Memorandum

Date: January 13, 2016

From: CDC, Division of Vector-Borne Diseases, Arboviral Diseases and Dengue Branches

Subject: Updated 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. For patients with acute fever, rash, myalgia, or arthralgia and have travelled within the previous 2 weeks to an area with ongoing transmission, Zika, chikungunya, and dengue virus infections should all be considered. 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 is strong between Zika and dengue viruses, so emphasis should be placed on molecular detection in acute specimens. Laboratory testing for all of these agents is currently available at CDC and several state 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 all three 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.

Virus-specific IgM antibodies may be detectable >3 days after onset of illness. However, serum collected within 7 days of illness onset may not have detectable virus-specific IgM antibodies and IgM testing should be repeated on a convalescent-phase sample to rule out infection in patients with a compatible clinical syndrome. IgM antibodies against Zika virus, dengue viruses, and other flaviviruses (e.g., yellow fever and West Nile virus) have strong cross-reactivity possibly generating false positive results in serological tests.

Laboratory assays for convalescent specimens

IgM antibodies typically persist for months. In patients with a compatible clinical syndrome, serum collected more than 8 days after illness onset should be tested by virus-specific IgM ELISA and positive results confirmed by testing for neutralizing antibodies.

There is substantial serological cross-reactivity between the flaviviruses and 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 discriminate between cross-reacting antibodies in primary flavivirus infections. For primary flavivirus infections, a fourfold or greater increase in virus-specific neutralizing antibodies between acute- and convalescent-phase serum specimens collected 2 to 3 weeks apart may be used to confirm recent infection. In patients who have been immunized against (e.g., received yellow fever or Japanese encephalitis vaccination) or 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.

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. 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.

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

CDC

Zika, chikungunya, and dengue virus RT-PCR, IgM ELISA, and plaque reduction neutralization tests (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 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, questions about laboratory testing or sending specimens to CDC should be directed tothe Arboviral Diseases Branch on-call epidemiologist 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 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 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 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.

For state health departments interested in obtaining the materials described above, please contact dvbid@cdc.gov. If your state 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 laboratory options described below.

Commercially-available testing
There are no commercially-available diagnostic assays or kits for Zika virus infection.

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

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

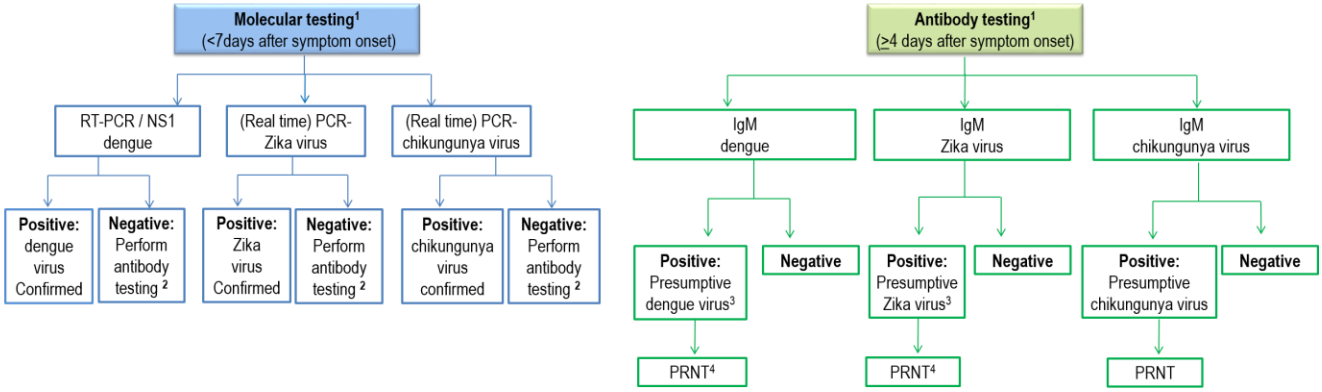
- Focus Diagnostics (http://www.focusdx.com/) performs a chikungunya virus RT-PCR and IgM and IgG IFA assays as well as an anti-DENV IgM ELISA.
- ARUP Laboratories (http://www.aruplab.com/) performs chikungunya virus and dengue virus IgG and IgM ELISA testing.
- Quest Diagnostics (http://www.questdiagnostics.com)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

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 not all are FDA-cleared:

- Anti-CHIKV IgM human ELISA kit (Abcam, UK)
- Anti-CHIKV ELISA (IgM) (Euroimmun, Germany)
- Anti-CHIKV IIFT (IgM) (Euroimmun, Germany)
- CHIKjj *Detect* MAC-ELISA (Inbios, USA)

Reporting
Dengue, chikungunya, and Zika are nationally notifiable conditions; state health departments should report cases to CDC according to standard CSTE case definitions. State 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 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).