Connecticut Department of Public Health

Testing Performed to Identify Zika Virus Infection

Available at the CT Public Health Laboratory (PHL)

RT-PCR - Test for viral genetic material. The test is best performed on serum and urine within first 2 weeks of symptom onset. It can also be performed on placental tissue to confirm maternal infection.

IgM antibodies - Test is for immunoglobulin M in serum by an enzyme-linked immunosorbent assay (ELISA). The best time to test is 2-12 weeks after exposure. May be positive for asymptomatic or symptomatic patients.

Specimens Sent to the Centers for Disease Control and Prevention

PRNT - A more specific antibody test that is performed on ***IgM presumptive positive*** specimens to detect the presence of anti-Zika neutralizing antibodies and rule out cross reactivity due to other flaviviruses such as dengue. This is considered when RT-PCR is either not performed or was negative. Important to note that while generally more specific than an IgM ELISA it often fails to distinguish serologic reactions to cross reacting flaviviruses.

Placental tissue –Testing by RT-PCR or immunohistochemical staining for Zika virus is performed to confirm maternal infection.

Definition of Zika Virus Exposure

- Travel to an area where Zika virus is spread by mosquitoes; or
- Unprotected sex with someone who has travelled within 2 weeks of their return

Zika Virus Testing at the CT PHL

For symptomatic patients (male or female):

- ***Submit serum and urine samples***.
- Criteria for testing include at least one of the following: fever, rash, arthralgia, conjunctivitis or Guillain-Barre' syndrome and an exposure.
- Serum and urine specimens collected within 2 weeks of symptom onset are tested by RT-PCR.
- Serum specimens collected between 2 - 12 weeks are tested for anti-Zika IgM antibodies first and if IgM is presumptive positive or equivocal then RT-PCR is performed to confirm Zika virus infection rather than a reaction to a related flavivirus. If RT-PCR is negative PRNT is considered.

For asymptomatic patients (female only):

- ***Submit serum and urine samples***
- Criteria for testing include pregnancy and an exposure.
- Serum and urine specimens collected within 2 weeks of last exposure are tested by RT-PCR.
- Serum specimens collected 2-12 weeks following the last exposure are tested for anti-Zika IgM antibodies first and if IgM is positive or equivocal then RT-PCR is performed to confirm Zika virus infection rather than a reaction to a related flavivirus. If RT-PCR is negative PRNT is considered.

Infants:

- ***Submit infant serum and urine.***
- Specimens should be collected in the first 2 days of life if possible.
- Note that cord blood is no longer a recommended specimen for testing.
- Infants are tested if the mother tested positive for Zika or an undetermined flavivirus.
- Serum and urine specimens are tested by RT-PCR, and serum is tested for IgM antibodies.
- If an infant is born with a birth defect characteristic of Zika virus and the mother has a history of exposure but was not tested previously then the mother is tested for IgM antibodies; if the mother is positive then the infant is also tested.
- However, if there is concern regarding compliance with recommended infant follow-up, then infant testing should be performed before hospital discharge.
- ***Submit placental tissue*** to confirm maternal infection when maternal and fetal testing of serum and urine is not definitive for Zika and concerns regarding the health of the infant remain.

Specimen Handling and Request for Testing at the State PHL

Testing of clinical specimens for viral RNA by RT-PCR is offered at the State PHL for all who meet clinical and exposure criteria ***and are pre-approved for testing by the DPH Epidemiology Program***. The PHL uses the Trioplex assay to detect Zika, Dengue and Chikungunya viruses in serum and Zika virus in urine. Providers must complete and fax the ***Zika Virus Report Form*** **to 860-509-7910.** An epidemiologist will review the request and contact the provider's office by the next business day. For questions regarding the approval process please call **860-509-7994**. The form is available at: http://www.ct.gov/dph/lib/dph/infectious_diseases/pdf_forms_/zika_virus_report_form_current_fnl.pdf

Serum (3-5 ml) and ***urine*** (at least 1 ml) must be collected within 14 days from symptom onset for testing using the RT-PCR assay; for infant serum provide at least 1 ml.. Specimens can be stored and shipped refrigerated (2-6°C) or frozen (-70°C). Urine should be collected in a sterile container with a tight fitting screw cap (possibly film secured). Refrigerated specimens may be shipped to the CT PHL with adequate ice packs, and for frozen specimens, ship on dry ice to ensure specimens remain frozen until received. Complete all required fields on the CT PHL Clinical Test Requisition ***OL-9B form***: http://www.ct.gov/dph/lib/dph/laboratory/labhome/forms/clinical_test_requisition_ol9b_fill.pdf. For questions regarding specimen handling please call **860-920-6635 or -6506**.

When submitting ***placental tissue*** include sections of the placental disk (3 full thickness pieces from the middle third and one from the margin), fetal membranes 5x12 cm strip), and pathologic lesions when possible. Fix specimens in formalin with a volume 10x the mass of tissue. Specimens can be shipped at room temperature.

Specimens may be packaged and shipped to the CT PHL as ***Category B Biological Substances*** in accordance with the Department of Transportation Hazardous Materials Regulations (49 CFR Part 171-180).

Commercial Laboratories Testing for Zika Virus

Please see ***Disclaimer*** at: http://portal.ct.gov/policies/disclaimer/

1. **Quest Diagnostics Laboratory**
   Currently offers testing of serum and urine specimens for Zika virus RNA by RT-PCR for patients satisfying the CDC recommendations.

   Information is available from the Quest Test Center for ***Zika Virus RNA, Qualitative, Real-Time RT-PCR Panel, Serum/Urine at:***
   http://www.questdiagnostics.com/home/physicians/testing-services/condition/infectious-diseases/zika

2. **Laboratory Corporation of America (LabCorp)**
   Currently offers testing of serum and urine specimens for Zika viral RNA by RT-PCR and testing of serum for anti-Zika IgM antibodies for patients satisfying the CDC recommendations. All IgM presumptive positive, equivocal or inconclusive results are confirmed by RT-PCR or by PRNT performed at the CDC.

   Information for the ***Zika Virus Comprehensive Profile, NAA, Serum and Urine*** and the ***Zika Virus, MAC-ELISA, IgM, Serum*** is available at: https://www.labcorp.com/wps/portal/provider/testmenu

3. **Mayo Medical Laboratory**
   Currently offers testing of serum, plasma and urine specimens for Zika viral RNA by RT-PCR and testing of serum for anti-Zika and anti-dengue IgM antibodies for patients satisfying the CDC recommendations. A presumptive positive result by this assay is not diagnostic. All IgM presumptive positive, equivocal or inconclusive results are confirmed by RT-PCR or by PRNT performed at the CDC.

   Information for the ***Zika Virus Real-time RT-PCR, Plasma, Serum, Urine*** and ***Zika and Dengue Virus Panel, IgM, Serum*** are available at:
   http://www.mayomedicallaboratories.com/test-catalog/alphabetical/Z