

8/24/2016

## U.S. Laboratories Testing for Zika Virus Infection

### Summary:

Unlike many viruses, Zika virus requires multiple tests performed at various stages of exposure and/or infection depending on the case. There are a number of commercially available Zika virus tests on the market today, public health anticipates increased testing in the private sector. While this will increase laboratory capacity, IDEpi wants to make sure our public health partners are aware of the following considerations.

1. If a physician suspects Zika, please contact IDEpi at 800-256-2748. This is the best option for rapid identification of Zika cases and immediate institution of prevention and control measures.
2. The Centers for Disease Control and Prevention (CDC) recommends that all specimens from Zika suspects that meet CDC testing criteria with a negative Zika PCR result receive IgM antibody testing. CDC advises providers requesting molecular testing (e.g. PCR) for Zika virus infection from a clinical or commercial testing laboratory to retain and store (refrigerate at 2°C -8°C) an aliquot of the patient's serum for subsequent Zika IgM ELISA testing if the rRT-PCR is negative. If a retained specimen is not available or was not collected within the appropriate time frame for antibody testing, another specimen must be collected.
3. Please keep in mind that if a sample is submitted to the state Office of Public Health (OPH) laboratory is Zika rRT-PCR negative, we automatically test for IgM.

As in most things related to Zika, testing guidance is complicated and evolving as experts learn more about this virus. For laboratory specific questions go to <http://www.cdc.gov/zika/laboratories/lab-guidance.html>

### Symptomatic individuals meeting epidemiological criteria:

Serum and urine collected from symptomatic **patients less than 14 days** post onset of symptoms should be tested by Zika virus real time reverse transcriptase-polymerase chain reaction (rRT-PCR). A positive Zika rRT-PCR result in either specimen is sufficient to diagnose Zika virus infection. If Zika virus rRT-PCR results are negative for both specimens, serum should be tested by antibody detection methods.

Serum that has been collected from **patients presenting 2 to 12 weeks** from onset of symptoms should be tested first by anti-Zika immunoglobulin (IgM) detection methods. Serum from symptomatic pregnant women should also be accompanied by a urine specimen. Because of the potential for cross-reactivity, all serum specimens obtained from symptomatic individuals for whom serological testing is recommended

should be tested for the detection of anti-Zika IgM, and anti-dengue IgM. For those with exposure risk and a clinically compatible illness, anti-chikungunya IgM testing should also be performed.

- For non-pregnant symptomatic patients, anti-Zika IgM positive or equivocal result is followed by plaque reduction neutralization test (PRNT) at CDC.
- For symptomatic pregnant women, anti-Zika IgM positive or equivocal result is followed by rRT-PCR on both serum and urine. Some pregnant women have been reported to have detectable RNA present in serum and/or urine beyond the acute phase. If the rRT-PCR is negative, PRNT is necessary to confirm the presence of anti-Zika antibodies.

### **Asymptomatic pregnant women meeting epidemiological criteria for testing:**

If serum and urine have been collected from a pregnant woman **patients less than 14 days** of her exposure, serum and urine should be tested by rRT-PCR. If negative, a second serum specimen should be collected 2 to 12 weeks following exposure and tested by antibody detection methods.

If serum from a pregnant woman first **presenting 2 to 12 weeks** following exposure is collected or living in areas of ongoing transmission, the serum should be tested for anti-Zika IgM. If positive or equivocal, rRT-PCR should be performed on the serum and urine. If rRT-PCR is negative, PRNT should be performed for confirmation of IgM result.

### **Asymptomatic non-pregnant women or males are not recommended to be tested for Zika.**

#### Who is performing PCR testing for Zika virus infections?

Because PCR is a commonly used method for looking for many viruses and bacteria, there is more laboratory capacity (many commercial laboratories) for this test than the other Zika tests. PCR is very specific and fast relative to the other tests available (results can often be given within one-two days). CDC recommends the use of the FDA-authorized Trioplex Real-time RT-PCR Assay (Trioplex rRT-PCR) for detection of Zika, dengue, and chikungunya viral RNA in specimens collected from individuals suspected of Zika infection and meeting CDC clinical and epidemiological criteria. The OPH Lab is running the Trioplex. As far as we know, no reference lab is running the Trioplex. Please refer to the attachment for more information.

#### Who is performing ELISA testing for Zika virus infections?

One large commercial laboratory and the state OPH Laboratory are performing ELISA testing for Zika virus. CDC has recently licensed their FDA-approved test for use by the four major commercial laboratories. One has started testing; three are expected to begin soon. ELISA is labor intensive and can take up to three days to perform a single test. This test tells us whether a person has IgM antibodies to Zika virus. The type of antibody the Zika virus test looks for is generated by the body's immune system within 7 to 10 days after infection and can last for several weeks. ELISA can cross-react with antibodies to dengue virus and West Nile virus, causing false positives for Zika virus in some cases. This is because Zika virus, dengue virus and West Nile virus all belong to the flavivirus family. Because these family members are very similar, ELISA can be positive if the person has a current flavivirus infection. For this reason, a positive ELISA test result requires follow-up testing with PRNT. Please refer to the following pages for more information.

Who is performing PRNT testing for Zika virus infections?

The CDC laboratory can perform this very specialized type of testing. PRNT is a highly complex test that requires laboratory scientists to grow the virus in their laboratory. PRNT is a different type of serology test that can detect antibodies from a recent or past Zika infection.

PRNT can be used to help determine if the initial ELISA test was positive due to Zika infection or if there was a cross-reaction with dengue virus or another flavivirus.

Agent	Method(s)	Laboratory	Specimen
Zika, chikungunya, and dengue RNA*	<b>FDA-authorized kit (EUA):</b> CDC Trioplex rRT-PCR	<b>CDC, OPH Laboratory</b>	<b>Serum and Urine (collected alongside a patient matched serum)</b>
Zika RNA only ( <i>this is not recommended for pregnant women</i> )	<b>FDA-authorized kit (EUA):</b> commercial laboratories: <ul style="list-style-type: none"> <li>· Focus Diagnostics Zika Virus RNA Qualitative Real-Time RT-PCR*</li> <li>· Altona RealStar Zika Virus RT-PCR</li> <li>· Hologic Aptima Zika Virus Assay (transcription-mediated amplification TMA test)</li> <li>· Viracor-IBT Zika Virus RT-PCR</li> <li>· Siemens VERSANT Zika RNA 1.0 Assay kit</li> <li>· Luminex xMap MultiFLEX Zika RNA Assay</li> </ul>	<p><i>*QUEST is the only lab running Focus kit</i></p> <ul style="list-style-type: none"> <li>-available at LABCORP and for purchase by other reference laboratories</li> <li>-available for purchase by other reference laboratories</li> <li>- only available at Viracor-IBT</li> <li>-available for purchase by other reference laboratories</li> <li>-available for purchase by other reference laboratories</li> </ul>	<ul style="list-style-type: none"> <li>Serum only</li> <li>Serum and Urine (collected alongside a patient matched serum)</li> <li>Serum and Plasma only</li> <li>Serum and Urine (collected alongside a patient matched serum)</li> <li>Serum and Urine (collected alongside a patient matched serum)</li> <li>Serum and Urine (collected alongside a patient matched serum)</li> </ul>
Dengue RNA only	FDA-cleared kit: CDC DENV-1-4 Real-Time RT-PCR Assay		

**Important Points:**

Co-infection is rare, but possible so it is important to note that a positive result for one of these viruses does not preclude infection with the others.

Serum: Zika virus is usually detected in serum during the acute phase of infection. Due to the difficulty in many cases of precisely determining the onset date of symptoms, as well as the general low level of Zika viremia in serum, all rRT-PCR negative serum specimens should be tested by serological methods as well.

Urine: Zika virus RNA has been detected in urine for a longer period of time than in serum (Bingham et al., 2016). Based on a limited number of cases, detection of Zika virus RNA has been demonstrated up to 14 days after onset of symptoms. Beyond 14 days, urine testing may still be useful, but there are limited data to determine Zika virus RNA persistence in urine. CDC recommends that, for symptomatic individuals presenting up to 14 days after onset of symptoms, urine be collected alongside serum and be tested by rRT-PCR (MMWR, 2016).

If Zika rRT-PCR testing was conducted for a patient and any of the patient’s specimens yielded positive results, the patient is positive for Zika infection. No antibody testing is required.

Please note that Zika, dengue and chikungunya virus infections are all on the 2016 list of nationally notifiable conditions. Therefore, results of testing should be reported back to IDEpi 800-256-2748 to facilitate investigation and classification of the case and reporting to CDC.

Agent	Method(s)	Laboratory
Anti-Zika IgM*	FDA-authorized kit (EUA):	CDC or OPH Laboratory Labcorp Laboratory
	CDC Zika MAC-ELISA *	
	ZIKV Detect IgM Capture ELISA (InBios, USA)	None yet
Anti-Dengue IgM	FDA-cleared kit (EUA):	OPH Laboratory Focus Diagnostics ARUP Laboratories Quest Diagnostics Mayo Medical Laboratories
	DENV Detect IgM Capture ELISA (InBios, USA)	
Anti-Chikungunya IgM	Genway Anti-Chikungunya Virus IgM	OPH Laboratory
	Anti-Chikungunya IgM	Focus Diagnostics ARUP Laboratories Mayo Medical Laboratories

<http://new.dhh.louisiana.gov/assets/oph/Center-PHCH/Center-CH/lab/Virology/ZikaMacELISA053116.pdf>

<https://www.labcorp.com>

<http://new.dhh.louisiana.gov/assets/oph/Center-PHCH/Center-CH/lab/Virology/DengueIgM031516.pdf>

<http://www.focusdx.com/>

<https://arupconsult.com>

<http://www.specialtylabs.com/>

<http://www.mayomedicallaboratories.com/>

<http://new.dhh.louisiana.gov/assets/oph/Center-PHCH/Center-CH/lab/Virology/ChikIgM031516.pdf>

<http://www.focusdx.com/>

<https://arupconsult.com>

<http://www.mayomedicallaboratories.com/>

**Important Points:**

Negative results do not preclude infection with Zika virus and should not be used as the sole basis of a patient treatment/management decision.

Please note that Zika, dengue and chikungunya virus infections are all on the 2016 list of nationally notifiable conditions. Therefore, results of testing should be reported back to IDEpi 800-256-2748 to facilitate investigation and classification of the case and reporting to CDC.

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