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Zika Virus: An Update on the Disease and Guidance for Laboratory Testing

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Highlights

- The primary route of Zika virus infection is through the bite of a mosquito.
- The World Health Organization (WHO) declared Zika virus a public health emergency of international concern in 2016.
- The clinical presentation in symptomatic patients is non-specific and overlaps with other arbovirus infections.
- Serologic and Nucleic Acid Amplification Test (NAAT) testing for Zika virus are available through the FDA Emergency Use Authorization (EUA) pathway.
- NAAT and serologic testing are currently available at reference laboratories, designated public health laboratories and the Centers for Disease Control and Prevention (CDC).
- Laboratories should perform a risk assessment before conducting in-house Zika virus testing.

Background

Zika virus is a flavivirus that is transmitted by *Aedes* spp. (*A. aegypti* and *A. albopictus*) mosquitoes, both of which are found in the United States (U.S.). *Aedes* spp. are also vectors for dengue and chikungunya viruses. Although vector-borne transmission is the primary mode of transmission, infection with Zika virus via sexual contact and blood transfusion has been documented. Zika virus was first identified in 1947 and prior to 2007 was only associated with sporadic cases in Asia and Africa. From 2013-2016 an outbreak of Zika occurred in French Polynesia affecting approximately 30,000 people. In 2015 a second, larger outbreak started in Central and South America, eventually spreading to the continental U.S. By February 1, 2016, WHO declared Zika virus a public health emergency of international concern. This outbreak lasted until 2017 and resulted in approximately 220,000 confirmed cases.

Since 2017 the worldwide incidence of Zika infections has dramatically declined. The reason for this decline is poorly understood, though it is partially attributed to the development of protective immunity (1). There have been no reports of Zika virus infection acquired from mosquitos in the continental U.S. since 2018. In Central and South America, it is estimated that cases have reduced 30-70-fold. Areas once highly affected by circulating Zika virus are now exhibiting high rates of infection with dengue virus, to the extent that dengue cases outnumber Zika cases by approximately 200 to 1 (2). The geographical shifts in epidemiology have important implications for diagnostic testing.



Clinical Presentation

The vast majority (~80%) of individuals who are infected with Zika virus remain asymptomatic. In symptomatic patients, the clinical presentation is non-specific and overlaps with other arbovirus infections, notably dengue and chikungunya viruses. When present, symptoms caused by Zika virus may 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 established between Zika virus infection during pregnancy and congenital disease. Specifically, an increased risk of microcephaly and intracranial calcifications in neonates born to mothers infected with Zika virus during pregnancy was noted. Additionally, Guillain-Barré syndrome has been associated with Zika virus infection.

Information regarding clinical presentation and appropriate laboratory testing are available from the CDC <https://www.cdc.gov/zika/index.html>.

General Guidance on Diagnostic Laboratory Testing

Current diagnostic testing recommendations for consideration:

- Most individuals suspected of infection with Zika virus do not require laboratory testing.
- In the absence of an ongoing outbreak, the pretest probability of Zika positivity in the U.S. is low.
- The clinical presentation of dengue and Zika virus overlap; dengue is more common in areas once highly affected by Zika.
- Serologic cross reactivity between Zika and dengue viruses can make test interpretation challenging.

In patients presenting with symptoms consistent with Zika virus infection, testing for dengue and chikungunya virus should also be prioritized. Zika virus testing may be warranted for patients who live in or have recently traveled to a Zika endemic region and are critically ill, hospitalized or pregnant, or for infants born to mothers positive for Zika virus. Serologic testing should be avoided in situations with low pre-test probability given potential difficulties in result interpretation.

- [CDC Guidance on Zika for Healthcare Providers](#)
- [Zika and Pregnancy](#)

Laboratory Testing Scenarios

Zika virus testing is **not** recommended for non-pregnant patients.

Zika virus testing should **not** be performed as a component of preconception screening.

For pregnant women *with* a clinical illness consistent with Zika virus infection:

- For those with travel to an area of increased Zika virus risk **with active dengue** virus transmission, testing should occur as soon as possible within the first 12 weeks of symptom onset.
 - Zika and dengue virus NAAT should be performed on a serum specimen.
 - Zika and dengue virus IgM should be performed on a serum specimen.



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- Zika virus NAAT should be performed on a urine specimen.
- If Zika IgM antibody is positive in the absence of Zika NAAT positivity, a plaque reduction neutralization (PRNT) assay for dengue virus should be performed to rule out cross-reactivity with dengue virus as a cause of Zika virus IgM positivity. If dengue virus PRNT is negative or four-fold less than Zika virus PRNT, Zika virus IgM positivity may indicate Zika virus infection.
- Zika virus NAAT positivity of either the serum or urine sample should be confirmed utilizing a second, separately collected serum or urine sample to rule out false positivity.
- Dengue virus NAAT or IgM positivity is adequate evidence of dengue virus infection and does not require confirmatory testing.
- For those with travel to an area of increased Zika risk **without active dengue virus** transmission or those who have had sex with someone who lives in or has traveled to these areas, testing should occur as soon as possible within the first 12 weeks of symptom onset.
 - Zika virus NAAT should be performed on serum and urine.
 - Zika virus NAAT positivity of either the serum or urine sample should be confirmed utilizing a second, separately collected serum or urine sample to rule out false positivity.
 - Zika virus serologic testing is not recommended given potential for persistence for years following infection and false positivity due to other flavivirus infection.

For pregnant women *without* symptoms of Zika: Zika virus serologic testing is not recommended given potential for persistence for years following infection and false positivity due to dengue virus or other flavivirus infection.

- Routine testing is not recommended for those living in or with recent travel to the U.S. and its territories.
- Routine testing is not recommended for those with travel to an area of increased Zika virus risk (as defined by the CDC), though NAAT may be considered up to 12 weeks after travel.

For cases in which fetal microcephaly or intracranial calcifications are observed on prenatal ultrasound AND the mother has traveled to or lived in an area with a risk of Zika virus infection during her pregnancy:

- Zika virus NAAT should be performed on serum and urine.
- Zika virus NAAT positivity of either the serum or urine sample should be confirmed utilizing a second, separately collected serum or urine sample to rule out false positivity.
- Zika virus NAAT should be performed on amniotic fluid if amniocentesis is performed as a component of patient care.
- Zika virus IgM antibody should be performed on serum. If Zika virus IgM is positive in the absence of NAAT positivity, a plaque reduction neutralization (PRNT) assay for dengue virus should be performed to rule out cross-reactivity with dengue virus as a cause of Zika virus IgM positivity. If dengue virus PRNT is negative or four-fold less than Zika virus PRNT, Zika virus IgM positivity may indicate Zika virus infection.
- Zika virus NAAT may be considered on infant serum or placental tissue. Histopathologic evaluation of the placenta and umbilical cord with Zika virus immunohistochemical staining may be considered. Immunohistochemical staining is available through the CDC <https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html>.



Available Laboratory Tests

Currently, serologic and NAAT testing for Zika virus are available through the FDA EUA pathway. In addition to assays cleared under EUA, there are currently four FDA-cleared IgM-based serologic assays.

NAAT and serologic testing are currently available at reference laboratories, designated public health laboratories and the CDC. Confirmatory PRNT for dengue and Zika viruses are currently available only at select public health laboratories and the CDC.

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 NAAT and serologic testing; population-based studies are lacking.
- Caution: Zika virus IgM-based assays are prone to cross-reactivity with antibodies produced in response to infection with other flaviviruses, such as dengue virus. Due to this inherent limitation, all Zika virus IgM positive results should be followed by dengue virus PRNT performed in parallel with testing for other flaviviruses. A comparative PRNT Zika virus titer ≥ 4 -fold higher than the PRNT titers for other tested flaviviruses (e.g., dengue virus) confirms reactivity for Zika virus. However, in a significant number of cases, especially in areas with high prevalence of dengue and Zika virus infections, PRNT may not distinguish between dengue and Zika virus infections.
- Laboratories considering the development and implementation of laboratory-developed tests for Zika virus (e.g., NAAT or serology) should perform a thorough clinical validation, including testing of 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 virus 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 viruses, 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. There is also risk of transmission through sexual contact and blood transfusion. Therefore, mitigating the risk of percutaneous exposure to Zika virus 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). Due to the association between Zika virus infection and microcephaly, laboratory workers who are pregnant or who may become pregnant should be educated about the risk so that an informed decision can be made by the individual in consultation with the medical provider and the occupational health physician.



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