



Memorandum

Date: February 7, 2016

From: CDC, Division of Vector-Borne Diseases

Subject: Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories

Background

Many countries in the Americas now have local transmission of multiple arboviruses that can cause febrile illness with rash, myalgia, or arthralgia. Therefore, laboratory testing has become even more important to confirm the etiology of these diseases. Zika, chikungunya, and dengue virus infections should all be considered for patients with acute fever, rash, myalgia, or arthralgia who have traveled within the previous 2 weeks to an area with ongoing transmission or are living in an area with ongoing transmission. In accordance with the Updated Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age During Ongoing Zika Virus Transmission – United States, 2016 (<http://www.cdc.gov/mmwr/volumes/65/wr/mm6505e2er.htm>), this test algorithm now includes a recommendation to offer serologic testing to asymptomatic pregnant women with a history of travel to areas with local transmission of Zika virus or are living in an area with ongoing transmission. Laboratory evidence of recent chikungunya, dengue, or Zika virus infection is generally accomplished by testing serum to detect viral nucleic acid or virus-specific immunoglobulin (Ig) M and neutralizing antibodies. However, serological cross-reactivity may occur between Zika and other flaviviruses (e.g., dengue, yellow fever, St. Louis encephalitis, Japanese encephalitis, West Nile), so emphasis should be placed on molecular testing (RT-PCR) in acute specimens received from individuals with clinically compatible illness. Laboratory testing for Zika, chikungunya, and dengue viruses is currently available at CDC and several state and territory health departments.

Laboratory assays for acute specimens

During the first 7 days of these illnesses, viral RNA can often be identified in serum, and RT-PCR is the preferred test for Zika, chikungunya, and dengue viruses. In addition, for dengue viruses, NS1 antigen can be detected by ELISA in acute phase specimens but this assay is not widely available in the US. Because viremia decreases over time, a negative RT-PCR collected 5-7 days after symptom onset does not exclude flavivirus infection and serologic testing should be performed.

Virus-specific IgM antibodies may be detectable ≥ 4 days after onset of illness. However, serum collected within 7 days of illness onset may not have detectable virus-specific IgM antibodies. IgM antibodies against Zika virus, dengue viruses, and other flaviviruses have strong cross-reactivity which may generate false positive results in serological tests.

Laboratory assays for convalescent specimens

IgM antibodies typically persist for approximately 2-12 weeks. In patients with a compatible clinical syndrome, serum collected as early as 4 days after illness onset can be tested by Zika, chikungunya, and dengue virus-specific IgM ELISA and positive results confirmed by testing for neutralizing antibodies (Figure 1).

Due to serological cross-reactivity between flaviviruses, current IgM antibody assays cannot reliably distinguish between Zika and dengue virus infections. Therefore, an IgM positive result in a dengue or Zika IgM ELISA test should be considered indicative of a recent flavivirus infection. Plaque-reduction neutralization tests (PRNT) can be performed to measure virus-specific neutralizing antibodies and may be able to determine the cause of primary flavivirus infection. In patients who have received yellow fever or Japanese encephalitis vaccination or infected with another flavivirus 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.

Serologic testing for Zika virus infection may be performed on serum specimens from asymptomatic pregnant women (Figure 2). Serologic test interpretation is complex; a positive IgM result can be difficult to interpret since cross-reactivity can occur with related flaviviruses. PRNT may be able to discriminate between cross-reacting antibodies in primary flavivirus infections. In addition, a negative Zika IgM result obtained 2-12 weeks after travel suggests that infection did not occur. Based on experience with other flaviviruses, we expect that antibodies will be present at least 2 weeks after virus exposure and persist for at least 12 weeks. Information about the performance of serologic testing of asymptomatic individuals is limited.

As with any diagnostic test, while a negative Zika IgM or RT-PCR test would suggest that an infection has not occurred, a negative Zika IgM or RT-PCR test result does not rule out infection with Zika virus.

For additional information, please see Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure — United States, 2016 (<http://www.cdc.gov/mmwr/volumes/65/wr/mm6505e2er.htm>).

Laboratory safety

Zika and dengue viruses are classified as biological safety level (BSL) 2 pathogens while chikungunya virus is classified as a BSL-3 agent. All should be handled in accordance with Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines and a risk assessment performed for each laboratory for the specific procedures utilized. Until the association between Zika virus infection and congenital microcephaly is better understood, pregnancy should be considered a significant factor in risk assessment for individuals working with Zika virus, and the involvement of pregnant workers in studies with Zika virus should be minimized. It is recommended that laboratories perform a risk assessment when bringing on new tests, and safety precautions should be based on each laboratory's risk assessment. In particular, because chikungunya virus produces such high levels of viremia, serum from suspected chikungunya virus cases should be treated as potentially infectious even for serological procedures. For further information, see: BMBL <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>, and

Healthcare Infection Control Practices Advisory Committee Standard Precautions Standard (http://www.cdc.gov/hicpac/2007IP/2007ip_part3.html)

Options for obtaining/conducting Zika, chikungunya, and dengue virus diagnostic testing

CDC

Zika, chikungunya, and dengue virus RT-PCR, IgM ELISA, and PRNT are performed at CDC. The specific tests performed will depend on the timing of the specimens relative to illness onset and clinical information as outlined in the algorithm figure. To determine the appropriate testing algorithm and interpret results, please provide the date of illness onset, dates of specimen collection, specimen type, description of clinical illness, travel history, flavivirus vaccination history, and contact information for the submitter. Testing will primarily be performed on serum or CSF but other specimen types, including urine, amniotic fluid, and tissues, can be submitted alongside a patient-matched serum specimen for evaluation of the utility of these specimen types.

Within Puerto Rico, please call 787-706-2399 for questions about testing. For submission of specimens, please submit a dengue case investigation report (DCIR) for each specimen which can be downloaded from: <http://www.cdc.gov/dengue/clinicalLab/index.html>

For all other states and territories, state health departments should contact the CDC Arboviral Diseases Branch at 970-221-6400. A completed DASH form should accompany submitted specimens. More information about submitting specimens to CDC is at: <http://www.cdc.gov/ncezid/dvbd/specimensub/arboviral-shipping.html>.

State and Territory Health Department Laboratories

RT-PCR: The CDC chikungunya virus and Zika virus RT-PCR protocols follow essentially the same protocol as the CDC West Nile virus RT-PCR assay. CDC will provide chikungunya and Zika virus primer/probe sequences, an RNA-positive control, and chikungunya and Zika virus RT-PCR proficiency panels to state and territory laboratories that have demonstrated proficiency at the CDC West Nile virus RT-PCR assay. Dengue virus RT-PCR kits can be ordered online using the following link: <http://www.cdc.gov/dengue/clinicalLab/realTime.html>

Zika virus IgM ELISA: The CDC Zika virus IgM ELISA is similar to the CDC West Nile virus IgM ELISA assay. State and territory laboratories that have demonstrated proficiency in performing the CDC West Nile virus IgM ELISA during the 2015 evaluation can request Zika virus antigen, conjugated antibody, and positive control serum for use in the CDC Zika virus IgM ELISA.

The CDC is currently working on a regulatory pathway to manufacture and distribute assays to support laboratory response to Zika. Further details will be shared when information becomes available.

For state and territory health departments interested in obtaining the materials described above, please contact eocevent278@cdc.gov. If your state or territory health department laboratory does not perform the CDC West Nile virus RT-PCR assay or IgM ELISA assay, consider sending specimens to CDC or using one of the commercial options described below.

Commercially available testing

There are no commercially available FDA-cleared diagnostic assays or kits for Zika virus infection in the United States at this time.

The following commercial reference laboratories perform testing for chikungunya and dengue viruses but none of the assays are FDA-cleared.

- Focus Diagnostics (<http://www.focusdx.com/>) performs a chikungunya virus RT-PCR and IgM and IgG IFA assays as well as an anti-DENV IgM ELISA.
- ARUP Laboratories (<http://www.aruplab.com/>) performs chikungunya virus and dengue virus IgG and IgM ELISA testing.
- Quest Diagnostics (<http://www.questdiagnostics.com>) performs dengue virus IgG and IgM immunoassays.

There is an FDA-cleared kit for anti-DENV IgM antibodies which can be purchased (InBios, USA).

The following chikungunya virus IgM antibody test kits are available for purchase in the United States and provide sensitivity and specificity comparable to that of the CDC assays but may not be FDA-cleared:

- Anti-CHIKV IgM human ELISA kit (Abcam, UK)
- Anti-CHIKV ELISA (IgM) (Euroimmun, Germany)
- Anti-CHIKV IIFT (IgM) (Euroimmun, Germany)

Specimen collection and shipping

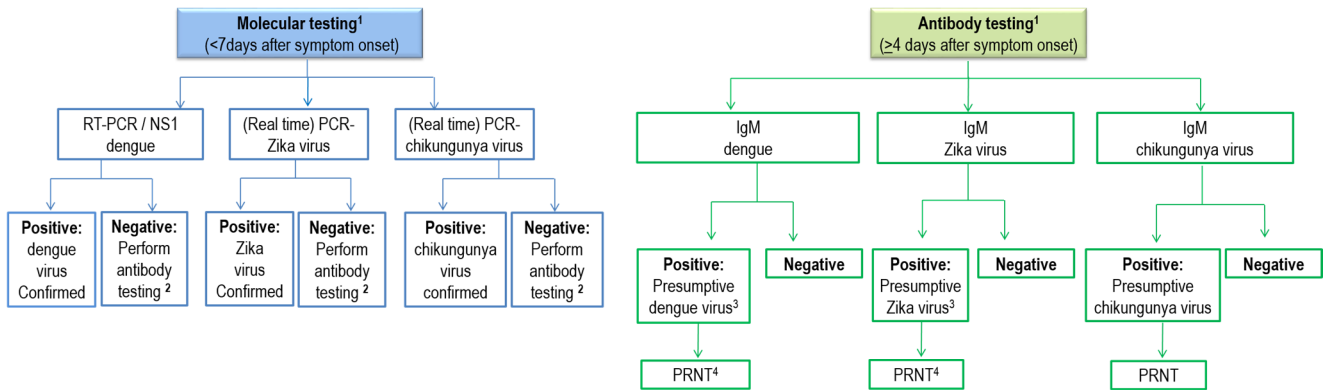
If a patient is suspected of having a Zika, chikungunya, or dengue infection, a serum specimen must be collected. Other specimens may also be collected for testing in addition to the serum specimen including CSF, urine, amniotic fluid and tissues. Information on the patient including the date of illness onset, description of clinical illness, travel history, and flavivirus vaccination history should be documented. Additional information on specimen collection can be found at: <http://www.cdc.gov/ncezid/dvbd/specimensub/arboviral-shipping.html>

Specimens collected from individuals for Zika virus studies may be transferred within the U.S. as Category B Biological substances in accordance with Department of Transportation Hazardous Materials Regulations (49 CFR Part 171-180). 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. Additional information about the Department of Transportation Hazardous Materials Transport Regulations may be found at <https://www.transportation.gov/pipelines-hazmat>.

Reporting

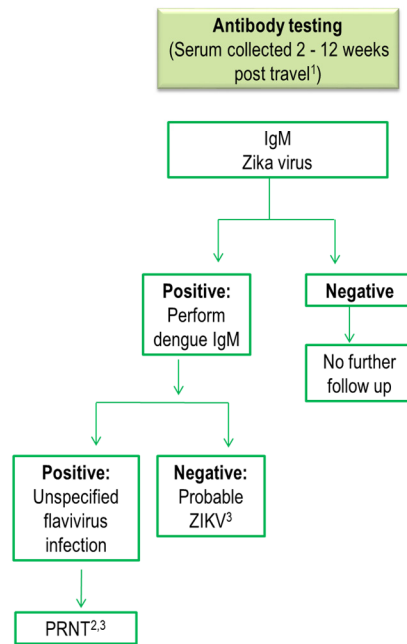
Zika, dengue, and chikungunya are all nationally notifiable conditions; state and territory health departments should report cases to CDC according to standard CSTE case definitions. State and territory health departments are requested to report laboratory-confirmed cases of any arbovirus to CDC through ArboNET, the national surveillance system for arboviral disease.

**Tiered algorithm for arbovirus detection for suspected cases of chikungunya, dengue, or Zika
(Testing only performed if patient symptomatic and travel history indicates travel to affected area.)**



- ¹ Due to extensive cross-reactivity in flavivirus serological assays, for samples collected <7 days post illness onset, molecular detection should be performed first.
- ² Perform if sample ≥ 4 days after symptom onset
- ³ Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).
- ⁴ PRNT should include any flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).

Testing algorithm for Zika virus detection in asymptomatic, pregnant women



- ¹ For women living in areas with ongoing transmission, refer to Updated Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age During Ongoing Zika Virus Transmission — United States, 2016 for timing of sample collection.
- ² Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM assay with both antigens should be followed up by using PRNT against both ZIKV and DENV as well as any other flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas). Depending on previous flavivirus exposure, resolution of infecting flavivirus may not be possible.
- ³ Follow up care should be undertaken as specified in the Interim Guidelines for Pregnant Women During a Zika Virus Outbreak – United States, 2016.