Recommendations for Zika Virus Testing and Follow-Up

Updated on May 8, 2017

Background

Zika virus is a flavivirus that is transmitted to humans primarily by *Aedes* species mosquitoes; in the Americas, *Aedes egypti,* 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 (http://wwwnc.cdc.gov/travel/page/zika-information).

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 http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e2.htm?s_cid=mm6529e2.

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 March 2016, CDC published outcomes of Zika virus infection among nine U.S. pregnant travelers; all of these women had one or more symptoms. Five of six women who reported symptoms during the first trimester had poor pregnancy outcomes, including miscarriages (2), elective terminations (2), and microcephaly (1) (http://www.cdc.gov/mmwr/volumes/65/wr/mm6508e1.htm).

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. http://www.cdc.gov/mmwr/volumes/65/wr/mm6512e2.htm http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm?s_cid=mm6529e1_e

In August 2016, the Florida Department of Health reported local mosquito-borne transmission of Zika virus by the *Aedes egypti* in two areas of Miami-Dade County; the Wynwood neighborhood and a section of Miami Beach. The CDC is recommending that pregnant women avoid travel to this area if at all possible. For more information about local transmission in Florida, visit http://www.cdc.gov/zika/intheus/florida-update.html.

In November 2016, the Texas Department of Health Services reported the state's first case of local mosquito-borne Zika virus infection located in the Brownsville. The CDC is recommending that pregnant women avoid travel to this area if at all possible. For more information about local transmission in Texas, visit https://www.cdc.gov/zika/intheus/texas-update.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 areas in the United States. Miami-Dade County, FL, and Brownsville, TX are designated as yellow areas. In the following Texas counties: Cameron, Hidalgo, Starr, Webb, Willacy and Zapata, it is recommended to test all pregnant residents in both the first and second trimester and in residents who have a rash plus at least one common Zika symptom: fever, joint pain or eye redness.

- **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, please 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 asymptomatic pregnant women living in or frequently traveling to areas with Zika virus transmission, CDC recommends that healthcare providers take these steps:

1. Screen pregnant women for risk of Zika exposure and symptoms of Zika. Promptly test pregnant women with NAT if they become symptomatic during their pregnancy or if a sexual partner tests positive for Zika virus infection.
2. Consider NAT testing at least once per trimester, unless a previous test has been positive.
3. Consider NAT testing of amniocentesis specimens if amniocentesis is performed for other reasons.
4. Counsel pregnant women each trimester on the limitations of IgM and NAT testing. For more information about Zika virus testing, see: https://www.cdc.gov/zika/pdfs/living_in.pdf. For more information about counseling before testing, see: https://www.cdc.gov/zika/pdfs/preconception-counseling.pdf
5. Consider IgM testing to determine baseline Zika virus IgM levels as part of preconception counseling.

For more information for testing recommendations, please visit:
https://emergency.cdc.gov/han/han00402.asp,
http://www.cdc.gov/zika/hc-providers/testing-for-zikavirus.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 http://www.cdc.gov/mmwr/volumes/65/wr/mm6533e2.htm. If the clinical provider has questions regarding further testing of pregnant women or infants, please 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 6 months if the man is symptomatic and 2 months if the man is asymptomatic, regardless of Zika test results. If the woman has traveled to an area with active Zika virus transmission, then pregnancy should be postponed for 2 months, regardless of Zika virus test results.

Zika Laboratory Testing Information

- Approved laboratory tests for Zika virus infection diagnosis include a combination of polymerase chain reaction (RT-PCR), Zika virus IgM antibody, and plaque reduction neutralization antibody tests (PRNT). The Trioplex RT-PCR test, available at some state laboratories and CDC, allows for testing of serum and cerebrospinal fluid (CSF) for Zika virus, chikungunya, and dengue. Urine and amniotic fluid can be used to detect Zika virus only. The Zika IgM-ELISA is also available at some state laboratories, commercial laboratories, and CDC, and can be used to test serum and CSF specimens. PRNT testing on serum is confirmatory and is available at CDC and some state laboratories; these tests can measure virus-specific neutralizing antibody titers to determine the cause of primary flavivirus infection. Given the overlap of symptoms and endemic areas with other arboviral illnesses, patients should also be evaluated for other possible flavivirus infections. There may be serological cross-reactivity among the flaviviruses and current IgM antibody assays may not reliably distinguish between Zika virus and dengue virus infections. CDC has been looking for cross-reactivity on recent samples submitted for Zika virus testing and has found that the Zika virus IgM MAC-ELISA test is performing better than expected.
- Currently, the Utah Public Health Laboratory (UPHL) performs the Zika virus IgM MAC-ELISA and the Trioplex RT-PCR tests. 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. CDC is currently performing dengue and chikungunya antibody tests on Zika virus IgM-positive specimens only. If clinicians need to rule out these infections regardless of Zika virus results, these tests are available through commercial laboratories.

- Acute serum collected within the first 14 days following symptom onset should be tested by RT-PCR. IgM antibodies may be detectable by day 4 of illness but this test is more reliable later in the course of infection. For persons whose infections are equivocal on IgM, paired acute and convalescent specimens, collected 2-4 weeks apart, may be necessary to confirm or rule-out infection.
- Serum collected between 2 to 12 weeks following symptom onset should be tested by IgM.
- Urine specimens may be collected within the first 14 days following symptom onset and should be tested by PCR. Urine specimens must always be accompanied with a serum sample.
- 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

- At this time, Zika virus testing for Utah residents will be performed at UPHL free of charge. However, testing capacity may be limited; therefore, UPHL and CDC are requesting that the State Epidemiologist, Medical Officer on-call at the UDOH, 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 http://www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm?s_cid=mm6521e1_w#T1_down 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 **(with in 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 **(with in 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.
Placenta and fetal membranes[†]	Collect a minimum of (3) full thickness pieces (0.5-1 x 3-4 cm in depth) from middle third of placental disk and at least 1 from the placental disk margin. 5 x 12 cm strip of fetal membranes Please include sections of placental disk, fetal membranes, and	Tissues should be placed into two sterile containers containing adequate formalin. Formalin should be about 10x mass of tissue. Place in 10% neutral buffered formalin for a minimum of 3 days. Once fully fixed, tissue can be transferred to 70%	At least one formalin fixed (wet) or formalin-fixed paraffin-embedded (FFPE) placental tissue sample should be stored and sent at room temperature to UPHL.

	pathological lesions when possible. Label all specimens to identify location of sample.	ethanol for long-term storage.	
Umbilical cord[†]	Collect a minimum of (4) 0.25 cm squares from the umbilical cord. Umbilical cord segments should be obtained proximal middle, and distal to umbilical cord insertion site on the placenta. Label all specimens to identify location of sample.	Fresh tissues should be placed into two sterile containers. Formalin should be about 10x mass of tissue. Place in 10% neutral buffered formalin for a minimum of 3 days. Once fully fixed, tissue can be transferred to 70% ethanol for long term storage.	At least one formalin fixed (wet) or formalin-fixed paraffin-embedded (FFPE) umbilical cord tissue sample should be stored and sent at room temperature to UPHL.

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.

- http://www.cdc.gov/zika/hc-providers/tissue-collection-submission.html
- http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.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 the 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.