



**Guidance for Clinical Laboratories Regarding Testing of Persons with Probable Exposure to Zika virus**  
**Aug 2, 2016**

**Testing for Zika virus at CDC must be coordinated through the local health jurisdiction.** Contact information for your local health jurisdiction can be found [here](#):

<http://www.doh.wa.gov/AboutUs/PublicHealthSystem/LocalHealthJurisdictions>

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

**Testing Suspect Cases:**

If a provider suspects Zika virus disease in their patient and is seeking testing through public health, they must consult with their Local Health Jurisdiction (LHJ), which must approve specimen submission. **Once LHJ approval is granted, specimens should be shipped, weekday arrivals only, using Styrofoam insulated Category B packaging and labels** to the Washington State Public Health Laboratory (PHL) with the appropriate [form](#).

	<b>Symptomatic Patient</b>	<b>Asymptomatic pregnant women</b>	<b>Fetal abnormalities present</b>
<b>When to test</b>	Ideally <14 days after onset, up to 12 weeks	Up to 12 weeks after exposure	Any time during pregnancy
<b>Which test to order</b>	RT-PCR (<14 days)*, ELISA, PRNT <b>Also order DenV and ChikV serologic testing commercially</b>	RT-PCR (<14 days)*, ELISA, PRNT	RT-PCR, ELISA, PRNT
<b>What specimen to collect</b>	2 mL <b>serum</b> , spun down, >1mL <b>urine</b> (if ≥14 days and not pregnant, only send serum) (≥ 1.0 mL CSF if available)	2 mL <b>serum</b> , spun down, >1mL <b>urine</b>	2 mL <b>serum</b> , spun down, >1mL <b>urine</b> , consider <b>amniotic fluid</b>
<b>How to store it</b>	Keep cold or freeze to -70°C, ship in insulated container with ice packs or on dry ice	Keep cold or freeze to -70°C, ship in insulated container with ice packs or on dry ice	Keep cold or freeze to -70°C, ship in insulated container with ice packs or on dry ice
<b>Vessel</b>	<b>Serum:</b> 1.8 mL cryotube or 2.0 mL microtube (red or tiger top serum separator tube) <b>Urine:</b> Sterile vial with tight fitting screw cap and O-ring ( <u>NOT urine collection cup</u> )	<b>Serum:</b> 1.8 mL cryotube or 2.0 mL microtube (red or tiger top serum separator tube) <b>Urine:</b> Sterile vial with tight fitting screw cap and O-ring ( <u>NOT urine collection cup</u> )	<b>Serum:</b> 1.8 mL cryotube or 2.0 mL microtube (red or tiger top serum separator tube) <b>Urine:</b> Sterile vial with tight fitting screw cap and O-ring ( <u>NOT urine collection cup</u> ) <b>Amniotic fluid:</b> Sterile container with tight fitting screw cap

**Specimen Collection and Testing Guidance:**

For information on testing for perinatal suspect cases, see “Testing Perinatal Suspect Cases” below.

- 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.**
- PHL will forward approved specimens to the Minnesota Public Health Laboratory or CDC.

- **Results will be faxed to the submitter listed on the specimen submission form**
- If dengue and/or chikungunya are possibilities, a separate serum specimen should be sent commercially.
- During the first 13 days of illness, viral RNA can often be identified in serum or urine, and RT-PCR is the preferred test (see CDC algorithms at the end of the document).
- **RT-PCR testing is also available from commercial laboratories.**
- RT-PCR testing is NOT indicated for asymptomatic non-pregnant individuals, or for patients who test negative by IgM  $\geq 2$  weeks after illness onset or exposure (exception: fetal abnormalities present).
- A negative RT-PCR result on a serum specimen collected  $\geq 4$  days after illness onset does not rule out infection.
- 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. The combination of a negative RT-PCR result and negative IgM antibody testing suggests that there was no recent infection.
- **IgM ELISA testing is also available from commercial laboratories.**
- 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 specimens collected 7 days to 12 weeks after onset of symptoms, a negative IgM antibody result to both Zika and dengue viruses rules out recent infection with either virus.
- A PRNT titer  $\geq 10$  against Zika virus, together with negative PRNTs (ie.  $< 10$ ) against other flaviviruses is confirmatory for recent infection with Zika virus
- A PRNT titer  $\geq 10$  for both Zika and dengue virus (or another flavivirus) provides evidence of a recent infection with a flavivirus but precludes identification of the specific infecting virus. Patients should be clinically managed for both infections.
- 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 Perinatal Suspect Cases:

- **For pregnant women who test positive or inconclusive for Zika virus infection, or for whom fetal ultrasounds detect microcephaly or intracranial calcifications**, Zika virus RT-PCR can be performed on serum, urine, and 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: Several full thickness pieces of placenta, including at least 3 full thickness pieces (0.5–1 cm x 3–4 cm in depth) from middle third of placental disk and at least 1 from the placental disk margin, should be submitted if available. Include sections of the placental disk, 5x12 cm strip of fetal membranes, and pathologic lesions when possible. Please include information about placenta weight and sample both maternal and fetal side of the placenta and label all specimens to identify location of sample. Umbilical cord segments should be obtained proximal, middle, and distal to umbilical cord insertion site on the placenta. Four or more, 2.5 cm segments of umbilical cord should be submitted, if available. All specimens should be fixed in formalin (volume of formalin about 10x mass of tissue) and held at ambient temperature. Also collect umbilical cord serum ( $> 1$  mL) AND infant serum ( $> 1$  mL) for serologic

testing and keep cold or frozen. 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, still birth, a terminated pregnancy, or death of an infant shortly after birth in a woman with history of possible exposure to Zika virus during pregnancy and with evidence of maternal or fetal Zika virus infection**, the following specimens should be collected if possible and fixed in formalin:
  - Brain tissue (most important to evaluate for possible Zika virus infection), and spinal cord, 5 or more specimens from different parts of the brain and spinal cord, 0.5-1 cm<sup>3</sup> each. Maintain tissue architecture to evaluate viral pathology. Please fix brain specimens 48-72 hrs.
  - Placenta should be sampled extensively or submitted intact if early in gestation. Include at least 3 full thickness pieces (0.5-1 cm x 3-4 cm), from the middle third of the placental disk and at least 1 from the placental disk margin. Also include one 5 x 12 cm strip of fetal membranes
  - Four or more umbilical cord specimens should be submitted, in 2.5 cm segments.
  - If individual organs or tissue types can be easily identified at autopsy, collect 1 representative 0.5-1.0cm<sup>3</sup> sample from: heart, lungs, liver, kidneys, skeletal muscle, eyes and bone marrow. Sampling of eyes is highly recommended.
  - For situations where individual organs or tissue types cannot be identified, provide any available tissue with minimal disruption (4 or more specimens if possible).

**Fixed tissues should be stored and shipped at room temperature.**

#### **Form**

Ship with the [serology form](#) to PHL. 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.”

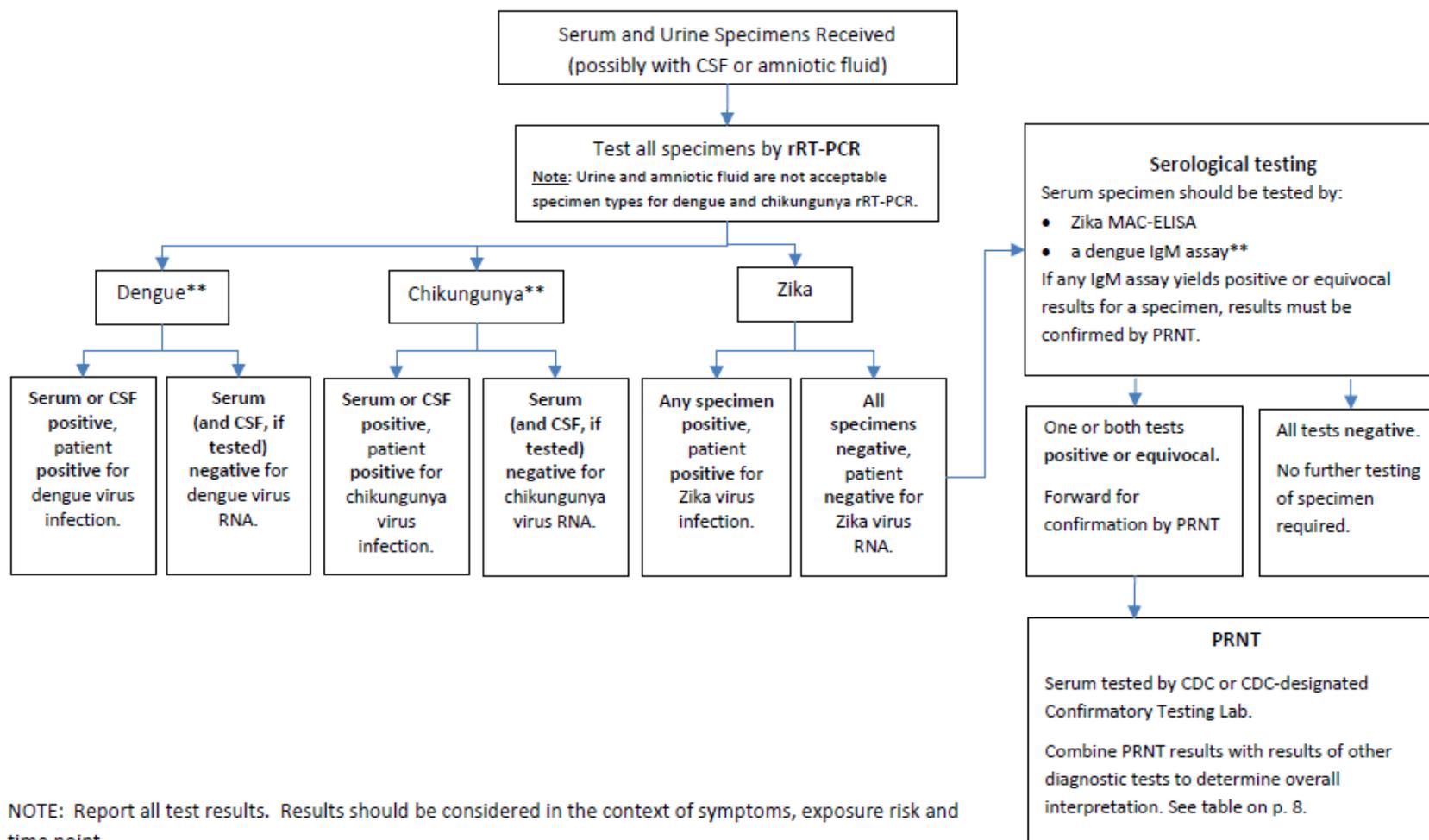
#### **Important Instructions**

Weekday arrivals only using Category B labels and packaging.

1)

### 2016 Zika Response: Algorithm for U.S. Testing of Symptomatic Individuals\*

Specimens Collected <14 days Following Symptom Onset



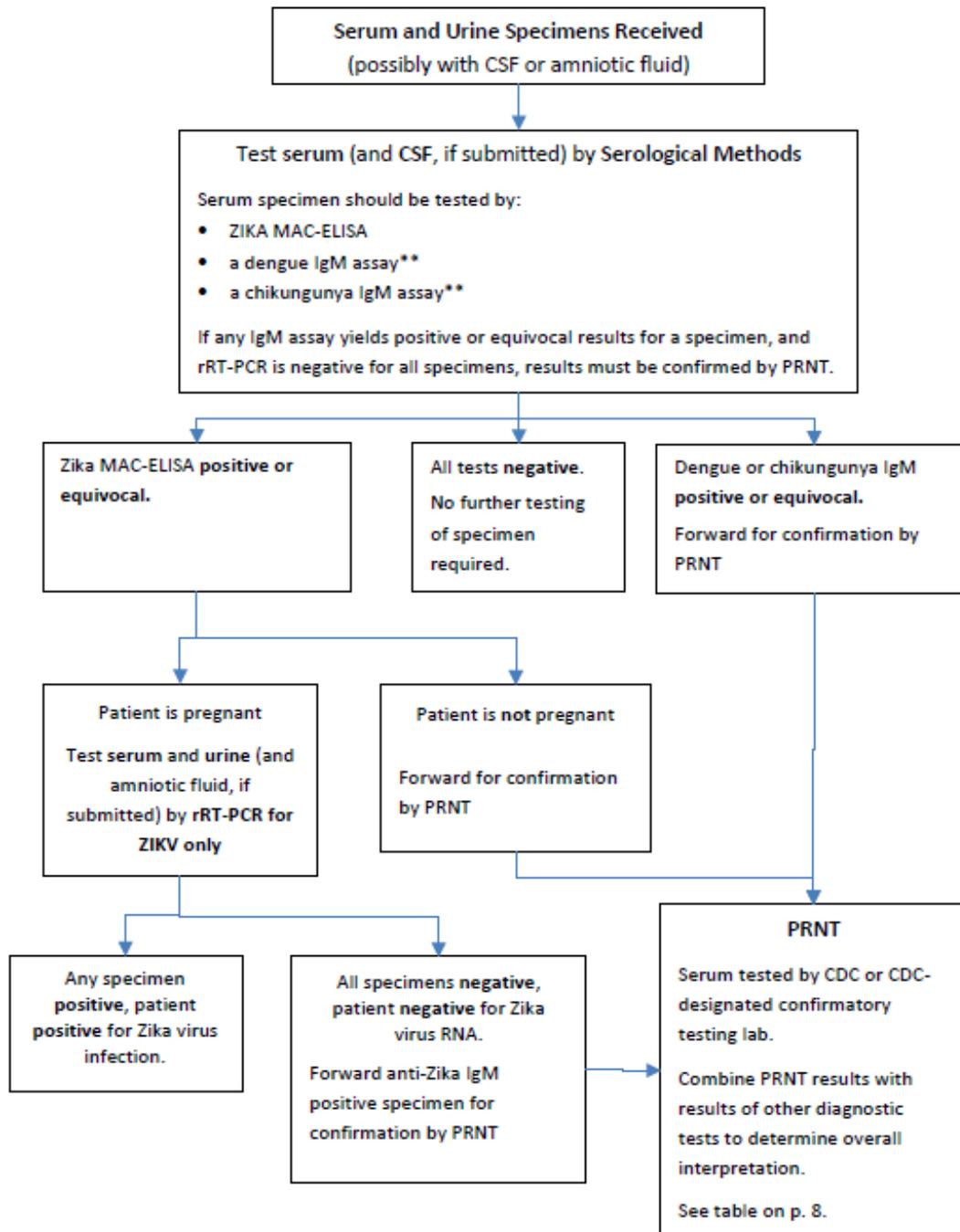
NOTE: Report all test results. Results should be considered in the context of symptoms, exposure risk and time point.

\*Pregnant and non-pregnant symptomatic individuals

\*\*For CDC guidance on patient management and follow-up for dengue or chikungunya virus infection, please refer to the CDC websites listed on p. 9 of this document.

2)

**2016 Zika Response: Algorithm for U.S. Testing of Symptomatic Individuals\***  
**Specimens Collected  $\geq$  14 Days Following Symptom Onset**



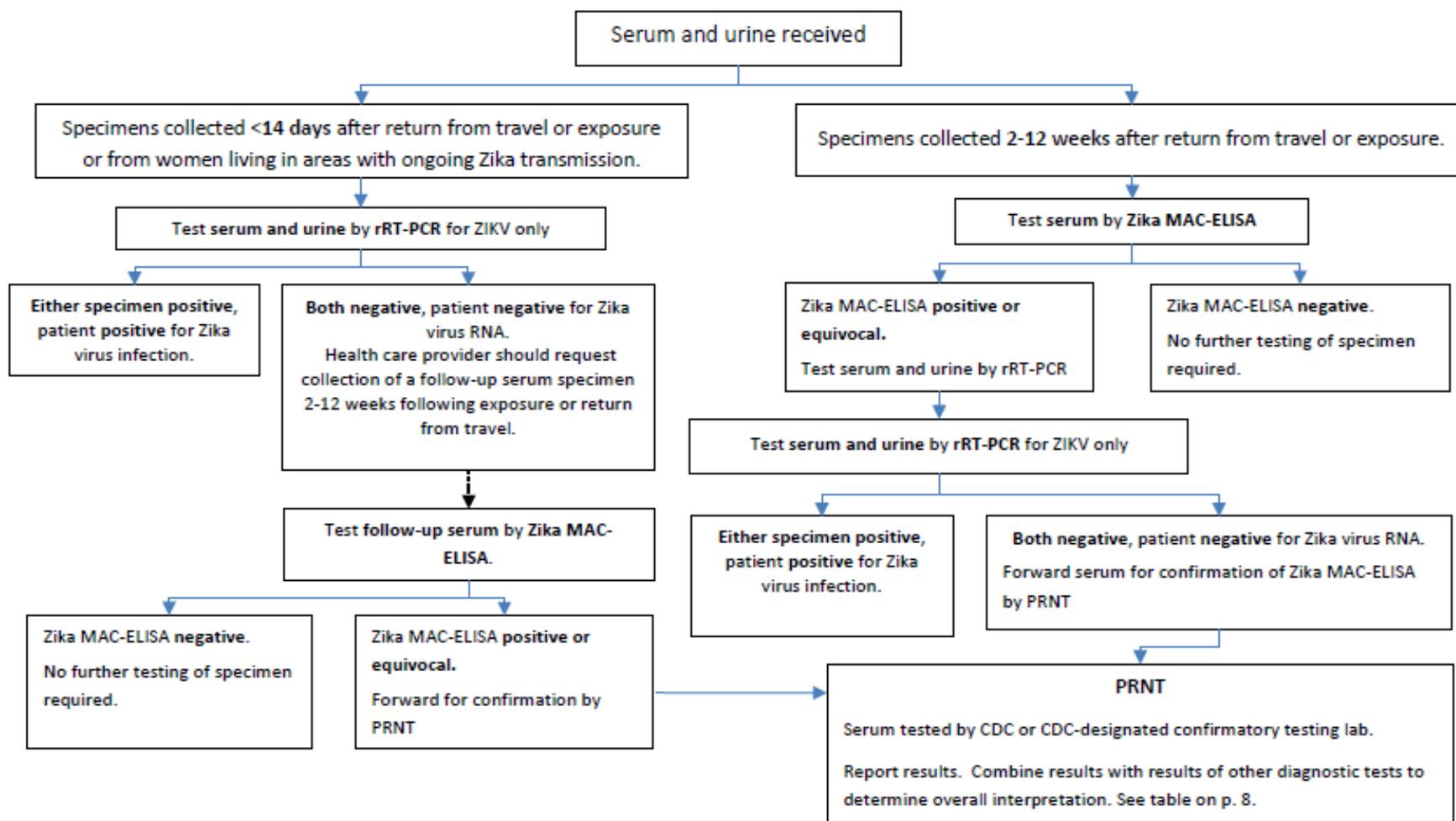
NOTE: Report all test results. Results should be considered in the context of symptoms, exposure risk and time point.

\*Pregnant and non-pregnant symptomatic individuals

\*\*For CDC guidance on patient management and follow-up for dengue or chikungunya virus infection, please refer to the CDC websites listed on p. 9 of this document.

3)

### 2016 Zika Response: Algorithm for U.S. Testing of Asymptomatic Pregnant Women Serum and Urine from Asymptomatic Pregnant Women Meeting Epidemiologic Criteria



NOTE: Report all test results. Results should be considered in the context of exposure risk and time point.

