Diagnostic Testing and Case Reporting for Zika Virus (October 2016)

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Background

The Centers for Disease Control and Prevention (CDC) released testing algorithms for Zika virus infection. The CDC recommends a combination of molecular and serologic testing in order to definitively exclude Zika virus infection. Molecular testing (RT-PCR) is recommended for serum and urine samples collected < 14 days after symptom onset. A positive RT-PCR result in either specimen is confirmation of Zika virus infection and no additional testing is required. A negative RT-PCR result for a specimen collected less than a week after the start of illness usually means that Zika virus did not cause a patient's recent symptoms. Most people with Zika virus infection have virus in their blood for up to a week following the start of illness. A negative result that is incorrect can happen if an individual fights a Zika virus infection faster than most other people do. Also since symptoms can be mild, illness/symptoms may have started earlier than the date that the patient noticed them. In these cases, the virus may have been cleared before the specimens were collected. If both serum and urine are negative by RT-PCR, the CDC recommends that serum be analyzed by IgM antibody (serologic) testing.

The serologic tests, such as the Zika MAC-ELISA developed by the CDC, can detect IgM antibodies against Zika virus in serum or cerebrospinal fluid. However, due to cross-reactivity with other flaviviruses and possible nonspecific reactivity, results may be difficult to interpret. Presumed positive, equivocal, or inconclusive IgM tests may be forwarded to the CDC for confirmation by plaque-reduction neutralization testing (PRNT).

In response to the recent travel-related cases in El Paso County, the City of El Paso Department of Public Health (DPH) is able to provide RT-PCR tests for Zika virus at our laboratory. Capacity for testing is limited. Patients should be assessed by a health care provider prior to testing. If a health care provider would like the DPH Laboratory to perform RT-PCR tests for a patient, the provider should contact DPH Epidemiology staff at (915) 212-6520. DPH Epidemiology staff will screen a health care provider's request for RT-PCR testing.

Currently, at least two large commercial laboratories offer RT-PCR testing for both serum and urine. Both of these laboratories also offer IgM assays with serum. The Texas Department of State Health Services (DSHS) Laboratory in Austin offers Zika MAC-ELISA assays for patients who meet specific testing criteria; however, the expected turn-around time is 4 – 8 weeks. DPH Epidemiology staff must screen patients for the DSHS IgM assay prior to shipping. PRNT assays are performed at the CDC, and the CDC is working with other laboratories to increase testing capacity.

As new information is made available about Zika virus, the CDC will provide updated guidance. Please continue to visit this page to obtain up-to-date information.

Clinical and Epidemiological Criteria for Zika RT-PCR Testing by City of El Paso Department of Public Health

If a health care provider would like the DPH Laboratory to perform RT-PCR tests for a patient, the provider should contact DPH Epidemiology staff at (915) 212-6520. DPH Epidemiology staff will determine if a patient meets clinical and epidemiological criteria for RT-PCR testing.

Clinical Criteria for RT-PCR Testing of Serum and Urine

- Pregnant women* who within the past 13 days have had one or more clinical symptoms of Zika virus infection (fever, rash, joint pain, or red irritated eyes) and have an epidemiologic link suggesting exposure. Pregnant women meeting these criteria may also be considered for amniotic fluid testing.
- Individuals who within the past 13 days have had one or more clinical symptoms of Zika virus infection (fever, rash, joint pain, or red irritated eyes) and have an epidemiologic link suggesting exposure.
- For symptomatic pregnant* women, positive or equivocal IgM antibody test results are followed by RT-PCR on both serum and urine. Some pregnant women have been reported to have detectable RNA present in serum and/or urine beyond the acute phase.
- Asymptomatic pregnant women* who within the past 13 days have an epidemiologic link suggesting exposure.
- Pregnant women* with epidemiologic risk factors and complications of pregnancy:
    - Fetal loss OR
    - *In utero* findings of microcephaly and/or intracranial calcifications
- A person who developed Guillain-Barre syndrome following onset of clinical symptoms of Zika virus infection and not known to be associated with another diagnosed etiology. Cerebrospinal fluid (CSF) from symptomatic individuals with neurological symptoms may also be submitted along with match serum and urine specimens.
- Infant with fever, rash, conjunctivitis, or arthralgia within the first 2 weeks of life and mother has an epidemiologic link suggesting exposure.
    - RT-PCR within 7 days of symptoms onset

- Evaluation of infants and children for acute Zika virus infection should include testing of serum and urine and may include cerebrospinal fluid (CSF) testing for Zika viral RNA, if samples were obtained as part of routine care. A CSF sample collected for the sole purpose of Zika RT-PCR testing is not recommended.

- Within two days of birth**, an infant whose mother had laboratory evidence or indications of Zika virus infection:
  - A positive RT-PCR test in any maternal specimen
  - A positive or equivocal maternal IgM test

- Within two days of birth** and regardless of maternal Zika virus test results, an infant with abnormal clinical or neuroimaging findings suggestive of congenital Zika syndrome and a maternal epidemiologic link suggesting possible transmission.

- A person with no known exposure and 3 or more clinical symptoms of Zika virus infection (fever, rash, joint pain, or red irritated eyes) that are not explained by other illness. These patients will be evaluated on a case-by-case basis.

* *Pregnant women includes women who were not pregnant during travel or sexual exposure, but became pregnant within 8 weeks of exposure (within 6 weeks of last menstrual period).*

***Infant laboratory testing for Zika virus should be performed within the first 2 days after birth; if testing is performed later, distinguishing between congenital, perinatal, and postnatal infection will be difficult. If the timing of infection cannot be determined, infants should be managed as if they have congenital Zika virus infection.*

Epidemiologic Links

- Travel to an area with ongoing Zika virus transmission OR
- Sex without barrier protection with a partner with possible Zika virus exposure (partner does not need to be symptomatic) OR
- Receipt of blood or blood products within 30 days of symptom onset OR
- Organ transplant recipient within 30 days of symptom onset OR
- Association in time and place with a known case

Areas with Active Zika Virus Transmission

Below is the CDC's list of countries and territories with active Zika transmission as of September 28, 2016. Updates are posted on CDC's webpage CDC's List of Countries and Territories with Active Zika Virus Transmission.

Americas

Anguilla	Aruba	Bolivia	Cayman Islands
Antigua and Barbuda	The Bahamas	Bonaire	Colombia
Argentina	Barbados	Brazil	Commonwealth of Puerto Rico
	Belize		

Costa Rica
Cuba
Curaçao
Dominica
Dominican Republic
Ecuador
El Salvador
French Guiana
Grenada
Guadeloupe
Guatemala
Guyana
Haiti
Honduras
Jamaica
Martinique
Nicaragua
Panama
Paraguay
Peru
Saba
Saint Barthélemy
Saint Lucia
Saint Martin
Saint Vincent and the Grenadines
Sint Eustatius
Sint Maarten
Suriname
Trinidad and Tobago
Turks and Caicos
United States
Venezuela

Oceania/Pacific Islands

American Samoa
Fiji
Kosrae, Federated States of Micronesia
Marshall Islands
New Caledonia
Papua New Guinea
Samoa
Tonga

Africa

Cape Verde

Asia

Singapore

Florida

In the United States, please consider Miami-Dade County, Florida, specifically Miami (Wynwood neighborhood) and Miami Beach. For detailed maps of the designated areas see Zika in Florida. Travel to the Wynwood neighborhood of Miami anytime from June 15, 2016 to September 18, 2016 may be considered an epidemiologic link. For Miami Beach travel to the area from July 14, 2016 to the present may be considered a risk of exposure. Florida health officials are investigating non-travel related cases outside of Miami-Dade County. The investigation includes sampling close contacts and community members around each case to determine if additional people are infected. If evidence that active transmission is occurring in additional areas, Florida health officials will notify the media and the public.

Other countries

It is possible that a traveler may have been in exposed in a few other countries. Countries with possible endemic transmission or evidence of local mosquito-borne Zika infection in 2016 may be found on a World Health Organization webpage (http://www.who.int/emergencies/zika-virus/en/). As of September 29, 2016, Indonesia, Thailand, Philippines, Vietnam, Maldives, and Malaysia were listed on the WHO Situation Report.

Specimen Requirements for RT-PCR Testing by City of El Paso Department of Public Health Laboratory

Acceptable specimen types

All specimens must be labelled with patient's name and at least one other identifier (e.g., DOB or medical record). Specimens from individuals suspected of having Zika virus infection should be handled in accordance with the Healthcare Infection Control Practices Advisory Committee Standard Precautions Standard. These include the use of gloves, a laboratory gown or coat, and eye protection when handling

these specimens. In general, Biosafety Level 2 precautions, as described in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories manual, 5th edition (BMBL5) are appropriate for the handling of these specimens.

Serum

- Collect at least 5.0 mL of blood in a serum tube (red top or serum separator tube—tiger top/gold) and refrigerate at 4°C as soon as possible.
- Options for serum specimen handling:

  OPTION 1: Facility has a centrifuge and refrigerator. (Preferred option)

  Centrifuge within 2 hours from time of collection, to separate the serum from the red blood cells. Transfer the serum from the collection tube into a serum transport tube with screw cap for shipment. (Use of gloves, lab coat, mask, and eye protection are recommended when transferring serum.)

  OPTION 2: Facility has no centrifuge.

  Hold specimens in a refrigerator at 4°C and arrange courier transportation on cold packs to DPH Laboratory within 1 1/2 hours of collection. Specimens must arrive at DPH Laboratory by 3 pm AND within 1 1/2 hours of collection (to allow time for centrifugation within 2 hours) on a weekday. Label the outer packaging: "STAT specimen." Samples not meeting handling criteria will be rejected.

- Urine matched with patient serum
  - Urine should be collected in sterile container without preservatives
  - Provide at least 0.5 mL
  - Please ensure a tight seal as leaking specimens cannot be accepted
  - Specimens should be kept cold (2-8°C) or frozen (-≤ 20 °C)

- CSF matched with patient serum and urine
  - At least 1.0 mL
  - Specimens should be kept cold (2-8°C) or frozen (-≤ 20 °C)

- Amniotic fluid matched with patient serum and urine
  - Provide at least 0.5 mL
  - Specimens should be kept cold (2-8°C) or frozen (-≤ 20 °C)

Required Test Requisition Form

A completed specimen submission form must accompany each specimen submitted. The DPH specimen form is available on the EPHealth.com Zika webpage (Zika, Chikungunya, and Dengue Specimen Submission Form).

Specimen Transport

- All healthcare facilities must arrange for transport of specimens from their facility to the DPH Laboratory; DPH will not pick up specimens from any submitters.

- All specimens should be stored and transported cold or frozen.
  - STAT serum specimens requiring centrifugation
    - Arrange shipping or courier transportation to DPH Laboratory with cold packs for arrival within 1 1/2 hours of collection (to allow time for centrifugation within 2 hours of collection), to arrive during business hours Monday–Friday, 8:00 am – 4:30 pm.
  - Other specimens
    - Arrange shipping or courier transportation to DPH Laboratory with cold packs for arrival within 24 hours of collection, to arrive during business hours Monday–Friday, 8:00 am – 4:30 pm.
    - If the specimen will be received at the DPH Laboratory more than 24 hours after collection, freeze serum and urine transport tubes at -20°C or -70°C and ship on dry ice. Specimens can be batched for shipping. Label the outer packaging: "Store at -70°C upon arrival."
- When transporting human specimens, ensure that all applicable regulations for transport of potentially infectious biological specimens are met. Follow packing and shipping instructions for Category B, Biological Substances (see Guidance for packaging samples in accordance with Category B Biological substance requirements can be found in the CDC/NIH Publication Biosafety in Microbiological and Biomedical Laboratories, 5th edition (BMBL5). Additional information may be found in the Department of Transportation Hazardous Materials Transport Regulations).
- Ship according to IATA (International Air Transport Association) guidelines using an overnight or same-day courier to arrive during business hours Monday–Friday, 8:00 am – 4:30 pm.
- DPH Laboratory delivery and shipping address:

  City of El Paso Department of Public Health Laboratory
  4505 Alberta Avenue, 2nd Floor
  El Paso, TX 79905
  Phone: (915) 543-3255

City of El Paso Department of Public Health Laboratory RT-PCR Test Results

- Turn-around time for RT-PCR will depend upon testing volume. Expected turn-around time is 3 days.
- As results are available, submitters will receive a faxed copy to the fax number noted on the submission form.
- For Health Care Provider Fact Sheets see Fact Sheet for Health Care Providers http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491588.pdf
- For Patient Fact Sheets see Understanding Results from the Trioplex Real-Time PCR Assay http://www.cdc.gov/zika/hc-providers/testresults.html. Two fact sheets are available; one is specifically for pregnant women. Both fact sheets for patients are available in English and Spanish.

City of El Paso Department of Public Health Laboratory RT-PCR Cost

There is no cost for Zika RT-PCR testing at the City of El Paso DPH laboratory, which can accept specimens from residents in El Paso County who meet specific testing criteria. Please call the City of El Paso Department of Public Health to request testing (915) 212-6520.

The DPH Laboratory can also accept specimens from residents of the following counties: Brewster, Culberson, Hudspeth, Jeff Davis, Pecos, Presidio, Reeves, and Terrell. Please call the Texas Department of State Health Services Region 9/10 to request testing (888) 847-6892.

Texas Department of State Health Services IgM AntibodyTests

If a health care provider would like DSHS Laboratory in Austin to perform IgM tests (Zika MAC-ELISA) for a patient, the provider should contact DPH Epidemiology staff at (915) 212-6520. DPH Epidemiology staff will determine if a patient meets clinical and epidemiological criteria for IgM testing. If a patient meets the criteria, DPH staff will coordinate testing with DSHS.
The Texas Department of State Health Services (DSHS) Laboratory in Austin perform IgM testing of serum. Please note that turn-around times for the DSHS Laboratory are longer than those for commercial laboratories. The DSHS Laboratory turn-around time is 4-8 weeks for the IgM assay.

To be eligible for testing by the DSHS Laboratory, the patient must meet DSHS's specific criteria. Below are the two most commonly used criteria for IgM testing. For the entire list of criteria please see Texas DSHS Zika Specimen Criteria Version 5.0 September 8, 2016 .

- Individuals who have a clinical illness consistent with Zika virus disease during or within 2 weeks of travel to areas with ongoing Zika virus transmission and who present 14 or more days after onset of symptoms
- Asymptomatic pregnant women (women who do not report clinical illness consistent with Zika virus disease) with possible Zika virus exposure and who present 14 or more days after onset of symptoms

DSHS requests payor source information for patients who have Medicaid, Medicare, or insurance. However, the DSHS Laboratory does not require patients who meet their criteria for Zika virus testing to have Medicaid, Medicare, or insurance.