



The Commonwealth of Massachusetts
Executive Office of Health and Human Services
Department of Public Health
Bureau of Infectious Disease and Laboratory Sciences
305 South Street, Jamaica Plain, MA 02130

CHARLES D. BAKER
Governor

KARYN E. POLITO
Lieutenant Governor

Clinical Advisory
Zika Virus
June 1, 2016

MARYLOU SUDDERS
Secretary

MONICA BHAREL, MD, MPH
Commissioner

1. Zika Virus

Zika virus is a mosquito-borne flavivirus (in the same family as yellow fever, dengue and West Nile viruses) previously found largely in Africa and Southeast Asia. In May 2015, the World Health Organization reported the first local transmission of Zika virus in the Western Hemisphere, in Brazil. Since 2015, local transmission has been identified in at least 47 countries or territories in the Americas, including Puerto Rico, as well as in the Pacific Islands and Cape Verde in Africa. Further spread is likely and the most current information about areas with Zika transmission can be found at <http://www.cdc.gov/zika/geo/active-countries.html>. Local mosquito transmission of Zika virus has not been documented in the continental United States. However, Zika virus infections have been reported in travelers returning to the United States. The number of Zika virus disease cases among travelers visiting or returning to the United States likely will continue to increase.

An estimated 80% of persons infected with Zika virus are asymptomatic. In symptomatic infection, the incubation period is 3-12 days. Symptomatic disease is generally mild and characterized by at least two of the following:

- acute onset of fever,
- maculopapular rash which may be pruritic,
- arthralgia, and/or
- nonpurulent conjunctivitis

Symptoms usually last from several days to 1 week. Severe disease requiring hospitalization is uncommon, and fatalities are rare.

The most significant concern associated with Zika virus infection is for pregnant women. In pregnant women who become infected with Zika virus, it is possible for the virus to spread to the developing fetus. When this happens, it can result in birth defects. The complete range of possible adverse birth outcomes associated with Zika virus infection is unknown at this time but has included: microcephaly; intracranial calcifications; fetal loss; and abnormalities in both vision and hearing in the neonate. Pregnant women can be infected with Zika virus in any trimester. No evidence exists to suggest that pregnant women are more susceptible to Zika virus infection or experience more severe disease during pregnancy. Maternal-fetal transmission of Zika virus has been documented throughout pregnancy; some evidence exists that infection during the first trimester results in the most significant risk to the fetus.

In addition, Guillain-Barré syndrome has been reported in a small proportion of patients following Zika virus infection.

2. Planning Travel to an Area with Zika Virus Transmission

Because there is neither a vaccine nor prophylactic medications available to prevent Zika virus infection, the **Centers for Disease Control and Prevention (CDC) and the Massachusetts Department of Public Health (MDPH) continues to recommend that all pregnant women, and those planning on becoming pregnant within two months, postpone travel to areas where Zika virus transmission is ongoing. Because sexual transmission is possible, the sexual partners of pregnant women or those planning on attempting conception within six months, should also strongly consider postponing their travel.**

If a pregnant woman, a woman who wishes to become pregnant, or the sexual partner of these women, **must** travel to an area with Zika virus transmission, they should be counseled that this poses a risk to the unborn child or may result in a recommendation to delay conception in couples trying to become pregnant. These individuals must be advised to strictly follow steps to avoid mosquito bites. Mosquitoes that spread Zika virus bite both indoors and outdoors, mostly during the daytime; therefore, it is important to ensure protection from mosquitoes throughout the entire day. Mosquito prevention strategies, recommended for all travelers to an area with transmission, include:

- wearing long-sleeved shirts and long pants;
- using U.S. Environmental Protection Agency (EPA)–registered insect repellents;
- using permethrin-treated clothing and gear; and
- staying and sleeping in screened-in or air-conditioned rooms.

When used as directed on the product label, insect repellents containing DEET, picaridin, and IR3535 are safe for pregnant women.

3. Recommendations for Men with Recent Travel to an Area with Zika Virus Transmission and Their Pregnant Partners

Sexual transmission of Zika virus from infected men to their sexual partner is possible. It is not known how frequently Zika virus is found in semen or how long it might persist. **Therefore, men who reside in or have traveled to an area of active Zika virus transmission who have a pregnant partner should abstain from sexual activity or consistently and correctly use latex condoms during each act of vaginal, anal, or oral sex for the duration of the pregnancy. Pregnant women should discuss their male partner’s potential exposures to mosquitoes and history of clinical illness consistent with Zika virus disease with their healthcare provider.**

Fact sheets on condoms and information on using condoms correctly are available from the U.S. Department of Health and Human Services and CDC at <https://www.aids.gov/hiv-aids-basics/prevention/reduce-your-risk/using-condoms/> and <http://www.cdc.gov/condomeffectiveness/brief.html#Condom>.

4. Understanding Laboratory Testing for Zika Virus

Two types of laboratory tests are available to assess infection with, or evidence of exposure to, Zika virus.

1. RT-PCR detection of Zika virus RNA indicates the presence of the virus itself. PCR for Zika RNA can be done on serum, urine, cerebral spinal fluid, and amniotic fluid. Evidence of Zika virus RNA may be found in serum up to 7 days after symptom onset but generally only lasts 3-4 days. Evidence of Zika virus RNA in urine lasts longer, and has occasionally been found as long as 3-4 weeks after symptom onset. Approval requirements, sample types and timing of collections for at-risk patients are detailed in the **Diagnostic Testing Guidance** table. **This test is available at the MA SPHL for all listed sample types.**

- Anti-Zika virus IgM antibodies provide evidence of recent exposure in patients meeting the designated testing criteria. Testing to detect anti-Zika virus IgM antibodies can be performed on serum samples from potentially exposed, symptomatic or asymptomatic patients as detailed in the **Diagnostic Testing Guidance** table. Anti-Zika virus IgM antibodies reliably appear by 2 weeks following exposure and can last up to 12 weeks. This test is available at the MA SPHL. Serum samples with a positive or equivocal anti-Zika virus IgM result must be confirmed by testing serum for the presence of neutralizing antibodies using the plaque reduction neutralization test (PRNT). Currently, PRNT assays are still performed at the CDC. However, the MA SPHL is working with the CDC to implement PRNT assays at the MA SPHL.

Ideally, detection of Zika virus RNA using RT-PCR on samples of serum and/or urine, early in infection, is optimal to confirm infection with Zika virus. However, the appropriate application of this test requires that the patient has been currently, or very recently, symptomatic with clinically consistent Zika-like disease. Unfortunately, 80% of individuals exposed to Zika virus remain asymptomatic making this type of laboratory test unsuitable since the likely viremic period of the patient cannot be determined. Even for symptomatic patients, most clinical samples are not collected within the ideal timeframe to detect Zika virus RNA.

For both asymptomatic patients and those whose specimens are drawn after the likely viremic period, serological testing is the only available diagnostic tool. Interpretation of serology tests may be complicated due to recent and/or prior exposure to another cross-reacting flavivirus (especially dengue virus), or a vaccination history with other flaviviruses such as yellow fever or Japanese encephalitis. Due to the extensive cross-reactivity of Zika and dengue viruses, and the persistence of both dengue and chikungunya viruses in areas with Zika virus transmission, additional testing for these other diseases may be required. Despite testing for additional viruses, in many cases, a positive Zika virus IgM from a single serum with laboratory evidence of neutralizing antibody in both the Zika and dengue PRNT assays will be indicative of “recent exposure to a flavivirus” and a definitive diagnosis may not be possible.

Selection and interpretation of the appropriate test by the laboratory requires specific clinical information including dates and location of travel, evidence of mosquito exposure, symptoms clinically consistent with Zika virus, date of symptom onset, and any previous exposure to dengue virus, yellow fever vaccine or Japanese encephalitis vaccine. This information must be provided when calling for testing approval AND on the specimen submission form that must accompany the sample to the MA SPHL.

5. Availability of Laboratory Testing for Zika Virus

The table below provides additional information about the types of laboratory testing for Zika virus and where they are currently available. Sample submission should not be based on this information. Specific guidance for individual circumstances is available below in the **Diagnostic Testing Guidance** table.

| Test Type | Symptoms? | Sample types | Availability | Turn-around Time |
|---------------------------|-------------|----------------|--|---------------------------------------|
| RT-PCR for Zika virus RNA | Symptomatic | serum | <ul style="list-style-type: none"> MA SPHL Commercially available† | MA SPHL < 7 days after sample receipt |
| | Symptomatic | urine | MA SPHL | MA SPHL < 7 days after sample receipt |
| | N/A | amniotic fluid | MA SPHL | MA SPHL < 7 days after sample receipt |
| | Symptomatic | CSF | MA SPHL | MA SPHL < 7 days after sample receipt |

| | | | | |
|--|------------------------------|-------|--|---------------------------------------|
| IgM MAC-ELISA for Zika virus antibodies | Symptomatic and Asymptomatic | serum | MA SPHL | MA SPHL < 7 days after sample receipt |
| Plaque reduction neutralization test (PRNT) for Zika virus neutralizing antibodies | Symptomatic and Asymptomatic | serum | CDC NOTE: MA SPHL implementing; not yet available | CDC – longer than 3 weeks |

† Please note that the currently available commercial PCR testing for Zika virus does not include serologic antibody testing and should only be used for currently or very recently symptomatic patients. In order to ensure comprehensive testing for high risk patients, all samples from pregnant women with potential exposure to Zika virus should be sent to the MA SPHL.

6. Diagnostic Testing Guidance Tables

To discuss/request testing, please contact the MDPH Epidemiology Line at 617-983-6800, available 24/7. If the testing requested meets the current guidelines and current laboratory testing capacity, testing can be approved. The information requested below MUST accompany the sample(s) either on the specimen submission form or on an attached form. **Samples that do not meet the criteria or do not include all the requested information will be rejected for testing.**

For any testing questions or to request testing approval for any of the other circumstances outlined in the **Diagnostic Testing Guidance** table, please contact the MDPH Epidemiology Line at 617-983-6800, available 24/7, with the following information:

- Date of onset of disease symptoms;
- Date of specimen collection;
- Unusual immunological status of patient (e.g., immunosuppression);
- Travel history with dates (e.g., travel to area with current transmission <http://wwwnc.cdc.gov/travel/notices/>);
- Vaccination history (e.g., vaccination against yellow fever, Japanese encephalitis);
- Disease history (e.g., previous history of chikungunya or dengue fever); and
- Brief clinical summary including suspected diagnosis and approximate gestational age

Specimens should be submitted using the Massachusetts State Public Health Laboratory (MA SPHL) clinical specimen submission form (<http://www.mass.gov/eohhs/docs/dph/laboratory-sciences/general-submission-form.pdf>) and should include the information provided above for consistency.

Specific information on specimen collection, storage and shipping is available on the companion document, “Specimen Collection, Storage and Shipment for Molecular and Serological Testing for Zika Virus”. This can be found at www.mass.gov/dph/zika under Information for Healthcare and Public Health Partners.

DIAGNOSTIC TESTING GUIDANCE TABLE

| Category | Symptomatic or Asymptomatic | Sample Type and Timing | Additional Notes About Testing | Patient Counseling Recommendations |
|--|-----------------------------|--|---|---|
| PREGNANT WOMEN - - TESTING REQUIRES PRE-APPROVAL BY MDPH, CONTACT 617-983-6800 | | | | |
| Women who were pregnant OR conceived during, or within 8 weeks, of travel to a place with ongoing Zika virus transmission | Symptomatic ¹ | <ul style="list-style-type: none"> • ≤28 days post-symptom onset: serum and urine³ • >28 days, but not more than 12 weeks, post-symptom onset: serum⁴ | <p>Convalescent specimen might be requested depending upon results.</p> <p>Testing is not recommended if more than 12 weeks have passed since symptom onset.</p> | <p>If the woman’s male sexual partner also has possible exposure to Zika virus because of travel, the couple should be counseled on using latex condoms, consistently and correctly, for all sexual contact for the duration of the pregnancy.</p> <p>Testing of men for the assessment of risk for sexual transmission is not recommended.</p> |
| | Asymptomatic ² | 2-12 weeks post-travel: serum ⁴ | Testing is not recommended if more than 12 weeks have passed since last possible exposure. Negative results after 12 weeks do not assure absence of exposure. | |
| <p>Women that became pregnant while residing in areas with ongoing Zika virus transmission</p> <p>AND</p> <p>Pregnant women that have been in and will be returning to an area with ongoing Zika virus transmission</p> | Symptomatic ¹ | <ul style="list-style-type: none"> • ≤28 days post-symptom onset: serum and urine³ • >28 days, but not more than 12 weeks, post-symptom onset: serum⁴ | <ul style="list-style-type: none"> • Convalescent specimen might be requested depending upon results. • Testing is not recommended if more than 12 weeks have passed since symptoms onset. Negative results after 12 weeks do not assure absence of exposure. • Providers may choose to do serial ultrasounds to monitor the development of the fetus during pregnancy in place of, or in addition to, testing. | <p>If the woman’s male sexual partner also has possible exposure to Zika virus because of travel, the couple should be counseled on using latex condoms, consistently and correctly, for all sexual contact for the duration of the pregnancy.</p> <p>Testing of men for the assessment of risk for sexual transmission is not recommended.</p> |
| | Asymptomatic ² | Testing recommended approximately once per trimester or after each trip, whichever is fewer: serum ⁴ | <ul style="list-style-type: none"> • Because residing in an area with ongoing Zika virus transmission presents a high risk for exposure AND the timing of that exposure is likely not identifiable, testing should be offered but may not be useful. Negative results do not assure absence of exposure. • For women repeatedly traveling to affected areas, testing can be repeated, approximately once per trimester, as long as exposure is recurring • Providers may choose to do serial ultrasounds to monitor the development of the fetus during pregnancy in place of, or in addition to, testing. | |

¹Symptomatic: One (except where otherwise noted) or more of the following - fever, rash, arthralgia, conjunctivitis - occurring no more than 14 days after last potential exposure

²Asymptomatic: absence of symptoms OR with clinical illness **not** characterized by one of the following signs: fever, rash, arthralgia, conjunctivitis

³RT-PCR on serum and urine with follow-up IgM ELISA if negative

⁴IgM ELISA with follow-up PRNT if equivocal or positive

⁵RT-PCR on serum and urine WITHOUT follow-up with IgM ELISA if negative

| PREGNANT WOMEN continued - - TESTING REQUIRES PRE-APPROVAL BY MDPH, CONTACT 617-983-6800 | | | | |
|--|--|--|---|--|
| Pregnant women, not otherwise exposed, with unprotected sexual contact with a man that had travel to or resided in an area with ongoing Zika virus transmission | At least one partner is symptomatic ¹ | <p>For symptomatic pregnant woman:</p> <ul style="list-style-type: none"> • ≤28 days post-symptom onset: serum and urine³ OR • >28 days, but not more than 12 weeks, post-symptom onset: serum⁴ <p>For asymptomatic pregnant woman: 2-12 weeks after last possible exposure (unprotected sexual contact): serum⁴</p> | <ul style="list-style-type: none"> • Recommendation is that pregnant women should be tested as long as either the male traveler or she is symptomatic. • Male may also be tested dependent upon laboratory capacity <ul style="list-style-type: none"> ○ Symptomatic¹ <ul style="list-style-type: none"> ▪ ≤28 days post-symptom onset: serum and urine³ ▪ >28 days, but not more than 12 weeks, post-symptom onset: serum⁴ ○ Asymptomatic² <ul style="list-style-type: none"> ▪ 2-12 weeks post-travel: serum⁴ | <p>The couple should be counseled on using latex condoms, consistently and correctly, for all sexual contact for the duration of the pregnancy.</p> <p>Providers should discuss with their patients the possibility of serial ultrasounds to monitor the development of the fetus during pregnancy, in addition to, testing.</p> |
| | Both individuals are asymptomatic ² | Testing not recommended – sexual transmission has been documented from symptomatic males to their sexual partners. | <ul style="list-style-type: none"> • The performance of the test in asymptomatic persons is unknown and the results are difficult to interpret. Negative results do not indicate absence of risk. • Providers should discuss with their patients the possibility of serial ultrasounds to monitor the development of the fetus during pregnancy. | The couple should be counseled on using latex condoms, consistently and correctly, for all sexual contact for the duration of the pregnancy. |
| COUPLES PLANNING CONCEPTION - - TESTING REQUIRES PRE-APPROVAL BY MDPH, CONTACT 617-983-6800 | | | | |
| Couples planning on attempting conception (naturally or IVF), after travel to an area with ongoing Zika virus transmission | Symptomatic ¹ female | <ul style="list-style-type: none"> • ≤7 days post-symptom onset: serum and urine⁵ • ≤14 days post-symptom onset: urine⁵ | <ul style="list-style-type: none"> • Testing should be done as soon as possible post-symptom onset to increase likelihood of detecting virus using RT-PCR. • Testing does not change clinical recommendation. | Conception should be delayed for 8 weeks after symptom onset REGARDLESS of the test result. |
| | Symptomatic ¹ male | <ul style="list-style-type: none"> • ≤28 days post-symptom onset: serum and urine³ • >28 days, but not more than 12 weeks, post-symptom onset: serum⁴ | <ul style="list-style-type: none"> • Testing should be done as soon as possible post-symptom onset to increase likelihood of detecting virus using RT-PCR. • If PCR test is negative and serologic test is preliminarily positive, time to final results is likely to be several months. | If the man is positive, conception should be delayed for 6 months and the couple should be counseled on using latex condoms, consistently and correctly, for all sexual contact for the 6 month duration. |
| | Asymptomatic ² | Testing not recommended | <ul style="list-style-type: none"> • The performance of the test in asymptomatic persons is unknown and the results are difficult to interpret. Negative results do not indicate absence of risk. | If both partners are asymptomatic, conception should be delayed for 8 weeks following the last possible exposure of both individuals. |
| <p>¹Symptomatic: One (except where otherwise noted) or more of the following - fever, rash, arthralgia, conjunctivitis - occurring no more than 14 days after last potential exposure</p> <p>²Asymptomatic: absence of symptoms OR with clinical illness not characterized by one of the following signs: fever, rash, arthralgia, conjunctivitis</p> <p>³RT-PCR on serum and urine with follow-up IgM ELISA if negative</p> <p>⁴IgM ELISA with follow-up PRNT if equivocal or positive</p> <p>⁵RT-PCR on serum and urine WITHOUT follow-up with IgM ELISA if negative</p> | | | | |

| FETUSES/INFANTS⁶ - - TESTING REQUIRES PRE-APPROVAL BY MDPH, CONTACT 617-983-6800 | | | | |
|---|--|--|---|---|
| Fetus with identified abnormalities on ultrasound whose mother had a positive or equivocal Zika virus test | Prior to delivery | Amniotic fluid can be submitted for RT-PCR testing ⁵ | Amniocentesis as a procedure has inherent risks and a decision to perform it should be made in the context of the whole situation. The ability of a negative amniotic fluid RT-PCR to exclude infection is not known. | Fetus with identified abnormalities on ultrasound whose mother had a positive or equivocal Zika virus test |
| Infant whose mother traveled to or resided in an affected area within 2 weeks prior to delivery | Symptomatic: <u>Two</u> or more clinical signs/symptoms within 2 weeks after delivery | <ul style="list-style-type: none"> • ≤28 days post-symptom onset: serum and urine³ • >28 days, but not more than 12 weeks, post-symptom onset: serum⁴ | | |
| Infant whose mother traveled to or resided in an affected area within 2 weeks prior to delivery | Asymptomatic ² | Testing not recommended | | |
| INDIVIDUALS NOT CAPTURED IN OTHER CATEGORIES - - TESTING REQUIRES PRE-APPROVAL BY MDPH, CONTACT 617-983-6800 | | | | |
| Women, men and children not addressed in other categories | Symptomatic ¹ | <ul style="list-style-type: none"> • ≤7 days past symptom onset: serum and urine⁵ • ≤14 days post-symptom onset: urine⁵ | ONLY IF LABORATORY CAPACITY EXISTS | Some commercial PCR testing capacity exists for serum; utilizing commercial testing is only recommended if the sample can be drawn ≤7 days post-onset |
| | Asymptomatic† | Testing not recommended | The performance of the test in asymptomatic persons is unknown and the results may be difficult to interpret. | |

¹Symptomatic: One (except where otherwise noted) or more of the following - fever, rash, arthralgia, conjunctivitis - occurring no more than 14 days after last potential exposure

²Asymptomatic: absence of symptoms OR with clinical illness **not** characterized by one of the following signs: fever, rash, arthralgia, conjunctivitis

³RT-PCR on serum and urine with follow-up IgM ELISA if negative

⁴IgM ELISA with follow-up PRNT if equivocal or positive

⁵RT-PCR on serum and urine WITHOUT follow-up with IgM ELISA if negative

⁶MDPH is working with provider to collect additional samples at delivery for:

- A fetus with identified abnormalities on ultrasound, or neonate with identified abnormalities at birth, whose mother was not tested OR had a negative test for Zika virus but had travel to or resided in an area with ongoing Zika virus transmission; OR
- Neonate whose mother had a positive or equivocal Zika virus test

These specimens are a priority and should consist of:

- Serum from mother if she was not tested or had a negative test for Zika virus.
- Serum from cord blood or serum from infant ≤ 2 days after delivery
- Multiple sections from placenta: some sections should be saved as formalin-fixed and some as fresh-frozen tissue. If unable to collect both sets, formalin-fixed tissue should be prioritized.

7. ADDITIONAL RESOURCES

CDC Zika virus web site: <http://www.cdc.gov/zika/index.html>

[All Countries & Territories with Active Zika Virus Transmission](#)

Caring for Pregnant Women and those of Reproductive Age

- UPDATE: [Interim Guidance for Health Care Providers Caring for Women of Reproductive Age with Possible Zika Virus Exposure – United States, 2016](#) (April 1, 2016)
- [Questions and Answers for Healthcare Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure](#)

Caring for Infants and Children

- UPDATE: [Interim Guidelines for Healthcare Providers Caring for Infants and Children with Possible Zika Virus Infection – United States, February 2016](#) (Feb. 26, 2016)
- [Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection — United States 2016](#) (January 29, 2016)
- [Questions and Answers for Healthcare Providers Caring for Infants and Children with Possible Zika Virus Infection](#)

Preventing Sexual Transmission

- UPDATE: [Interim Guidance for Prevention of Sexual Transmission of Zika Virus – United States, 2016](#) (April 1, 2016)
- [Questions and Answers for Providers – Zika and Sexual Transmission](#)

Laboratory Guidance

- [Interim Guidance for Interpretation of Zika Virus Antibody Test Results](#) (May 31, 2016)
- [Interim Guidance for Zika virus testing of urine - United States, 2016](#) (May 13, 2016)