MISSISSIPPI STATE DEPARTMENT OF HEALTH

November 30, 2016
Zika Virus Testing Information

Diagnostic Testing

The Mississippi Public Health Laboratory (MPHL) is currently performing the following tests:

1. Reverse transcription polymerase chain reaction (Trioplex RT-PCR) assays for the detection of Zika, dengue, and chikungunya viral nucleic acid in serum, urine, whole blood and cerebral spinal fluid (CSF).
2. IgM antigen-capture MAC-ELISA assay for detection of Zika, dengue, and chikungunya IgM antibody in serum. CSF specimens may be submitted for IgM testing if they are accompanied by a serum specimen.

NOTE: Specimens that test positive or equivocal for Zika virus IgM will be forwarded to the Centers for Disease Control and Prevention (CDC) for confirmatory plaque-reduction neutralization (PRNT) testing due to significant cross-reactivity between Zika and other Flaviviruses in the MAC ELISA assay.

Pre-approval Required for Zika virus testing at MPHL:

Healthcare providers should contact the Mississippi State Department of Health (MSDH) Office of Epidemiology at (601) 576-7725 during normal business hours to arrange for Zika virus testing (601-576-7400 after hours, weekends or holidays). After testing is approved, the MSDH Office of Epidemiology will provide the requesting physician or laboratory with a form that must be completed and accompany the specimen.

MSDH Testing Recommendations:

- Symptomatic persons (at least one of the following symptoms within 2 weeks of exposure – rash, fever, arthralgia, or conjunctivitis) that live in or have a history of travel to an area with active Zika transmission OR sexual contact with a traveler or resident of an area with active Zika transmission.
- Asymptomatic pregnant women who, while pregnant, have lived in or traveled to an area with active Zika transmission or have had sexual contact with a traveler or resident of an area with active Zika transmission.
  Please see the following for up to date information on areas with active Zika transmission,
  http://www.cdc.gov/zika/intheus/florida-update.html or http://www.cdc.gov/zika/geo/active-countries.html

Refer to http://msdh.ms.gov/msdhsite/_static/resources/6804.pdf for the MSDH Zika testing algorithm for healthcare providers.

Currently available MPHL Zika Virus tests and Indications

Test	***Test Description***	***Required specimens per patient***	***Optional specimen types****
Zika TrioPlex RT-PCR	Detects Zika virus, dengue virus, and chikungunya virus RNA	Serum, urine and whole blood(EDTA)	Cerebral Spinal Fluid Amniotic fluid
Zika IgM MAC-ELISA	Detects Zika virus IgM antibodies.	Serum	Cerebral Spinal Fluid

*Optional specimens MUST be submitted with a Serum specimen

Acceptable Specimens, Required Volumes, and storage requirements:

Specimen Type	***Specimen Collection***	***Specimen Storage***
Serum	Collect 5-10 ml of blood in a red top or serum separator tube (tiger top or speckle top). Allow blood to clot for 30 minutes at room temperature. Promptly separate (centrifuge) serum from cells and transfer serum to a plastic tube with screw cap prior to shipment.	Store the separated serum at 2-8°C.
Whole Blood	Collect 3-5ml of blood in a EDTA blood collection tube.	Store at 2-8°C
Cerebral Spinal Fluid (Must be submitted with a serum sample)	Collect 1-2 mL in sterile, leak-proof, screw cap plastic tube.	Store at 2-8°C
Urine (For PCR testing only. Must be submitted with a serum sample)	Collect urine in a clean container and pour off 3mLs into two clean plastic, leak proof, screw capped tubes. Do not collect urine in a container with preservative. Urine should be collected no later than 14 days after symptom onset.	Store at 2-8°C
Amniotic fluid (For PCR testing only. Must be submitted with a serum sample)	Submit a minimum of 1mL of amniotic fluid in a sterile, leak-proof, screw cap plastic tube.	Store at 2-8°C

Label each specimen with the patient's full name, Date of Birth, and specimen type (urine, serum, CSF, or whole blood).

Shipping Instructions and Required Documents:

- Specimens must be shipped in an insulated container with cold packs. Specimens should be triple packaged as a Category B Biological Substance.
- A MPHL Zika Virus test request form must be completed for each specimen submitted. The patient's name and DOB must match the information on the specimen container. The form can be printed from http://msdh.ms.gov/msdhsite/_static/resources/6667.pdf.
- Specimens must be shipped to the MPHL within 24 hours of specimen collection. After Office of Epidemiology approval is obtained, specimens may be delivered to a local health department for MSDH courier delivery to the MPHL or can be delivered directly to the MPHL.
- **Specimens without MSDH Office of Epidemiology approval and/or a completed test request form will not be tested.**

Turnaround Time (TAT):

MPHL testing: Specimen results are normally reported within 3 to 5 working days.
CDC confirmatory testing: Specimen results are normally reported within six weeks.

Reference Range:

RT-PCR= Normally Not Detected; IgM Serology= Normally Negative

MPHL Zika Virus Testing Algorithm

Blood and urine collected <14 days post exposure/onset of symptoms	Trioplex RT-PCR →	**Positive for Zika Virus** (Serum, Urine and Whole Blood) and **Positive or Negative for Dengue and Chikungunya Virus** (Serum, Whole Blood) by Trioplex RT-PCR. Testing final; No further testing performed A follow-up serum may be collected from asymptomatic pregnant women 2-12 weeks post exposure for Zika IgM serolgoy.
	→	**Negative for Zika Virus** (Serum, Urine and Whole Blood) and **Positive or Negative for Dengue and Chikungunya Virus** (Serum, Whole Blood) by Trioplex RT-PCR. Reflex Serum to Zika IgM serology. Testing follows Zika MAC ELISA algorithm below. Serum may also be reflexed to dengue and chikungunya IgM serology testing based on exposure history.
Blood and urine collected ≥ 14days to 12 weeks post exposure/onset of symptoms	Zika MAC-ELISA →	**Presumptive Positive** or **Equivocal** for Zika Virus IgM antibodies. If patient is not pregnant, serum is referred to the CDC for IgM confirmation by Plague Reduction Neutralization Test. If patient is pregnant, serum is reflexed to Zika Virus RT-PCR. If serum is positive for Zika Virus, testing is complete but if serum is negative for Zika Virus, serum is referred to the CDC for IgM confirmation by Plague Reduction Neutralization Test
	→	**Negative** for Zika Virus IgM antibodies. Testing Final; No further testing performed.