by both culture and PCR, while the PCR HSV positivity rate was 0.7% (9/1,290). When indicated, CSF samples can also be tested by PCR for Epstein-Barr virus (EBV), CMV, adenovirus, and JC Virus.



**LABORATORY TESTING**

The enterovirus PCR (EVPCR) assay is a FDA approved qualitative test for the direct detection of enterovirus from CSF samples (see [Marshfield Labs, Laboratory News, Vol. 33, No. 8 - Sept. 28, 2010](#)). The assay amplifies a region in the viral RNA that is highly conserved between all enteroviruses, allowing for 63 serotypes to be detected. The EVPCR assay at Marshfield Labs is available 24 hours daily. This real-time PCR test is completed within three hours of sample arrival in the lab.

To identify the cause of viral meningitis, rapid PCR methods for HSV (Test code-HS12PCR) and VZV (Test code-VZVPCR) are also available at Marshfield Labs (see [Marshfield Labs, Laboratory News, Vol. 33, No. 4 - May 21, 2010](#), and [Vol. 33, No. 7 - July 30, 2010](#)). The HS12PCR test will differentiate between HSV 1 and HSV 2. Both HS12PCR and VZVPCR can be performed on the same 0.5 mL CSF sample. EVPCR also requires 0.5 mL, therefore 1.0 mL of CSF is required to perform all three tests. HS12PCR and VZVPCR are performed daily Monday through Friday and can also be performed on weekends if necessary.

When clinically indicated, PCR amplification tests are available for EBV, CMV, Adenovirus, and JC Virus. Marshfield Labs will facilitate sending such testing to a reference laboratory. An important exception is West Nile virus (WNV), which is detected very poorly by PCR with a sensitivity as low as 10%. The gold standard for WNV diagnosis remains serology (Test code-WNVAB). The only remaining indication for a CSF viral culture is when PCR testing is not available for CSF and the virus is cultivable. Detection of unusual viruses in the CSF often requires special cell lines for culture. Please consult the laboratory in such in these cases.

**HOW TO ORDER ENTEROVIRUS DETECTION BY PCR***Lab Test Code: EVPCR*

- COM: Enterovirus PCR
- Centricity: Enterovirus CSF, PCR
- Downtime: Write-in (Form II)

*Specimen:*

1.0 mL (0.5 mL minimum) cerebral spinal fluid in a sterile transport container; can be stored for 24 hours at room temperature or up to three days refrigerated.

*Storage:*

Refrigerate up to 72 hours. Frozen CSF is acceptable - avoid freeze/thaw cycles.

*Available:* Set up 7 days/week, 24 hours/day; Turnaround Time: Analytic time is 3 hours after arrival in lab.

*Qualitative Interpretation:*

- Negative result
- Positive result
- Indeterminate with commentary recommending repeat testing if clinically indicated

*CPT Code: 87498*

### HOW TO ORDER HERPES SIMPLEX VIRUS (HSV)1/2 BY RAPID PCR

*Lab Test Code:* HS12PCR

- COM: HSV by PCR
- Centricity: HSV by PCR
- Downtime: Write-in (Form II)

*Specimen:*

CSF: 0.5 mL spinal fluid collected in a sterile vial.

*Storage:*

Refrigerate. Frozen CSF is acceptable - avoid freeze/thaw cycles.

*Available:* Set up Monday through Friday; one day analytical time.

*Qualitative Interpretation:*

- Negative result
- Positive result
- Indeterminate with commentary recommending repeat testing if clinically indicated

*CPT Code:* 87529

### HOW TO ORDER VARICELLA ZOSTER VIRUS BY RAPID PCR

*Lab Test Code:* VZVPCR

- COM: VZV by PCR
- Centricity: VZV by RAPID PCR
- Downtime: Write-in (Form II)

*Specimen:*

CSF: 0.5 mL spinal fluid collected in a sterile vial.

*Storage:*

Refrigerate. Frozen CSF is acceptable - avoid freeze/thaw cycles.

*Available:* Set up Monday through Friday; one day analytical time.

*Qualitative Interpretation:*

- Negative result
- Positive result
- Indeterminate with commentary recommending repeat testing if clinically indicated

*CPT Code:* 87798

### INTERPRETIVE QUESTIONS:

Contact any of the following at 715-221-6700 (or ext. 1-6700):

Timothy Uphoff, PhD; Thomas Fritsche, MD, PhD; Thomas Novicki, PhD; Mary Stemper, MS.

**REFERENCES**

1. Gorgievski-Hrisoho, M., Schumacher, J.-D., et al. (1998). *Detection by PCR of Enteroviruses in Cerebrospinal Fluid during a Summer Outbreak of Aseptic Meningitis in Switzerland*. *Journal of Clinical Microbiology*, 36(9): 2408-2412.
2. Polage, C. R. and Petti, C. A., (2006). *Assessment of the Utility of Viral Culture of Cerebrospinal Fluid*. *Clinical Infectious Diseases*, 43(12): 1578-1579. 

**Evolution of Herpes Simplex and Varicella Zoster Viral Testing: Move to Molecular Diagnostics and the Discontinuation of Culture**

Thomas Novicki, PhD, DABMM; Timothy Uphoff, PhD, DABMG, MLS(ASCP)<sup>CM</sup>; Thomas Fritsche, MD, PhD

Effective September 5, 2012, Marshfield Labs will retire the Herpes Simplex 1/2 (HSV 1/2) viral culture. Providers should instead order herpes simplex virus (HSV) 1/2 and/or varicella zoster virus (VZV) rapid polymerase chain reaction (PCR) tests for any specimen type other than fluids and tissues. Both HSV and VZV PCR tests should be considered since dermal lesions caused by these two viruses may be difficult to distinguish clinically. (Note that historically a VZV-only culture has not been offered due to the reduced ability for recovery of this virus in culture.) The HSV 1/2 PCR will detect and differentiate HSV 1 and HSV 2.

The HSV 1/2 and VZV PCR tests have been in use at Marshfield Labs for several years. The reasons behind retiring the HSV culture are several:

- HSV 1/2 PCR was found in an internal study to be approximately 20% more sensitive than HSV culture. The added sensitivity of HSV 1/2 PCR allows for improved viral detection during asymptomatic shedding, from very early lesions and from healing lesions, all scenarios where HSV culture is often negative. However, PCR positivity rapidly drops off once shedding has ceased.
- HSV 1/2 PCR does not require viable viral particles, making optimal transport less of an issue.
- HSV 1/2 PCR has a one day analytical time whereas the HSV culture takes from 1-3 days to complete.

HSV and VZV antigen detection by direct fluorescent antibody (DFA) will remain available for vesicular ulcers and external lesions. While being slightly lower in cost than the corresponding PCR tests, these DFAs are significantly less sensitive, and require scrupulous attention to collection from a fresh lesion. They are not sensitive enough to reliably detect virus that is being asymptotically shed, nor virus in very early or healing lesions.

**ORDERING AND AVAILABILITY**

See previous article.

### INTERPRETIVE QUESTIONS:

Contact any of the following at 715-221-6700 (or ext. 1-6700):

Timothy Uphoff, PhD; Thomas Fritsche, MD, PhD; Thomas Novicki, PhD

### REFERENCES

1. Ramaswamy, M., et al. 2004. *Diagnosis of Genital Herpes by Real Time PCR in Routine Clinical Practice*. Sex. Transm. Infect. 80:406.
2. Wald, A., et al. 2003. *Polymerase Chain Reaction for Detection of Herpes Simplex Virus (HSV) DNA on Mucosal Surfaces: Comparison with HSV Isolation in Cell Culture*. J. Infect. Dis. 188:1345.
3. Stránská, R., et al. 2004. *Routine use of a Highly Automated and Internally Controlled Real-time PCR Assay for the Diagnosis of Herpes Simplex and Varicella-zoster Virus Infections*. J. Clin. Virol. 30:39. 📄

## Change in Specimen Requirements for the Dilute Russell's Viper Venom Time

Michael J. Sanfelippo, MS, MT(ASCP), Technical Director of Coagulation Services

The Dilute Russell's Viper Venom Time is a test frequently used to identify the lupus anticoagulant. Until recently, there was an impression that the use of warfarin by a patient being tested with this procedure would interfere with the test and result in false positives. Recent studies in this laboratory have proved that the use of warfarin does not result in falsely abnormal results with this test. Our findings were supported by a recent publication. Therefore patients receiving warfarin can be tested for the lupus anticoagulant provided their INR is in the therapeutic range of 2-3. The warning regarding use of warfarin under specimen requirements for the Dilute Russell's Viper Venom Time has been removed.

Please address any questions or concerns regarding this change to the technical director of coagulation services at 715-221-6320.

### REFERENCE

Olteanu H, Downes K, Patel J, Praprotnik D, Sarode R. *Warfarin Does Not Interfere with Lupus Anticoagulant Detection by Dilute Russell's Viper Venom Time*. Clin Lab 2009, 55:138-142. 📄