



Laboratory *News*

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Molecular Diagnostic Testing for Varicella-Zoster Virus Infections Expands to Swab Samples from Skin and Mucosal Lesions

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Beginning August 2, 2010, Marshfield Labs will accept swab samples from genital, skin and mucosal lesions for the detection of varicella-zoster virus (VZV) by PCR. We encourage providers to order PCR testing in place of viral culture or VZV antigen when applicable because the PCR test offers substantially better sensitivity for these viruses. In-house testing of culture/antigen negative samples revealed that 10% were, in fact, 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 (VZVPCR).

If the patient has a herpetic appearing rash with a differential diagnosis including both VZV and Herpes Simplex virus (HSV-1 and HSV-2), PCR testing for each can be performed using the same swab sample. Both VZVPCR and HS12PCR must be ordered, however, a single swab specimen can be submitted. In the same manner, a single CSF specimen can be submitted when both VZVPCR and HS12PCR are ordered.

BACKGROUND

The family Herpesviridae includes several viruses that infect humans. In addition to varicella-zoster virus (VZV), the family also includes herpes simplex virus (HSV) serotypes 1 and 2, Epstein-Barr virus, cytomegalovirus, and human herpes viruses 6-8. VZV causes both varicella (chickenpox) and herpes zoster (HZ or shingles).



HOW TO ORDER THIS TEST

VARICELLA ZOSTER VIRUS
BY RAPID PCR
TEST CODE: VZVPCR

Specimen

Swabs: Vesicular fluid and cellular material from the base of a lesion (skin). 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 inches 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 varicella-zoster virus.

Indeterminate results are inconclusive due to inhibition of the PCR reaction. Repeat testing with a new specimen is recommended.

CPT Code

87798

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

Varicella or Chickenpox

VZV produces a generalized vesicular rash on the dermis in normal children, usually before the age of 10 years. It is a highly contagious rash illness that is transmitted from person to person by direct contact with patients or by airborne spread (from respiratory secretions or fluid from skin lesions). The average incubation period is 14-16 days (range: 10-21 days). People with varicella are considered infectious from 1-2 days before the rash appears and until all lesions are crusted over (average range: 4-7 days after rash onset). In most cases, chickenpox is generally a mild disease; however, infants, adolescents, adults, and immunocompromised persons are at higher risk for complications. Severe complications include secondary bacterial infections, dehydration, pneumonia, encephalitis, and cerebellar ataxia.

Herpes Zoster or Shingles

After primary infection with VZV, the virus persists in latent form and may emerge (typically in adults over age 50) clinically to cause a unilateral vesicular eruption, generally in a dermatomal distribution. People with HZ are infectious during the vesicular stages of rash; the rash typically crusts over within 7-10 days but may take from 2-6 weeks to heal completely. Localized HZ is generally less infectious than varicella. HZ is generally not life threatening, but can cause painful postherpetic neuralgia.

DIAGNOSTIC TESTING FOR VARICELLA ZOSTER VIRUS INFECTIONS

Laboratory confirmation of varicella-zoster cases is becoming more important, as fewer cases are seen and a higher proportion of these occur among those individuals vaccinated. PCR detection offers several advantages over traditional virus culture:

- more rapid test results
- improved sensitivity
- small sample size required

Testing Specifics

Laboratory testing presently includes molecular, culture, antigen detection, and immunoserologic studies.

- VZV detection by PCR:
 - PCR is the test of choice for the diagnosis of VZV infection of the central nervous system using a cerebrospinal fluid sample. CSF testing by PCR from suspected VZV disseminated disease of the neonate is also recommended.
 - PCR testing for VZV is also recommended for swab collected secretions or tissue biopsy specimens from skin and mucosal sites.

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- If the patient has a herpetic appearing rash with a differential diagnosis including both VZV and Herpes Simplex virus (HSV-1 and HSV-2), PCR testing for each can be performed using the same swab sample. Both VZVPCR and HS12PCR must be ordered, however, a single swab specimen can be submitted. In the same manner, a single CSF specimen can be submitted when both VZVPCR and HS12PCR are ordered.
- Cell Culture:
Cell culture isolation of VZV, while available, has a relatively long turnaround time, and is frequently insensitive compared to VZV antigen or nucleic acid detection. Its use should be reserved for those specimen sources that are not validated by the PCR or antigen tests (e.g., autopsy tissues).
- Direct Fluorescent Antibody (DFA):
Direct fluorescent antibody methodology detects specific VZV antigens. The optimal specimen is a cellular scraping of a lesion that is obtained within a few days of onset. Cellular scrapings from the base of vesicular lesions are collected and spread over the three wells on the slide provided in the HSV/VZ Collection Kit (supplies and instructions provided in kit). While the turnaround time for this assay may be faster than PCR, the PCR test is considered more sensitive.
- Serologic Testing:
Serologic testing allows detection of IgG or IgM anti-VZV antibodies. The presence of antibodies to VZV indicates exposure to the virus. VZV infected individuals may not be seropositive in the early stage of primary disease.
 - A positive IgG result coupled with a positive IgM result indicates recent infection with VZV.
 - A positive IgG result coupled with a negative IgM result indicates previous exposure to VZV and immunity.
 - A negative IgG result coupled with a negative IgM result indicates the absence of prior exposure to VZV and nonimmunity. However, a negative result does not rule out a VZV infection. The time required to seroconvert following primary infection varies and the serum may have been obtained before the occurrence of detectable antibodies.


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

Encephalitis, Meningitis, and Neonatal Disease			
Specimen Type	Specific Assay	When it is Most Useful	Test Code
Cerebrospinal fluid	Varicella-zoster Virus DNA Detection by PCR	Sensitive assay useful for detecting varicella-zoster virus DNA in CSF in disseminated disease. Generally performed in suspected VZV neonatal disease. A negative result does not exclude a diagnosis of VZV infection.	VZVPCR

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Skin Infections			
Specimen Type	Specific Assay	When it is Most Useful	Test Code
Cellular material from base of lesion	Varicella-zoster Virus DNA Detection by PCR	Sensitive assay useful for detecting varicella-zoster virus DNA in active lesions. PCR has been shown to be more sensitive than culture.	VZVPCR
Serum	Varicella-zoster Virus Antibody, IgG	Detects type-specific IgG antibodies in patients with recent or past, symptomatic or asymptomatic, infection.	VZI
Serum	Varicella-zoster Virus Antibody, IgG & IgM Assay performed by Mayo Medical Laboratories	Detects type-specific IgG and IgM antibodies in patients with recent or past, symptomatic or asymptomatic, infection. A positive result is generally predictive of immunity to VZV infection.	VZDSO
Cellular material from base of lesion	Virus Culture	Culture may be useful for isolating VZV virus from active tissue lesions when the PCR and antigen tests cannot be used (autopsy).	VIR
Cellular material from base of lesion	Varicella-zoster Virus Antigen, DFA	Antigen detection identifies VZV from cellular material of an active tissue lesion.	VAG

REFERENCES:

1. Varicella Vaccination: Centers for Disease Control and Prevention. Updated 10/20/2009. Retrieved 7/16/2010. Available from: <http://www.cdc.gov/vaccines/vpd-vac/varicella/default.htm#clinical>
2. Varicella Disease Questions & Answers: Centers for Disease Control and Prevention. Updated 6/13/2007. Retrieved 7/16/2010. Available from: <http://www.cdc.gov/vaccines/vpd-vac/varicella/dis-faqs-gen.htm> 

New Reference Ranges for Cardiolipin Antibody and Beta 2 Glycoprotein 1 Antibody

Michael J. Sanfelippo, MS, MT(ASCP)

New assay kits for cardiolipin antibody and beta 2 glycoprotein 1 antibody will be put into use on August 9, 2010. The new reference ranges will be as follows:


Cardiolipin Antibody		
IgG	<9.4 - 14.9 Units	Negative
	15.0 - 20.0 Units	Indeterminate
	20.1 - 80.0 Units	Low-moderate positive
	> 80 Units	High positive
IgM	<9.4 - 12.4 Units	Negative
	12.5 - 20.0 Units	Indeterminate
	20.1 - 80.0 Units	Low-moderate positive
	>80 Units	High positive
IgA	< 9.4 - 11.9 Units	Negative
	12.0 - 20.0 Units	Indeterminate
	20.1 - 80.0 Units	Low-moderate positive
	>80 Units	High positive

Beta 2 Glycoprotein 1 Antibody		
IgG	<9.4 - 20.0 Units	Negative
	20.1 - >150 Units	Positive
IgM	<9.4 - 20.0 Units	Negative
	20.1 - >150 Units	Positive
IgA	<9.4 - 20.0 Units	Negative
	20.1 - >150 Units	Positive

Please direct any questions regarding these changes to:

Michael J. Sanfelippo, MS, MT(ASCP)
 Technical Director of Coagulation
 715-221-6320

or

Mary C. Baldauf, MD
 Medical Director Immunodiagnostics
 715-221-6313 

Test Changes for the Diagnosis of *Cryptosporidium* and *Giardia* Infection

Thomas Novicki, PhD, DABMM, Jason Campbell MS, MT(ASCP), and Thomas Fritsche, MD, PhD

Effective August 2, 2010, the tests available for the laboratory diagnosis of intestinal parasites will be revised. Laboratory testing for the diagnosis of *Cryptosporidium* sp. and *Giardia lamblia*, the two most prevalent intestinal parasites in the United States (U.S.), will be requested using OVA AND PARASITE, ROUTINE (Test code: OPRCG). This testing will detect both parasites using a rapid Enzyme Immunoassay (EIA) method. *Cryptosporidium* sp.-only and *Giardia lamblia*-only EIA tests will also be available for those instances when only one organism need be detected (e.g., outbreaks). These tests will replace OVA AND PARASITE, ROUTINE (Test code: OPR). If other intestinal parasites are suspected (e.g., in foreign-born individuals or after recent travel abroad), OVA AND PARASITE, SPECIAL (Test code: OPS) should be used. This test remains unchanged.

EIA studies for the fecal antigens of *Cryptosporidium* sp. and *G. lamblia* are now known to have superior performance to other methods of diagnosis. Marshfield Labs has used such an EIA assay for many years, and will continue to do so. Our test menu for the diagnosis of intestinal parasitic infections will change to the following:

1. As a first line test for individuals with symptoms consistent with cryptosporidiosis or giardiasis, and no significant travel history (i.e., no travel outside the U.S. and Canada), the *Cryptosporidium* sp. and *G. lamblia* EIA, (Test code: OPRCG) should be considered (see sidebar for ordering information). Due to the high sensitivity of this test, most cases will be detected with a single specimen. However, a second test may be required if the first is negative by this test and clinical suspicion for these diseases remains high. This test code replaces test code OPR.
2. *Cryptosporidium* sp. (Test code: OPRC) and *G. lamblia* (Test code: OPRG) EIA's and will be available separately for those cases when only one parasite is suspected (e.g., during outbreaks).
3. The traditional O&P exam (OPS) should be ordered in cases where the clinical index of suspicion is high for an intestinal parasite and/or there is a recent history of travel outside of the U.S. and Canada. Three specimens, collected 1-3 days apart, should be submitted for maximum sensitivity. This test code remains unchanged.

Repeat testing for a test of cure is not routinely recommended. If repeat testing is indicated following treatment for *Cryptosporidium* sp. or *G. lamblia* due to unremitting or recurrent symptoms, the traditional O&P exam (OPS) should be ordered with a comment requesting a review for the original parasite. The *Cryptosporidium* sp./*G. lamblia* EIA should not be used in these cases since parasite antigens may persist in the stool for several weeks following successful eradication of the organism.

BACKGROUND

Cryptosporidiosis and giardiasis are the most common intestinal parasite diseases in the U.S.; they are also found worldwide. Between 2003 and 2005, 20-21,000 cases per year of giardiasis were reported to the Centers for Disease Control and Prevention (CDC). Similarly, between 2006 and 2008, 6-12,000 cases of cryptosporidiosis were reported annually to the CDC. Many more cases likely go undetected or are not reported since these diseases are typically self-limiting and may be asymptomatic. Because of asymptomatic shedding and because the infectious dose of both *Cryptosporidium* sp. and *G. lamblia* is low, these organisms have a strong potential to cause outbreaks. Yet, many cases of both cryptosporidiosis and giardiasis are thought to occur sporadically.

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HOW TO ORDER THESE TESTS

Cryptosporidium sp. and *G. lamblia* EIA
OVA AND PARASITE,
ROUTINE
Test code: OPRCG
CPT coding: 87328, 87329

G. lamblia EIA
OVA AND PARASITE
ROUTINE, GIARDIA
Test code: OPRG
CPT coding: 87329

Cryptosporidium sp. EIA
OVA AND PARASITE
ROUTINE, CRYPTO
Test code: OPRC
CPT coding: 87328

Full O&P exam
OVA AND PARASITE, SPECIAL
Test code: OPS
CPT coding: 87209, 87177

Specimen

Fresh stool specimen placed into a two vial Para-Pak Formalin and Zinc PVA Ova and Parasite collection kit. Add sufficient stool specimen to bring liquid in the vial to the fill line. Specimens left unpreserved for more than 30 minutes may produce misleading results.

Storage

Room temperature.

Available

Set up Monday through Friday.
Results available within 24 hrs.

Please direct questions to the microbiology laboratory,
Dr. Thomas Fritsche or
Dr. Thomas Novicki at
800-222-5835.

C. parvum, *C. hominis*, and *G. lamblia* are the parasitic species in humans; other species of both *Cryptosporidium* and *Giardia* are known to infect various animals. Transmission occurs through contact with infected persons or animals, or by ingestion of fecally contaminated food or water. These protozoans are hardy, being resistant to routine chlorine-based drinking and recreational water treatments, and also to alcohol-based hand sanitizers.

Most cases of giardiasis are asymptomatic or present as an acute self-limited diarrhea. However, chronic diarrhea or frequent loose stools, steatorrhea, abdominal cramps, bloating, fatigue, malabsorption, and/or weight loss can also occur. Jejunal and duodenal mucosal cell damage or reactive arthritis have infrequently been reported. HIV infection and other forms of immunosuppression do not predispose individuals to giardiasis, nor do they modify the severity or course of the disease.

Cryptosporidiosis occurs asymptotically, but also presents as a profuse watery diarrhea accompanied by cramping abdominal pain. Malaise, fever, anorexia, nausea and/or vomiting can also be present. In otherwise healthy persons, the infection generally clears within one month of onset. In contrast, the disease in immunosuppressed patients, especially HIV-positive ones, typically has a more fulminant course that can lead to death.

The laboratory diagnosis of intestinal parasites has classically relied on the "ova and parasite" (O&P) exam. This test is composed of a concentration step for helminth eggs and protozoan cysts, and a Wheatley trichrome stain for the trophozoite stage of protozoans; a wet preparation for motile parasites may also be included if the specimen is under an hour old. The O&P exam, while still the test of choice for helminthic and many protozoan intestinal infections, is not the best choice for *Cryptosporidium* sp. and *G. lamblia*: *Cryptosporidium* sp. is not readily observed in the concentrate, trichrome or wet prep components of the O&P, and while *G. lamblia* is an inhabitant of the upper small intestine, it can have a low concentration in feces, the material of choice for the O&P exam.

Select References

1. Yoder, JS, C. Harral, and MJ Beach. Cryptosporidiosis Surveillance - United States, 2006 - 2008. In Surveillance Summaries, June 11, 2010. MMWR 2010, 59(SS06); 1-14.
2. Yoder, JS and MJ Beach. Giardiasis Surveillance - United States, 2003 - 2005. In Surveillance Summaries, Sept. 7, 2007. MMWR 2007, 56(SS07); 11-18. 