Many countries in the Americas are experiencing simultaneous outbreaks of arboviral diseases that can cause febrile illness with rash, myalgia, or arthralgia. Agents include dengue, chikungunya, and Zika viruses. As a result, laboratory testing has become even more important to confirm the etiology of a case. Because of the similar geographic distribution and clinical presentation of Zika, dengue, and chikungunya virus infections, patients with symptoms consistent with Zika virus disease should also be evaluated for the other two agents. No commercial assay is currently available for detecting Zika virus, and serologic cross-reactivity is strong among Zika, dengue, and other flaviviruses. **Testing at CDC must be coordinated through the local health jurisdiction.** Contact information for your local health jurisdiction can be found [here](#).

**Laboratory Testing through Washington State Public Health Laboratories (PHL)**

**Testing Suspect Cases:**
If a provider suspects Zika virus disease in their patient, they must consult with their [Local Health Jurisdiction (LHJ)](#), which must approve specimen submission to CDC and notify DOH of inbound specimens. Once LHJ approval is granted, specimens should be shipped to the Washington State Public Health Laboratory (PHL) with the appropriate form.

- PHL does not test for these arboviruses but will forward approved specimens to CDC.
- If dengue and/or chikungunya are possibilities, a separate specimen should be sent commercially if possible to assist in decreasing the workload at CDC.
- During the first 7 days of illness, viral RNA can often be identified in serum, and RT-PCR is the preferred test (see CDC algorithm below).
- Virus-specific IgM antibodies by ELISA may be detectable >3 days after onset of illness, however, serum collected within 7 days of onset may not have detectable virus-specific IgM antibodies. For negative tests on specimens collected early in illness, IgM testing should be repeated on a convalescent sample to rule out infection in patients with compatible clinical syndromes. IgM typically persists for months.
- Positive IgM results by ELISA should be confirmed by testing for neutralizing antibodies. IgM antibodies against Zika virus, dengue virus, and other flaviviruses (e.g. yellow fever and West Nile virus) have strong cross-reactivity, possibly generating false positive results. Current IgM antibody assays cannot reliably distinguish between Zika and dengue virus infections. 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 four-fold or greater increase in virus-specific neutralizing antibodies between acute- and convalescent serum specimens collected 2-3 weeks apart confirms recent infection.
- In patients who have been immunized against 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.

**Testing is arranged through the local health jurisdiction, which must approve specimen submission to CDC.**

**Collect 2mL serum (0.25mL serum minimum), refrigerate and transport cold.** **Ship with the serology form to PHL, weekday arrivals only using Category B labels and packaging.** All specimens sent to PHL require two patient identifiers, both on the specimen label and on the submission form. Make sure to fill out the field “specific agent suspected” with “Zika virus,” and complete as many of the vaccination history and travel history fields as possible.
Testing Perinatal Suspect Cases:

- For pregnant women who test positive for Zika virus infection, or for whom fetal ultrasounds detect microcephaly or intracranial calcifications, Zika virus RT-PCR can be performed on amniotic fluid. The sensitivity and specificity of this test are currently unknown for congenital infection. It is also unknown if a positive result is predictive of a subsequent fetal abnormality.

- For a live birth with evidence of maternal or fetal Zika virus infection, collect the following specimens if possible: fixed placenta and umbilical cord tissue for histopathologic examination, frozen placental tissue and umbilical cord tissue for RT-PCR, and umbilical cord serum for serologic testing. Serum can alternatively be collected directly from the infant within 2 days of birth. CSF obtained for other studies can also be tested. If testing was not completed for the mother, also collect maternal serum.

- For pregnancies resulting in fetal loss in a woman with history of travel to an area of Zika virus transmission and with symptoms consistent with Zika virus disease during or within 2 weeks of travel or findings of fetal microcephaly, collect placental and umbilical cord tissue: fixed tissue for histopathologic examination and frozen tissue for RT-PCR.

Ship with the serology form to PHL, weekday arrivals only using Category B labels and packaging. All specimens sent to PHL require two patient identifiers, both on the specimen label and on the submission form. Make sure to fill out the field “specific agent suspected” with “Zika virus.”
Tiered algorithm for arbovirus detection for suspected cases of chikungunya, dengue, or Zika
(Testing only performed if travel history indicates travel to affected area.)

**Molecular testing**
(<7 days after symptom onset)

- **RT-PCR/NS1**
  - Positive: dengue
  - Negative: Perform antibody testing

- **(Real-time) PCR**
  - Positive: Zika virus
  - Negative: Perform antibody testing

- **(Real-time) PCR**
  - Positive: chikungunya virus
  - Negative: Perform antibody testing

**Antibody testing**
(4 days after symptoms)

- **IgM**
  - Positive: dengue
  - Negative: Perform antibody testing

- **IgM**
  - Positive: Zika virus
  - Negative: Perform antibody testing

- **IgM**
  - Positive: chikungunya virus

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1 Due to extensive cross-reactivity in flavivirus serological assays, for samples collected <7 days post illness onset, molecular detection should be performed first.

*Perform sample 4 days after symptom onset.

*Extensive cross-reactivity would be expected in samples from OENV/ZIKV circulation areas. A positive IgM assay with either antigen should be confirmed by using PRNT against both ZIKV and OENV as well as any other flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).

- **PRNT** should include any flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).