

Recommendations for Zika Virus Testing and Follow-Up

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Background

Zika virus is a flavivirus that is transmitted to humans primarily by *Aedes* species mosquitoes; in the Americas, *Aedes aegypti*, is the most common vector. Other documented modes of transmission include intrauterine resulting in congenital infection, intrapartum from a viremic mother to her newborn, sexual, blood transfusion and laboratory exposure. Only about 1 in 5 people who are infected with Zika virus show symptoms. In those that do, the most common symptoms are fever, rash, joint pain, and conjunctivitis. Human disease has been seen in Africa, Asia, and the Pacific islands. In May 2015, the first locally-acquired cases in the Americas were reported in Brazil. Since then, local transmission has been reported in many countries in the Americas and several U.S. territories, including Puerto Rico, the U.S. Virgin Islands, and American Samoa. (<https://www.cdc.gov/zika/geo/index.html>)

The first case of sexual transmission documented in the United States occurred in Dallas, Texas, in February 2016. Since that time, the U.S. Centers for Disease Control and Prevention (CDC) has reported additional cases from both men and women to their sexual partners. For guidance on prevention of sexual transmission of Zika virus, visit

https://www.cdc.gov/mmwr/volumes/67/wr/mm6731e2.htm?s_cid=mm6731e2_e.

In Brazil, a substantial increase in the number of infants born with microcephaly was noted in 2015, and Zika virus infection has been identified in several infants born with microcephaly and other fetal losses. In August 2018, CDC published outcomes of Zika virus infection among infants from Zika positive mothers reported to the U.S. Zika Pregnancy and Infant Registry. One out of seven infants born to laboratory confirmed mothers had a Zika-associated birth defect, a neurodevelopmental abnormality possibly associated with congenital Zika virus infection, or both.

(https://www.cdc.gov/mmwr/volumes/67/wr/mm6731e1.htm?s_cid=mm6731e1_e)

In May 2016, the CDC reviewed the evidence that Zika virus causes birth defects and determined that there is a causal association between Zika virus infection and adverse pregnancy outcomes (Rasmussen SA et al. *N Engl J Med* 2016;374:1981-1987). Therefore, CDC is recommending that pregnant women avoid traveling to areas with a risk of Zika. Women who traveled to these areas while pregnant should be evaluated according to the guidance found at the following websites. The websites include recommendations for women who want to get pregnant after recent travel to an area with active Zika virus transmission. (<https://www.cdc.gov/zika/prevention/sexual-transmission-prevention.html>)



Zika-Affected Areas/Travel Information

Pregnant women should avoid traveling to areas with a risk of Zika. However, if a pregnant woman must travel, she should consult with her doctor and strictly follow steps to prevent mosquito bites during the trip. Testing recommendations differ based on symptom status and where the pregnant woman traveled. There are no restrictions for travelers entering the United States who have contracted Zika virus. CDC has issued travel notices (level 2 alert, “practice enhanced precautions”) for people traveling to international destinations and overseas US territories where Zika virus is spreading. These notices include maps that show elevation levels in countries with Zika. Prolonged local transmission of Zika virus within the continental United States and Hawaii is unlikely due to environmental conditions (e.g., temperate climate, lower population density, widespread use of air conditioning, and screens, and reduced mosquito habitat). CDC’s approach to domestic travel guidance differs from international travel guidance because of the low likelihood of local transmission. There are two types of geographic areas: Zika active transmission (designated as red on map) and Zika cautionary areas (designated as yellow on map). There are currently no red or yellow areas in the United States.

- **Zika active transmission area (red area):** Zika virus transmission presents a significant risk to pregnant women.
- **Zika cautionary area (yellow area):** Local transmission has been identified, but evidence is lacking that the intensity of transmission is comparable to that in a red area. Although the specific level of risk in yellow areas is unknown, there is still a risk to pregnant women. Additionally, areas adjacent or close to red areas may have a greater likelihood of local Zika virus transmission and are considered to pose a risk to pregnant women.

For more information, visit: <https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika>.

Recommendations for Diagnostic Testing for Zika

Diagnostic testing for Zika virus is recommended for the following persons who have traveled to an area with Zika virus transmission or have had unprotected sex with a person who has recently traveled to such an area: 1) a person who is experiencing symptoms of Zika virus; and 2) a pregnant woman (with or without symptoms) who may have been exposed. Symptoms only occur in about 1 in 5 people and include fever, rash, joint pain, conjunctivitis (red eyes), muscle pain, and headache (<http://www.cdc.gov/zika/symptoms/>). Symptoms typically begin within a few days after being bitten by an infected mosquito. Diagnostic testing is not recommended for asymptomatic men, asymptomatic non-pregnant women, and children.

For more information for testing recommendations, visit: <https://www.cdc.gov/pregnancy/zika/testing-follow-up/pregnant-woman.html> and <https://www.cdc.gov/zika/laboratories/lab-guidance.html>.



Follow-up of Pregnant Women and Infants

For pregnant women where exposure to Zika virus is a real concern, the clinician should follow the pregnancy with serial fetal ultrasounds and other tests to detect abnormalities regardless of the initial Zika virus test results. If fetal abnormalities are detected later in pregnancy, then Zika virus testing should be repeated. Interim guidance for evaluation and testing of infants with microcephaly or intracranial calcifications whose mothers traveled to or resided in an area with Zika virus transmission during pregnancy can be found at <https://www.cdc.gov/pregnancy/zika/testing-follow-up/zika-in-infants-children.html>. If the clinical provider has questions regarding further testing of pregnant women or infants, contact the **UDOH, Bureau of Epidemiology at 801-538-6191**.

If a pregnant woman has a partner who lives in or traveled to an area with active Zika virus transmission, the couple should correctly and consistently use condoms or abstain from sex ***for the duration of the woman's pregnancy, regardless of Zika test results***. Sex includes vaginal, anal and oral sex and the sharing of sex toys. Zika virus has been detected in semen long after the virus is no longer present in blood.

Pregnant women who test positive for Zika virus will be followed up by public health at labor and delivery to determine pregnancy outcomes. The infant will also be followed to determine outcomes that may not have been readily apparent at birth.

Couples Planning Pregnancy

Couples in which the man has traveled to an area with active Zika virus transmission should postpone pregnancy for three months, regardless of Zika test results. If the woman has traveled to an area with active Zika virus transmission, pregnancy should be postponed for two months, regardless of Zika virus test results.

Zika Laboratory Testing Information

- Laboratory tests for Zika virus infection diagnosis include a combination of nucleic acid amplification testing (NAAT) using polymerase chain reaction (RT-PCR) technology, Zika virus IgM antibody testing, and plaque reduction neutralization antibody testing (PRNT).
- Multiple assays and sample types are often needed to establish a definitive diagnosis of Zika. Viral ribonucleic acid (RNA) detected by NAAT is the first analyte that can be detected on a person infected with Zika; it is most informative during the first six weeks after symptom onset. RNA may persist longer in pregnant women (up to 12 weeks) and in some tissues (e.g., semen or fetal tissues). IgM antibodies are present from about 2 to 12 weeks after symptom onset, but may persist longer. Neutralizing antibodies appear shortly after IgM antibodies and consist primarily of IgG antibodies; these are expected to persist for many years after infection.



- Cross-reactivity between flaviviruses (e.g., dengue, chikungunya) may occur with IgM testing. The Trioplex RT-PCR test is available at some commercial laboratories, state laboratories and CDC and can be used to test serum and cerebrospinal fluid (CSF) for Zika virus, chikungunya, and dengue. PRNT testing can also be used to distinguish infection with different flaviviruses. PRNT testing may be done as a confirmatory test for persons with IgM-positive results.
- Currently, the Utah Public Health Laboratory (UPHL) performs the InBios Assay for Zika virus IgM assay and the Trioplex RT-PCR tests. UPHL charges \$45 for the InBois IgM test. Trioplex RT-PCR testing will continue to be free of charge and will need approval from Bureau of Epidemiology before testing (http://health.utah.gov/epi/diseases/zika/Zika_IgM_Testing_Fee.pdf). Equivocal or inconclusive IgM test results will be sent to the CDC laboratory in Fort Collins, CO, for confirmation, including PRNT testing. If testing cannot be confirmed at UPHL, the specimen will be sent to CDC in Fort Collins for confirmatory testing.
- In patients who have been immunized against yellow fever or Japanese encephalitis virus or who have been infected with another flavivirus (e.g., West Nile or St. Louis encephalitis virus) in the past, cross-reactive antibodies in both the IgM and neutralizing antibody assays may make it difficult to identify which flavivirus is causing the patient's current illness. Because antibody tests may cross-react with other flaviviruses (e.g., dengue, yellow fever, or Japanese B encephalitis) and produce false positives, it is recommended the patient be tested for these viruses as well. If clinicians need to rule out these infections regardless of Zika virus results, these tests are available through commercial laboratories and through the CDC.
- Paired serum and urine are the primary diagnostic specimens for Zika virus infection. Other specimens such as plasma, whole blood, cerebrospinal fluid (CSF), and amniotic fluid may also be tested depending on the situation.
- Symptomatic non-pregnant persons should have an acute serum and urine collected ≤ 7 days after symptom onset and should be tested by RT-PCR.
- IgM antibodies may be detectable by day four (4) of illness, but typically detectable within the first two weeks after symptom onset and is more reliable later in the course of infection. For persons whose infections have occurred >7 days after symptom onset, the serum sample may be tested for IgM antibodies and PRNT if positive.
- Pregnant persons (regardless of symptoms) with recent exposure, but not ongoing exposure, should have serum and urine specimens collected as soon as possible and up to 12 weeks after last exposure or onset of symptoms to be tested by the PCR and IgM methods. Urine specimens must always be accompanied with a serum sample. Serum specimens collected after 12 weeks can be tested by IgM serology followed by PRNT, if positive.
- Asymptomatic women with ongoing possible exposure to Zika virus should be tested by RT-PCR, three times during pregnancy. *Ongoing exposure* is defined as persons with ongoing possible Zika virus exposure including those who reside in or frequently travel (daily or weekly) to an area with

risk for Zika virus transmission. It is recommended that testing is started at initial prenatal care visit, followed by two additional PCR tests performed during pregnancy.

- Staining placental and fetal tissues specimens for viral antigens or RT-PCR on fixed tissues may be conducted by the CDC. However, routine testing of placental tissues is not recommended.
- Consultation about laboratory testing is available through the Utah Department of Health (UDOH) State Epidemiologist, Medical Officer on call at the Utah Department of Health, or local public health department (see contact information below).

Requesting laboratory testing in Utah

- The InBios Zika IgM assay will cost \$45 and will not require prior approval. Approval is still required for the Triplex RT-PCR testing. RT-PCR testing may be limited; therefore, UPHL is requesting that the Bureau of Epidemiology at the Utah Department of Health or the local public health department approve testing requests. **To discuss testing, please contact your local health department or UDOH, Bureau of Epidemiology at 801-538-6191.** Visit <https://www.cdc.gov/zika/laboratories/lab-guidance.html> for Interim Guidance for Interpretation of Zika Virus Antibody Test Results.

Serum specimen collection and transport

General Instructions	Storage	Shipping
Collect serum (≥ 3 mL) in a large serum separator tube.	Samples collected and shipped with expected arrival the same day can be shipped on cold packs (4°C); not frozen.	If storage/transport will exceed 24 hours, serum should be frozen at -20°C or lower. Ship samples on dry ice to UPHL.

Urine specimen collection and transport

General Instructions	Storage	Shipping
Provide 1.0 mL of urine in a 1.8 mL cryotube or 2.0 mL microtube with sterile screw capped vial secured with thermoplastic, self-sealing lab film.	For RT-PCR testing, specimens should be kept cold (2–6 °C) if shipped within 24 hours or frozen (-70 °C) for storage and shipping greater than 24 hours. For virus isolation testing, specimens should be frozen (-70°C) as soon as possible.	Urine specimens should always be accompanied with a serum specimen.



Collecting & submitting specimens for Zika virus testing at time of birth

Specimen Type	General Instructions	Storage	Shipping
Infant serum (within first 2 days of life)	At least 1.0 ml Transfer serum to a plastic tube measuring approximately 50 mm tall and 15 mm in diameter (e.g., 1.8 mL cryotube or 2.0 mL microtube) with screw cap and secure with thermoplastic, self-sealing lab film.	For cold specimens, the sample should be placed in an insulated container with adequate ice packs to ensure specimen (cold chain) integrity. For frozen specimens, ship the sample on enough dry ice to ensure specimens remain frozen until received.	If storage/transport will exceed 24 hours, serum should be frozen at -20°C or lower. Ship samples on dry ice to UPHL.
Infant urine (within first 2 days of life)	Provide 0.5-1.0 mL of the specimen in a sterile screw capped vial secured with thermoplastic, self-sealing lab film. Please ensure a tight seal as leaking specimens cannot be accepted.	For RT-PCR testing, specimens should be kept cold (2–8 °C) if shipped within 24 hours or frozen (-70 °C) for storage and shipping greater than 24 hours. For virus isolation testing, specimens should be frozen (-70°C) as soon as possible. For frozen specimens, ship the sample on enough dry ice to ensure specimens remain frozen until received.	Urine specimens should always be accompanied with a serum specimen.

Notes

[¶]Generally considered at less than 12 weeks gestational age

[†]Considered at any gestation for which placenta is available

[‡]Considered upon fetal demise

Refer to the following websites for more information.

- <https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html>, and
- <https://www.cdc.gov/pregnancy/zika/testing-follow-up/evaluation-testing.html>.

Follow packaging and shipping instructions for Category B, Biological Substances.

Laboratory Forms Required for Testing by UPHL and CDC

The Infectious Disease Test Request Form should be securely emailed or faxed to UDOH and accompany the original with the specimen to Utah Public Health Lab (UPHL). The UPHL form is available at <http://health.utah.gov/epi/diseases/zika>. If a provider needs assistance with completing the form, work with local health department (LHD) or UDOH epidemiology staff. Additional forms may be required if



confirmation testing is necessary. Samples with incomplete information will result in delayed testing and reporting of results. Answers to questions about specimen types or shipping can be found at:

<http://www.cdc.gov/ncezid/dvbd/specimensub/arboviral-shipping.html>

- Arrangements must be made with the UDOH or LHD for specimen shipping and delivery to the UPHL in advance.
- Turnaround time for preliminary results is 7-10 days. If the samples must be sent to CDC for confirmation, turnaround time is 21-28 days.

