Zika Virus Detailed Laboratory Ordering Guidance

Serum and urine are the primary diagnostic specimens for Zika virus infection. For all diagnostic testing conducted on specimen types other than serum, it is also necessary to concurrently obtain a serum specimen for reflex IgM testing.

If testing is being performed at Public Health Laboratories, please review the PHL Specimen Collection and Submission Instructions using this link: [http://www.doh.wa.gov/ForPublicHealthandHealthcareProviders/PublicHealthLaboratories/MicrobiologyLabTestMenu](http://www.doh.wa.gov/ForPublicHealthandHealthcareProviders/PublicHealthLaboratories/MicrobiologyLabTestMenu)

Detailed Testing Guidance by Patient Type

(Symptomatic, Pregnant, Asymptomatic, Infant)

For testing infants and specimen collection at delivery – go to Detailed Testing Guidance by Patient Type #5.

1. I’ve reviewed the Zika virus testing criteria and I have a symptomatic non-pregnant patient with possible Zika virus exposure. What now?

   All symptomatic patients with travel to an area where dengue and/or chikungunya may also be circulating should also be tested for these viruses.

   - **<14 days since symptom onset:** Obtain serum and urine, and order molecular testing (RT-PCR or NAT). RT-PCR is available at commercial labs or through the Public Health Laboratories (PHL). An RT-PCR positive result is confirmation of infection but an RT-PCR negative result does not by itself rule out infection. If RT-PCR is negative, antibody testing is required. IgM antibody testing is also available at commercial labs or through PHL. If IgM antibody tests are also negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available through public health reference laboratories.

   - **≥14 days – 12 weeks since symptom onset:** Obtain serum and urine¹ and order IgM antibody testing. IgM antibody testing is available at commercial labs or through PHL. If IgM antibody tests are negative, then there is no evidence of Zika virus infection, and no further testing is indicated. If IgM antibody tests are positive or equivocal for Zika or dengue, confirmation by molecular testing (RT-PCR) or PRNT is required.

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¹ Urine should only be tested if serum IgM is positive. Serum and urine RT-PCR can be performed for confirmation of positive IgM findings.
2. I’ve reviewed the Zika virus testing criteria and I have a pregnant symptomatic patient with possible Zika virus exposure. What now?

All symptomatic patients with travel to an area where dengue and/or chikungunya may also be circulating should also be tested for these viruses.

- **<12 weeks since symptom onset:** Obtain serum and urine, and order molecular testing (RT-PCR or NAT) and IgM serology. RT-PCR and IgM tests are available at commercial labs or through PHL. An RT-PCR positive result is confirmation of infection but an RT-PCR negative result does not by itself rule out infection. If IgM antibody tests are also negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available at public health reference laboratories. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), consider testing of additional specimens*, to be collected at delivery.

- **>12 weeks since symptom onset – delivery:** Testing can be considered but results from specimens obtained >12 weeks after symptom onset may not be definitive. If testing, obtain serum and urine, and order IgM antibody testing on serum. IgM antibody testing is available at CDC or commercial labs. A negative IgM result does not rule out infection. Consider testing of additional specimens*, to be collected at delivery. If IgM antibody tests are positive or equivocal, test available specimens using RT-PCR. An RT-PCR positive result is confirmation of infection. If RT-PCR is negative, PRNT testing is required. PRNT tests are only available at public health reference laboratories. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), testing of additional specimens* collected at delivery and serial ultrasounds should be considered.

3. I’ve reviewed the Zika virus testing criteria and I have an asymptomatic pregnant patient with possible Zika virus exposure. What now?

Zika virus testing should be considered for asymptomatic pregnant women with possible Zika virus exposure, but a routine recommendation for testing is no longer encouraged. Healthcare providers should assess risk to their patients (considering location, timing and duration of travel, use of prevention measures), patient preferences, and use clinical judgment to determine whether testing should be pursued. If testing is pursued:

- **<12 weeks since possible exposure:** Obtain serum and urine, and order molecular testing (RT-PCR or NAT) and IgM serology. RT-PCR and IgM tests are available at commercial labs or through PHL. An RT-PCR positive result is confirmation of infection but an RT-PCR negative result does not by itself rule out infection. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available at public health reference laboratories. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), consider testing of additional specimens*, to be collected at delivery. If IgM antibody tests are negative and specimens were collected <14 days, testing of a follow-up specimen collected ≥14 days since last exposure can be
considered. Whether or not to pursue additional testing should be determined by the patient together with the provider through a shared decision-making dialog, taking into consideration the patient/provider’s preferences and concerns, and the amount of risk to which the patient was exposed.

- >12 weeks since possible exposure – delivery: Testing can be considered but results from specimens obtained >12 weeks after symptom onset may not be definitive. If testing, obtain serum and urine, and order IgM antibody testing on serum. IgM antibody testing is available at commercial labs or through PHL. A negative IgM result does not rule out infection; consider testing of **additional specimens***, to be collected at delivery. If IgM antibody tests are positive or equivocal, test available specimens using RT-PCR. An RT-PCR positive result is confirmation of infection. If RT-PCR is negative, PRNT testing is required. PRNT tests are only available through public health reference laboratories. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), consider testing of **additional specimens***, to be collected at delivery.

4. I’ve reviewed the Zika virus testing criteria and I have a pregnant patient with possible Zika virus exposure and fetal abnormalities present on ultrasound. What now?

- If fetal abnormalities are present on ultrasound, testing should be performed. Women who originally tested negative or who were not tested for Zika virus infection following exposure should be tested/retested for Zika virus infection. Obtain serum, urine, and if desired, amniotic fluid, and order molecular testing (RT-PCR). RT-PCR is available at commercial labs or through PHL. 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. If RT-PCR is negative, antibody testing should be performed. If IgM antibody tests are also negative and specimens were collected within 12 weeks of first exposure, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available public health reference laboratories. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), **additional specimens** should be collected at delivery for further testing.
5. I’ve reviewed the Zika virus testing criteria and I have an infant with laboratory evidence of maternal Zika virus infection, OR
An infant with abnormal clinical or neuroimaging findings suggestive of congenital Zika syndrome AND a maternal epidemiology link, OR
An infant with fever, rash, arthralgia, or conjunctivitis within 2 weeks of delivery and possible maternal exposure* occurred within 2 weeks of delivery:

- **Within 2 days of birth**, collect infant serum and urine for RT-PCR and serologic testing. Collection of cord blood is not generally recommended. CSF obtained for other studies can also be tested. If this initial infant sample is RT-PCR negative and IgM positive, but PRNT was not performed on the mother’s sample, PRNT should be performed on the infant’s initial sample. However, PRNT cannot distinguish between maternal and infant antibodies.
- For circumstances in which maternal testing was not previously performed, was performed more than 12 weeks after exposure, or was not definitive (e.g. unspecified flavivirus), **additional specimens** should be collected at delivery for further testing. If an infant appears clinically well and maternal testing has not yet been completed, Zika virus testing can be deferred until maternal test results are available.

6. I’ve reviewed the testing criteria and I have a pregnant patient experiencing fetal loss, still birth, a terminated pregnancy, OR
Death of an infant shortly after birth in a woman with laboratory evidence of maternal Zika virus infection:

The following specimens should be collected for RT-PCR 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 cm³ 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 0.5-1.0cm³ 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).

Additional specimens to be collected at delivery:

- May be useful when maternal testing was not previously performed, was performed more than 12 weeks after exposure, or was not definitive (e.g. unspecified flavivirus), AND the fetus or infant presents with symptoms of congenital Zika syndrome, OR the mother has ongoing possible exposure to Zika OR was symptomatic. Specimens should be submitted to PHL.
- 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 sides 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.

For more information, go to Zika Virus for Healthcare Providers and Clinical Labs.