Zika virus: An update on the disease and guidance for laboratory testing

Committee on Laboratory Practices, ASM and Advocacy/Public Relations Committee, PASCV

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Background:

Zika virus is a flavivirus that is transmitted by *Aedes* spp. (particularly *A. aegypti*) mosquitoes, which are found in the U.S. *Aedes* spp. are also vectors for dengue and chikungunya viruses. Zika was first identified in 1947, but has recently been associated with a large outbreak of disease in Central and South America, especially in Brazil. On February 1, 2016, the World Health Organization (WHO) declared Zika virus a public health emergency of international concern.


Clinical Presentation:

The vast majority (~80%) of individuals who are infected with Zika virus remain asymptomatic. In symptomatic patients, common symptoms are nonspecific and include: fever, rash, headache, arthralgia, and/or conjunctivitis. In most cases, the illness is mild and symptoms resolve without further complications in approximately 1 week. The period of viremia is believed to be brief, typically up to a week after symptom onset.

Following the 2015 outbreak in South America, an association was postulated between Zika virus infection during pregnancy and congenital disease. Specifically, a potential increased risk of microcephaly and intracranial calcifications in neonates born to mothers infected with Zika virus during pregnancy was noted. Another possible association with Zika virus is Guillain-Barré syndrome.


Laboratory Testing:

Most individuals that are infected with Zika virus will not require laboratory testing. Both Dengue and Chikungunya viruses should also be included in the differential diagnosis and testing for these viruses may be appropriate.

Diagnostic testing may be warranted for patients who live in or have recently travelled to an endemic region and are critically ill, hospitalized, or pregnant, or infants born to Zika virus positive mothers.
The following laboratory testing is recommended, based on patient risk factors:

For pregnant women with a clinical illness consistent with Zika within 2 weeks of travel to areas with documented Zika virus transmission:

- Zika virus reverse transcription polymerase chain reaction (RT-PCR) on maternal serum. This is recommended during the first 7 days following symptom onset; however, the sensitivity of RT-PCR decreases significantly after 5-7 days. Urine PCR testing may extend the period during which RNA is reliably detected up to 2 weeks after symptom onset. Gourinat, et. al. 2015. EID 21:84, http://www.cdc.gov/mmwr/volumes/65/wr/mm6518e1.htm
- IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA IgM) testing with confirmation by a neutralizing antibody assay on maternal serum. Sera should be collected ≥4 days after symptom onset.

For pregnant women without symptoms, but with travel history within 2 weeks to areas with documented Zika virus transmission, or sexual contact with an individual with an appropriate travel history and clinical illness consistent with Zika infection:

- MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on maternal serum. Sera should be collected ≥4 days after symptom onset.

For cases where fetal microcephaly or intracranial calcifications are observed on prenatal ultrasound:

- MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on maternal serum.
- An amniocentesis may be considered, depending on gestational age. Zika virus RT-PCR can be performed on amniotic fluid.

For infants with microcephaly or intracranial calcifications or infants whose mothers have positive or inconclusive test results for Zika virus (potential congenital infection):

- Zika virus RT-PCR on infant serum.
- MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on infant serum.
  - The initial sample should be collected either from the umbilical cord or directly from the infant within 2 days of birth, if possible.
- If cerebrospinal fluid is obtained for other studies, test for Zika virus RNA, Zika virus IgM and neutralizing antibodies.
- If not already performed during pregnancy, test mother’s serum for Zika virus IgM and neutralizing antibodies.
• Consider histopathologic evaluation of the placenta and umbilical cord with Zika virus immunohistochemical staining on fixed tissue and Zika virus RT-PCR on fixed and frozen tissue.

**Available laboratory tests:**

Currently, laboratory testing for Zika virus is only available at designated public health laboratories and the Centers for Disease Control and Prevention (CDC).

• MAC-ELISA IgM with confirmation by plaque-reduction neutralization test (PRNT); no IgG testing is being performed at this time.
• RT-PCR on serum, CSF, urine and amniotic fluid.

**Table 1. Tests used based on when the specimen is collected after symptom onset**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Days Post-onset</th>
<th>Test(s) Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>≤4</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>4-7*</td>
<td>RT-PCR, IgM, Flavivirus PRNT</td>
</tr>
<tr>
<td></td>
<td>&gt;7</td>
<td>IgM, Flavivirus PRNT</td>
</tr>
<tr>
<td>Urine, Saliva, Amniotic fluid</td>
<td>Currently being evaluated</td>
<td>Zika RT-PCR</td>
</tr>
</tbody>
</table>

* IgM negative specimens collected ≤7 days should be repeated ≥21 days post-onset.

RT-PCR: reverse transcription-PCR
PRNT: plaque reduction neutralization test

**Table 2. Specimen collection, testing and required volume**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Test performed</th>
<th>Specimen Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum and CSF</td>
<td>chikungunya RT-PCR and IgM</td>
<td>2-5 mL</td>
</tr>
<tr>
<td></td>
<td>Zika and dengue RT-PCR and virus-specific IgM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flavivirus PRNT</td>
<td></td>
</tr>
<tr>
<td>Urine, Saliva, Amniotic Fluid</td>
<td>Zika RT-PCR</td>
<td>1-3 mL</td>
</tr>
<tr>
<td>Cord Blood</td>
<td>Zika RT-PCR and IgM Flavivirus PRNT</td>
<td>0.5-3mL</td>
</tr>
<tr>
<td>Placental Tissue, fresh</td>
<td>Zika RT-PCR</td>
<td>2-5 grams</td>
</tr>
<tr>
<td></td>
<td>Viral Culture</td>
<td></td>
</tr>
<tr>
<td>Placental tissue/umbilical cord, paraffin-embedded formalin fixed</td>
<td>Immunohistochemical staining Zika RT-PCR</td>
<td>2-5 grams or paraffin blocks</td>
</tr>
</tbody>
</table>
Additional considerations for laboratory testing:

- Check with local and state public health authorities regarding the requirement for approval and necessary paperwork prior to initiating specimen collection and testing.
- Viral culture is not recommended for the diagnosis of Zika virus infection.
- Little is known about the positive and negative predictive values of Zika virus RT-PCR and IgM capture MAC-ELISAs; population based studies are lacking.
- Caution: IgM-based assays are prone to cross-reactivity with antibodies produced in response to infection with other flaviviruses such as dengue. Due to this inherent limitation, all IgM positive results should be confirmed by PRNT, with a reciprocal titer ≥ 4-fold higher than other flaviviruses (e.g., dengue virus).
- Laboratories considering the development and implementation of lab developed tests (LDTs) for Zika virus (i.e., real-time PCR or serology) should perform a thorough clinical validation, including testing known positive samples collected from patients with clinically and laboratory-confirmed disease. Furthermore, extensive specificity studies should be performed using clinical samples known to be positive for other flaviviruses, such as dengue and West Nile virus.

Safety and Laboratory Precautions:

Laboratories should perform a risk assessment before conducting Zika virus testing in house. Presenting symptoms and epidemiology of Zika virus overlap with both dengue and Chikungunya virus, and as such these viruses must also be considered in the risk assessment. Chikungunya virus produces high levels of viremia, therefore, the appropriate laboratory biosafety level and precautions for handling these samples must be considered. The primary route of Zika virus infection is through the bite of a mosquito, but it can be transmitted from mother to child during pregnancy and at birth, and there is also risk of transmission through sexual contact and blood transfusion. Therefore, mitigating the risk of percutaneous exposure to Zika is essential. Laboratory staff working with clinical samples collected from patients under investigation for Zika virus should follow standard precautions (e.g., eye protection, gloves, and gown) and, based on the risk assessment, consider enhanced PPE if needed (ex. back closing gown, double gloves, disposable sleeve covers and disposable full face shield). Due to the association between Zika virus infection and microcephaly, pregnant workers or those who may become pregnant should be educated about the risk so that an informed decision can be made by the individual in consultation with their medical provider and the occupational health physician.