

Guidance for U.S. Laboratories Testing for Zika Virus Infection

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Note: On May 5, 2017, CDC issued a Health Alert Notice (HAN) (<https://emergency.cdc.gov/han/han00402.asp>) to share emerging evidence about interpreting Zika IgM antibody test results of women who may have been exposed to Zika virus, particularly women who live in or frequently travel to areas with a CDC Zika travel notice (<https://wwwnc.cdc.gov/travel/page/zika-information>), before conception. It is possible that some women who are currently pregnant may have been previously infected and developed antibodies against Zika prior to pregnancy. New data suggest that Zika virus infection, similar to some other flavivirus infections, may result in Zika antibodies staying in the body for months after infection, which may make it difficult to use these tests to determine whether women might have been infected before or after they became pregnant. This HAN has specific recommendations not currently a part of the existing laboratory guidance, which should be considered for these women: 1. that nucleic acid testing is considered at least once per trimester unless a previous test has been positive, and on amniocentesis specimens, if amniocentesis is performed for other reasons and 2. that IgM testing may be considered as part of pre-conception counseling. CDC recommends other diagnostic methods, such as nucleic acid testing and ultrasounds, which may provide additional information to help healthcare providers know if antibody test results might represent a recent infection. For women planning to become pregnant who might have been exposed to Zika previously, healthcare providers can consider testing for Zika antibody before pregnancy. Antibody test results before pregnancy should not be used to determine if it is safe for a woman to become pregnant. Rather, testing before pregnancy can help determine whether a woman becomes infected during pregnancy. CDC is currently updating its webpages with this information.

Overview

Testing of specimens within the United States and U.S. territories to determine possible Zika virus infection should be limited to specimens collected from patients meeting CDC’s clinical and epidemiological criteria for testing¹. Clinical signs and symptoms associated with Zika virus infection are discussed here: <http://www.cdc.gov/zika/symptoms/index.html>. It is important to note that Zika virus infection can cause signs and symptoms similar to those seen in patients with other arthropod-borne virus (arbovirus) infections,

¹ The term “clinical and epidemiological criteria” refers to factors such as symptoms, pregnancy and exposure risk. Please refer to current CDC clinical guidance: <http://www.cdc.gov/zika/hc-providers/index.html>

including dengue virus, a related flavivirus, and chikungunya virus, an unrelated alphavirus. It is also important to note that a positive result for one of these viruses does not preclude infection with the others. Co-infection with Zika virus and dengue or chikungunya viruses is rare, but possible.

Current general information and guidance for the U.S. Zika response is available on CDC's Zika website at <http://www.cdc.gov/zika/index.html>. Information specific to laboratories is available at <http://www.cdc.gov/zika/laboratories/index.html>.

Current information and guidance specific to Zika virus in Puerto Rico can be found at the Puerto Rico Department of Health website: <http://www.salud.gov.pr/Sobre-tu-Salud/Pages/Condiciones/Zika.aspx>.

Testing for Zika virus infection is complicated by the temporal appearance and disappearance of biologic analytes in the infected person and thus multiple tests and sample types are often needed to establish a definitive laboratory diagnosis of Zika virus infection. Viral RNA is the first analyte that can be detected in an infected person in multiple specimen types. In blood, as the immune response develops (IgM titers rise), levels of viral RNA decline. However, viral RNA may be detectable in some infected persons for longer periods in certain specimen types.

Full testing algorithms are presented at the end of this document. **Serum and urine are the primary diagnostic specimens for Zika virus infection.** Other specimen types such as plasma, whole blood, cerebrospinal fluid (CSF), and amniotic fluid are authorized for use with some tests that have received a Food and Drug Administration (FDA) Emergency Use Authorization (EUA). **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.** Please review test instructions to determine acceptable specimen types for a given test. Testing algorithms presented at the end of this document should be used to determine test order based on presence of symptoms, pregnancy status, and time between symptom onset or exposure and specimen collection. For symptomatic persons, time between symptom onset and specimen collection dictates test order. For asymptomatic pregnant women meeting epidemiologic criteria for testing, time between exposure or return from travel dictates test order. Instructions for use for each EUA authorized test can be found under the "Labeling" bullet on the FDA website at: <http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika>

Biological Safety

To ensure laboratory safety when working with Zika virus, please review CDC guidance on Transport and Handling of Diagnostic Specimens and Working with Zika Virus in the Laboratory:

<http://www.cdc.gov/zika/laboratories/lab-safety.html>. See Biosafety in Microbiological and Biomedical Laboratories (BMBL) for additional biosafety information about arboviruses and laboratory biosafety practices: <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>.

Testing Methods

Molecular testing

Detection of Zika virus RNA in any acceptable specimen type should be interpreted as sufficient evidence that an individual is infected with Zika. Specimens collected from symptomatic individuals early in the course of illness (less than 14 days after illness onset), can be effective in diagnosing a recent Zika virus infection. However, failure to detect Zika virus RNA (i.e., a negative result on a molecular test) does not exclude Zika virus infection, and therefore serum should be analyzed by reflex IgM antibody (serological) testing. In situations where there is an increased risk of Zika, dengue, and chikungunya viral infections, the use of the FDA-authorized Trioplex Real-time RT-PCR (Trioplex) assay, which permits simultaneous detection and differentiation of RNA from all of these viruses, may be advantageous.

Multiple nucleic acid tests (NATs) have received Emergency Use Authorization (EUA) from FDA. FDA maintains a list on its website of all Zika virus EUAs. Please refer to the FDA website for a current list of available assays and associated letters of authorization, fact sheets and product labeling. Additional assay-specific information (e.g., performance characteristics) is included in the labeling.

<http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>.

Information about molecular tests that have been cleared by FDA for detection of arboviruses other than Zika virus can be found in the searchable database at this link:

<http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/510kClearances/ucm089319.htm>

Antibody detection methods:

Antibodies (IgM) directed against Zika virus typically emerge after viral RNA becomes undetectable. If the serum sample being tested was collected ≥ 14 days after onset of symptoms (for symptomatic persons) or defined virus exposure (for asymptomatic pregnant women), tests that detect anti-Zika IgM are performed first in the testing algorithm.

Zika and dengue viruses have similar clinical presentations, transmission cycles, and geographic distributions, and cross-reactivity on serologic assays for these viruses is common. Dengue IgM testing should be performed in any symptomatic person with potential dengue exposure. Currently, one FDA-authorized EUA Zika IgM test recommends follow-up testing with a FDA cleared dengue IgM device when the final interpretation is “Presumptive Other Flavivirus Positive” due to the inclusion of a cross-reactive control that includes a dengue virus antigen. This same test also recommends follow-up testing with an FDA cleared West-Nile virus IgM device when the final interpretation is “Presumptive Other Flavivirus Positive” as the other component of the cross-reactive control is West Nile Virus antigen. For persons who were in regions with known endemic flavivirus activity (e.g., West Nile virus, St Louis encephalitis virus) during their potential exposure period, IgM testing for those viral infections should be considered using an FDA cleared assay, if available. For more information about West Nile virus, please reference the following link: <http://www.cdc.gov/westnile/index.ht>. Because infections with other arboviruses, including chikungunya virus, can also produce symptoms similar to Zika virus infection,

additional testing for other arboviruses is often required to reach a diagnosis. For persons with chikungunya exposure risk and a clinically compatible illness², anti-chikungunya IgM testing should also be performed.

FDA maintains on its website a list of all Zika virus EUAs. Please refer to the FDA website for a current list of available assays and associated letters of authorization, fact sheets and product labeling. Assay-specific information (e.g., performance characteristics) is included in the labeling.

<http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>

² Clinical information about chikungunya virus infection, including clinical evaluation guidance, may be found on CDC's website: www.cdc.gov/chikungunya/hc/index.html

Serologic assays for arboviruses other than Zika virus that have been FDA cleared and/or are commercially available as of November 16, 2016.

Assay	Acceptable Specimen Type
<i>Anti-Dengue IgM</i> DENV Detect IgM Capture ELISA (InBios, USA) Commercial laboratories with capability: Focus Diagnostics (http://www.focusdx.com) ARUP Laboratories (http://www.aruplab.com) Quest Diagnostics (http://www.questdiagnostics.com) Mayo Medical Laboratories (http://www.mayomedicallaboratories.com/)	Serum
<i>Anti- Chikungunya IgM</i> Commercial laboratories with capability: Focus Diagnostics (http://www.focusdx.com/) ARUP Laboratories (http://www.aruplab.com/) Mayo Medical Laboratoires (http://www.mayomedicallaboratories.com/)	Serum
<i>Anti-West Nile IgM</i> Euroimmun Anti-West Nile Virus ELISA (IgM)	Serum, Plasma
Spectral West Nile Virus IgM Status Test	Serum, Plasma
West Nile Detect IgM ELISA (InBios, USA)	Serum
West Nile Virus IgM Capture ELISA, Model E-WNV02M (Panbio Limited, AU)	Serum
West Nile Virus IgM Capture ELISA (Focus Technologies, Inc. USA) West Nile Virus IgM Capture ELISA, Model EL0300M (Focus Technologies, Inc. USA)	Serum

Confirmation of anti-Zika IgM reactive results and anti-dengue IgM reactive results by plaque reduction neutralization test (PRNT):

Currently, within the United States and most U.S. territories, when enzyme-linked immunosorbent assay (ELISA) IgM antibody testing indicates the presence of anti-Zika IgM antibodies (positive, equivocal, presumptive or possible Zika virus positive result), the plaque reduction neutralization test (PRNT), which measures virus-specific neutralizing antibodies to Zika virus and other endemic flaviviruses, is required for diagnosis. PRNT must be conducted by CDC or a laboratory qualified by CDC. If ELISA testing indicates a positive or equivocal result for dengue infection, confirmatory testing should be performed as indicated in the IgM assay labeling.

Given the high degree of antibody cross-reactivity observed with Zika and dengue infections, results of Zika/dengue PRNT testing should be interpreted alongside initial IgM assay results to assess the status and timing of infection. The CDC Interim Guidance for Interpretation of Zika Virus Antibody Results (Rabe et al., 2016, <http://www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm>) contains specific information that guides the overall interpretation of combined results from Zika virus and dengue virus ELISA and PRNT. However, PRNT

is not always able to provide a definitive determination of the specific flavivirus causing a recent infection, particularly in persons with a prior history of flavivirus infection. For this reason, PRNT confirmation is not currently routinely recommended in Puerto Rico, where dengue virus is endemic and cross-reactivity is likely to occur in most cases. Puerto Rico surveillance data regarding current circulating flaviviruses should be used in conjunction with serologic laboratory results to guide clinical patient management.

For additional information, please refer to CDC guidance on clinical management of patients with positive test results: <http://www.cdc.gov/zika/hc-providers/index.html>.

The following result interpretations from PRNT testing are possible for Zika and dengue based on the detection of IgM by ELISA testing and the levels of neutralizing antibody titers identified in the PRNT. The results below will be determined by the laboratories performing PRNT.

Interpretation of Antibody Testing^{*,**}
Recent Zika virus infection [†]
Recent dengue virus infection [†]
Recent flavivirus infection; specific virus cannot be identified [†]
No evidence of Zika virus or dengue virus infection
Evidence of Zika virus infection; timing cannot be determined [§]
Evidence of dengue virus infection; timing cannot be determined [§]
Evidence of flavivirus infection; specific virus and timing cannot be determined [§]
Presumptive recent Zika virus infection ^{†,§}
Presumptive recent dengue virus infection ^{†,§}
Presumptive recent flavivirus virus infection ^{†,§}
Equivocal results [§]
Inconclusive results [§]
No evidence of recent Zika virus or dengue virus infection

*For persons with suspected Zika virus disease, Zika virus RNA NAT should be performed on specimens collected <14 days after onset of symptoms.

†In the absence of RNA nucleic acid testing, negative IgM or neutralizing antibody testing in specimens collected <7 days after illness onset might reflect collection before development of detectable antibodies and does not rule out infection with the virus for which testing was conducted.

§Zika IgM positive result is reported as “presumptive positive” to denote the need to perform confirmatory PRNT.

¶Report any positive or equivocal IgM Zika or dengue results to state or local health department.

**To resolve false-positive results that might be caused by cross-reactivity or nonspecific reactivity, presumptive positive Zika IgM results should be confirmed with PRNT titers against Zika, dengue, and other flaviviruses to which the person might have been exposed.

Adapted from CDC Interim Guidance for Interpretation of Zika Virus Antibody Results (Rabe et al., 2016)

Note: PRNT is not currently routinely recommended in Puerto Rico for specimens that have “Positive, Presumptive, Equivocal or Possible” Zika interpretations based on testing with current EUA authorized Zika IgM tests.

Detailed information for specimen types acceptable for Zika Testing

The information below describes more detailed characteristics of the various specimen types that have been validated for use with Zika virus diagnostic tests. **It is important to note that serum is required for all diagnostic algorithms and thus a paired serum specimen must be submitted alongside all other sample types listed below.**

Note to Health Care Providers: *To determine which specimen types can be tested and for specific specimen collection, handling and storage requirements, please consult the testing laboratory or the labeling information for current tests with Emergency Use Authorization.*

<http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>

Serum

Serum must be submitted for all persons tested for Zika virus infection. Serum may be tested for the presence of both viral nucleic acid (e.g., RNA) and anti-viral antibody for Zika, dengue, and chikungunya viruses. See algorithms on pages 13-15 for clarification. RNA from Zika, dengue, and chikungunya viruses is often detectable in serum during the acute phase of infection (<14 days after illness onset). There are data to indicate that Zika virus RNA may be detectable in serum of some patients for a longer period of time (Bingham et al., 2016). Levels of anti-Zika IgM antibodies in infected individuals typically appear within the first week after symptom onset and persist for approximately 8-12 weeks (Rabe et al., 2016).

Note to Health Care Providers: *Serum should be collected in serum separator tubes and centrifuged to prevent hemolysis. Serum should then be decanted into a plastic vial for transport as described in the CDC guidance for specimen collection and transport.*

Urine

Zika virus RNA has been detected in urine for a longer period of time than in serum (Bingham et al., 2016). Based on a limited number of cases, detection of Zika virus RNA has been demonstrated up to 14 days after onset of symptoms. Data are currently lacking to support a recommendation to test urine beyond 14 days after symptom onset in non-pregnant persons. For symptomatic persons presenting < 14 days after onset of symptoms, both urine and serum should be collected and tested by Zika RNA NAT (MMWR, 2016).

Note to Health Care Providers: *Please do not submit urine in urine collection cups for Zika virus testing. Urine should be transferred to a clean vial with screw cap and O-ring to prevent leakage in transport. For infant testing, please contact the laboratory performing the testing for collection requirements. Please see considerations for RNA testing in pregnant women and infants below.*

Whole Blood (EDTA or other anticoagulant in accordance with EUA labeling).

Recent evidence suggests that Zika virus RNA can be detected for a longer period of time in whole blood versus serum of infected persons (Lustig et al., 2016). Systematic data describing Zika virus RNA persistence in whole blood are limited. At this time, CDC recommends that, for symptomatic persons presenting up to 14 days after onset of symptoms and asymptomatic pregnant women presenting within 14 days of possible Zika virus

exposure, whole blood can be collected alongside serum and be tested by Zika RNA NAT in accordance with EUA labeling.

Note to Health Care Providers: *Please confirm with your testing laboratory that they can accept whole blood specimens prior to collecting and submitting this sample type.*

Cerebrospinal Fluid

Cerebrospinal fluid is not a primary diagnostic specimen for Zika virus testing. However, if CSF is obtained during evaluation for other reasons (e.g. abnormalities/symptoms present in an infant <http://www.cdc.gov/mmwr/volumes/65/wr/mm6533e2.htm>), the specimen may be tested for the presence of anti-Zika IgM antibodies by ELISA and for the presence of Zika virus RNA by some molecular methods. CSF, along with a paired serum specimen, should be tested by Zika RNA NAT if collected <14 days following onset of symptoms. CSF and serum should be tested by antibody detection methods if collected >14 days after symptom onset, or if PCR is negative in samples collected <14 days after onset of symptoms.

Amniotic Fluid

If indicated, amniotic fluid may be tested by some emergency use authorized molecular methods, alongside paired serum and urine specimens. Consideration of amniocentesis should be individualized, because data regarding sensitivity and specificity of Zika virus testing at different time points during pregnancy to diagnose congenital Zika virus infection are limited. The presence of Zika virus RNA in the amniotic fluid might indicate fetal infection; however, a negative result does not exclude congenital Zika virus infection. Please see Oduyebo et al, 2016, http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm?s_cid=mm6529e1_e for additional information regarding testing of amniotic fluid.

Tissue Specimens

There are currently no FDA authorized tests for Zika virus testing of tissue specimens, however, Zika, dengue, and chikungunya virus testing on fixed and frozen tissue at CDC may be considered on a case-by-case basis. Fixed tissues are preferred. Requests for testing should be coordinated through your state or local health department and pre-approval is required before submission to CDC. Additional information about specimen collection and submission procedures is available on CDC's website: <http://www.cdc.gov/zika/laboratories/test-specimens-tissues.html>.

Other Specimens Types

Laboratories have performed testing on specimen types such as semen and saliva, but there are currently no FDA authorized tests for which performance with these specimen types has been established (Bingham et al., 2016, <http://dx.doi.org/10.15585/mmwr.mm6518e2>).

Specimen Referral

Health care and laboratory professionals are instructed to direct Zika virus testing requests to their local or state public health laboratory or to a commercial laboratory that performs Zika testing using an authorized assay with demonstrated analytical and clinical performance. Health care and laboratory professionals should follow state or local public health department guidance on notification procedures for suspect cases of Zika virus infection.

Public health laboratories that are not CDC-designated Zika virus testing laboratories should work with their state, local or territorial public health department for testing of suspect specimens or referring specimens to CDC.

Information regarding submission of specimens to CDC from locations within the 50 states and the District of Columbia is at: <http://www.cdc.gov/ncezid/dvbd/specimensub/arboviral-shipping.html>.

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>

Overview of Testing Algorithms for detection of Zika Virus Infection

Testing algorithms were designed to accommodate the temporal nature of the appearance and disappearance of markers of Zika virus infection and to optimize testing for pregnant women. **Information regarding testing of infants at the time of birth can be found at: <http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html> and also in the Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection which can be found at: http://www.cdc.gov/mmwr/volumes/65/wr/mm6533e2.htm?s_cid=mm6533e2_w.**

Algorithms for testing based on presence of symptoms, pregnancy status, and time between symptom onset or exposure and specimen collection are presented at the end of this document.

Specimens collected from all symptomatic individuals < 14 days after the onset of symptoms:

- Test serum and urine with a Zika virus RNA NAT. A RNA-positive Zika virus NAT result in any specimen is sufficient to diagnose Zika virus infection.
- If Zika virus RNA NAT results are negative, serum should be tested for the presence of anti-Zika IgM. Testing for anti-dengue IgM should also be performed if the patient is pregnant or potentially exposed to dengue virus. Currently, one EUA anti-Zika IgM assay recommends that specimens with a result of presumptive positive for other flavivirus have follow-up testing with a FDA cleared anti-dengue IgM assay.

Serum collected from symptomatic individuals presenting ≥ 14 days following symptom onset:

Initial testing should be done with an anti-Zika IgM detection method. For non-pregnant symptomatic patients, a reactive (Equivocal, Presumptive Positive or Possible Zika Positive) anti-Zika IgM result is followed by PRNT to confirm the diagnosis.*

Note to Health Care Providers: *There are limited data that indicate RNA may persist longer in urine and whole blood, so collection of these specimen types, in addition to serum, may be beneficial to conduct RNA testing ≥ 14 days following symptom onset.*

Additional criteria and testing strategies apply for pregnant women:

- If a positive anti-Zika IgM result is obtained in specimens collected ≥ 14 days after onset of symptoms or potential exposure, testing by Zika RNA NAT (on all appropriate specimen types available) should be performed. If the Zika RNA NAT test results are negative, testing should proceed to PRNT to test for the presence of neutralizing anti-Zika antibodies.
- Anti-dengue IgM testing is recommended for symptomatic pregnant women.

Asymptomatic pregnant women meeting epidemiological criteria for testing:

- Specimens collected from a pregnant woman presenting < 14 days from exposure should be tested by RNA NAT for Zika virus. If negative, a second serum specimen should be collected 2-12 weeks following exposure and tested by Zika virus IgM detection methods.
- Serum specimens collected from asymptomatic pregnant women 2-12 weeks following a potential exposure or from asymptomatic pregnant women living in an area of ongoing transmission should be tested for anti-Zika IgM. If reactive, Zika RNA NAT should be performed on all appropriate specimen types available. If Zika RNA NAT is negative, PRNT* should be performed for confirmation of the IgM result.

The following link contains additional clinical guidance for healthcare providers caring for pregnant women.

<http://www.cdc.gov/zika/hc-providers/pregnant-woman.html>

*** PRNT is not currently routinely recommended for testing of any specimens in Puerto Rico**

Criteria and testing strategies for infants with possible congenital Zika virus infection

Information regarding testing of infants at the time of birth can be found at: <http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html> and also in the Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection which can be found at: http://www.cdc.gov/mmwr/volumes/65/wr/mm6533e2.htm?s_cid=mm6533e2_w. Below are recommendations specific to the testing of infants with possible congenital Zika virus infection:

- Infant laboratory testing for Zika virus RNA NAT should be performed within the first 2 days after birth, if possible.
- Zika virus RNA NAT should be performed on both infant serum and urine, and Zika virus IgM ELISA should concurrently be performed on infant serum. Information regarding collection of samples at time of birth can be found at: <http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html>.
- Whole blood can be collected alongside serum and be tested by RNA NAT in accordance with EUA labeling.
- If cerebrospinal fluid (CSF) is obtained from the infant for other clinical indications, RNA NAT for Zika virus RNA and Zika virus IgM should be performed on CSF.

- Detection of Zika virus RNA in the placenta can confirm the presence of maternal infection, but cannot distinguish between maternal and congenital infection.
- If the infant's initial serum sample is negative for RNA but is IgM-positive, then PRNT should be performed on the infant's initial sample if it was not performed on the mother. However, PRNT cannot distinguish between maternal and infant antibodies at birth.*

For infants with an initial sample that was negative for Zika virus RNA, serologic testing at ≥ 18 months of life, when the antibody responses are those of the child and maternal antibody has waned (Ades et al., 2016) can assist with diagnosis for congenital Zika virus infection. Currently PRNT testing is used for this purpose.

- PRNT should be performed on a sample collected from a child aged ≥ 18 months whose initial sample was anti-Zika IgM positive.
- If the infant's initial sample is negative by IgM ELISA, PRNT at age 18 months can be considered based on clinical and epidemiologic circumstances.

Local health departments should determine when to implement testing of infants at ≥ 18 months of life based on local context, including circulating flaviviruses as well as clinical and epidemiologic circumstances. For information about the implications of the PRNT results at ≥ 18 months for diagnosing congenital Zika virus infection, please refer to the Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection: <http://www.cdc.gov/mmwr/volumes/65/wr/mm6533e2.htm>.

The following link contains additional information for submitting specimens from infants for testing.
<http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html>

***PRNT is not currently routinely recommended in Puerto Rico for either maternal or infant specimens.**

Use of CDC Assays by Qualified Laboratories

The CDC Trioplex RNA NAT and CDC Zika MAC-ELISA (testing for anti-Zika IgM) are available to qualified laboratories in the United States and its territories. Eligible public health laboratories are those that have demonstrated proficiency with ELISA-based serological methods (for CDC Zika MAC-ELISA) or with RNA NAT (for CDC Trioplex RNA NAT) and that have facilities, personnel and equipment appropriate to the safe handling of specimens suspected of containing Zika, dengue, or chikungunya viruses. State, local, and territorial public health departments interested in obtaining the materials described above should contact LRN@cdc.gov for an application.

CDC-designated laboratories that perform the CDC Zika MAC-ELISA and/or Trioplex RNA NAT are first required to demonstrate proficiency with the assay(s) by successfully testing verification panels for each assay. Only

laboratories that have been notified by CDC that they have successfully completed the verification testing are authorized to use the CDC assays for diagnostic testing.

Reporting

Each test result generated for each specimen should be reported to clinicians as specified in the assay instructions for use. Pregnancy status, if available, should also be reported to allow health care providers to readily identify these women. Results generated by methods used under FDA EUA must be accompanied by the appropriate fact sheets when reported back to providers and patients. Fact Sheets have been prepared for health care providers and patients to help each understand the results of testing. Authorized Fact Sheets for each assay under EUA are posted to the FDA website:

<http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>

Tables and algorithms in this document are intended to assist laboratories in combining results from multiple specimens/methods to make appropriate decisions about next testing steps.

Guidance documents are available to assist in applying laboratory results to determine patient care and patient follow-up decisions:

Zika clinical guidance for health care providers caring for pregnant women, women of reproductive age, infants, children or other symptomatic individuals:

<http://www.cdc.gov/zika/hc-providers/index.html>

Dengue clinical guidance:

<http://www.cdc.gov/dengue/clinicalLab/index.html>

Chikungunya clinical guidance:

<http://www.cdc.gov/chikungunya/hc/index.html>

Please note that Zika, dengue, West Nile, and chikungunya virus infections are all on the 2016 list of nationally notifiable conditions: <https://www.cdc.gov/nndss/conditions/notifiable/2016/>. Therefore, results of testing should be reported back to state or local health department staff to facilitate investigation and classification of the case and reporting to CDC.

References

Ades AE, Newell ML, Peckham CS, et al. European Collaborative Study. Children born to women with HIV-1 infection: natural history and risk of transmission. *The Lancet*. 1991; 337(8736):253–260.

Bingham AM, Cone M, Mock V, et al. Comparison of Test Results for Zika Virus RNA in Urine, Serum, and Saliva Specimens from Persons with Travel-Associated Zika Virus Disease — Florida, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65. DOI: <http://dx.doi.org/10.15585/mmwr.mm6518e2>.

Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis*. 2015; 21(1):84–86.

Interim Guidance for Zika Virus Testing of Urine — United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65. DOI: <http://dx.doi.org/10.15585/mmwr.mm6518e1>.

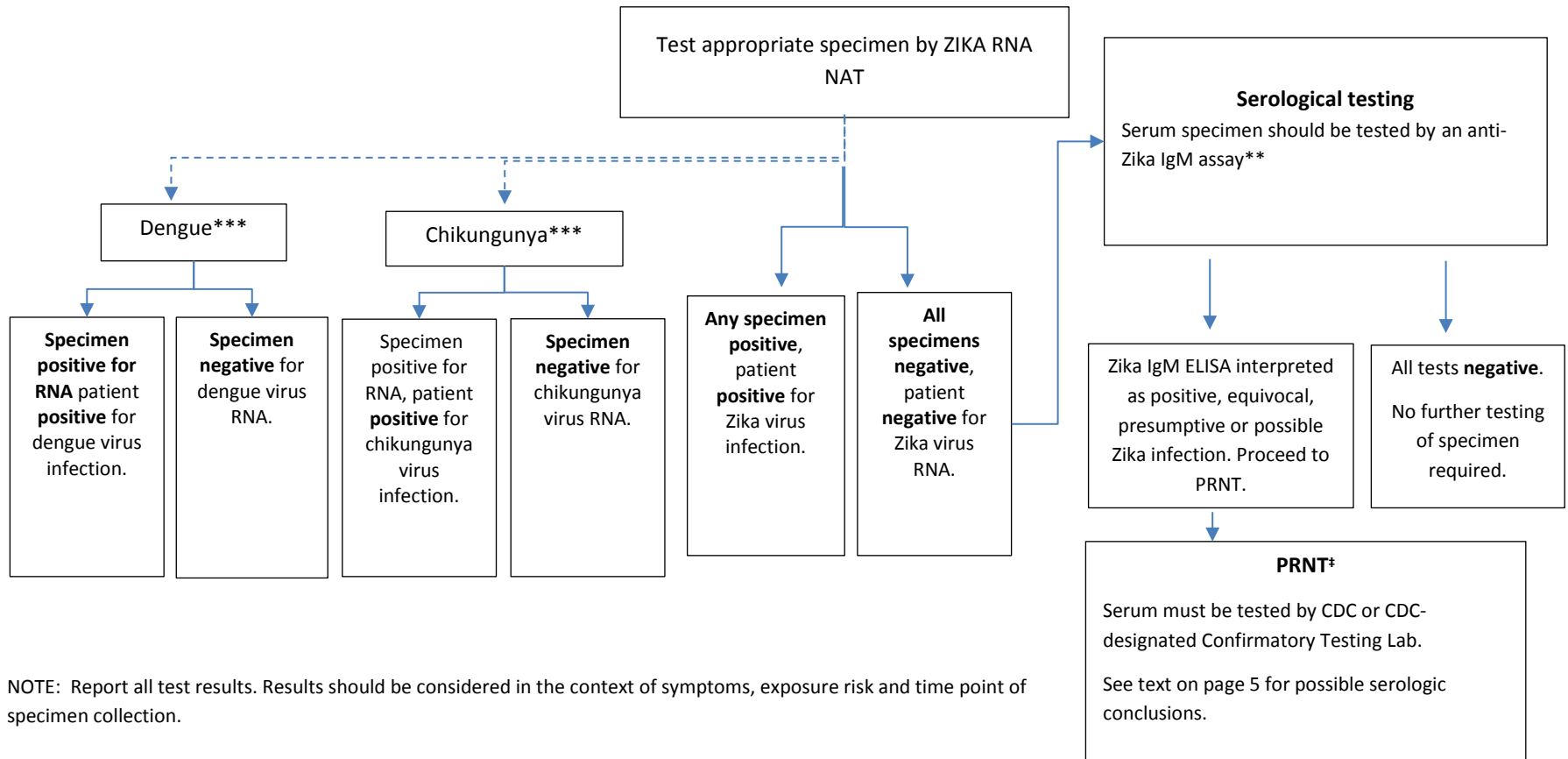
Rabe IB, Staples JE, Villanueva J, et al. Interim Guidance for Interpretation of Zika Virus Antibody Test Results. *MMWR Morb Mortal Wkly Rep* 2016;65. DOI: <http://dx.doi.org/10.15585/mmwr.mm6521e1>.

Lustig Y, Mendelson E, Paran N, Melamed S, Schwartz E. 2016. Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016. *Euro Surveill*. 2016; 21(26).

Russell, K, Oliver S, Lewis, L et al. Update: Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection — United States, August 2016. *MMWR Morb Mortal Wkly Rep* 2016;65 (33);870–878. DOI: https://www.cdc.gov/mmwr/volumes/65/wr/mm6533e2.htm?s_cid=mm6533e2_w

Oduyebo T, Igbinsosa, I, Petersen EF et al. Update: Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus Exposure — United States, July 2016. *MMWR Morb Mortal Wkly Rep* 2016;65(29);739–744. DOI: http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm?s_cid=mm6529e1_e

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 of specimen collection.

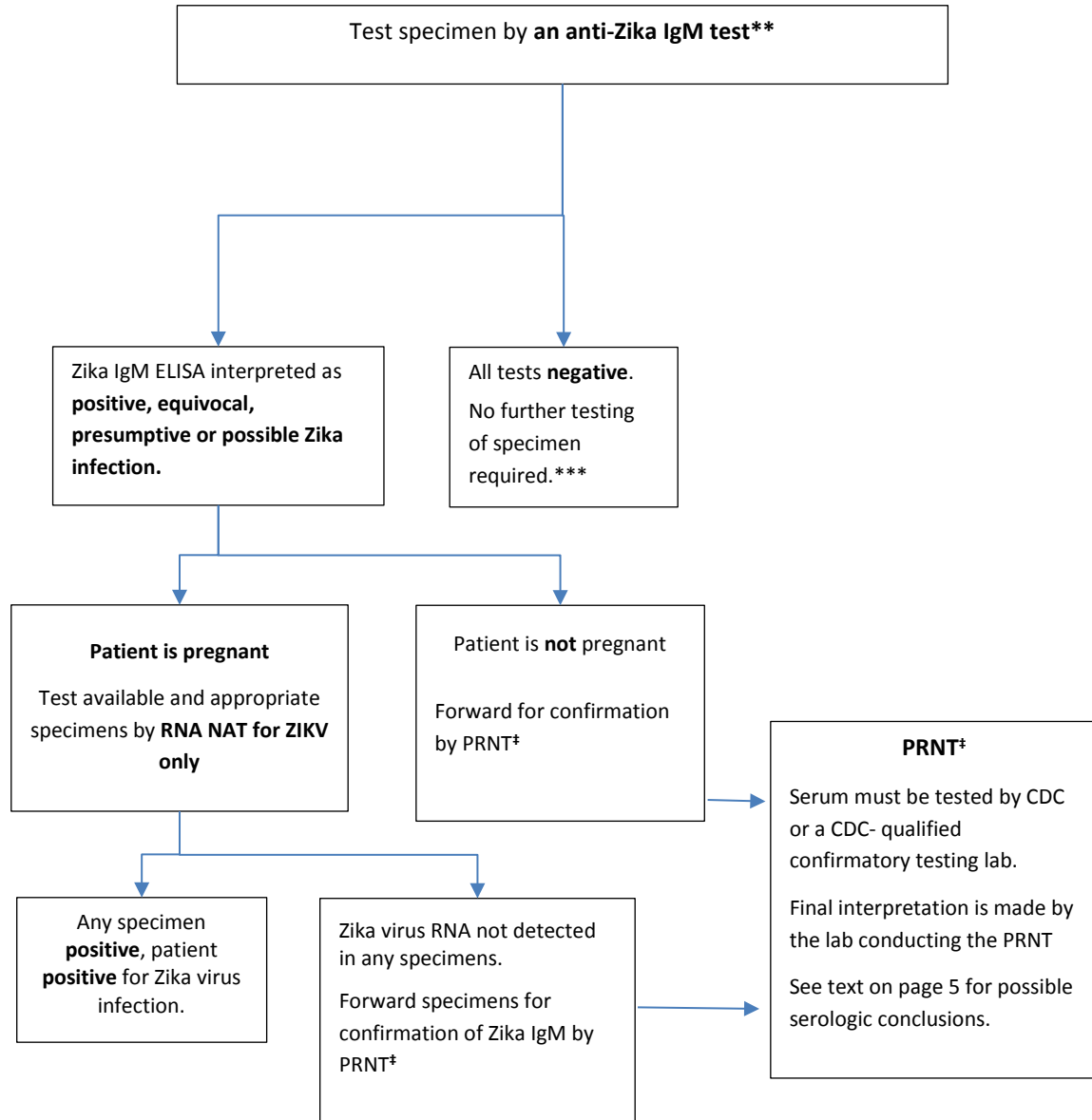
*Pregnant and non-pregnant symptomatic individuals

** Note antibody cross-reactivity to other flaviviruses complicates interpretation of the current anti-Zika IgM tests. Dengue IgM testing should be conducted for symptomatic pregnant women, individuals with a potential dengue exposure and when a presumptive other flavivirus result is obtained. See text on page 3-4 for additional information.

*** Indicates testing and interpretation for the CDC Trioplex assay. Note when testing urine and amniotic fluid with the CDC Trioplex assay, only report the Zika result.

‡PRNT confirmation is not currently routinely recommended for Puerto Rico. See page 5 for more information.

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



NOTE: Report all test results to the appropriate health authorities. Results should be considered in the context of symptoms, exposure risk and time point of specimen collection.

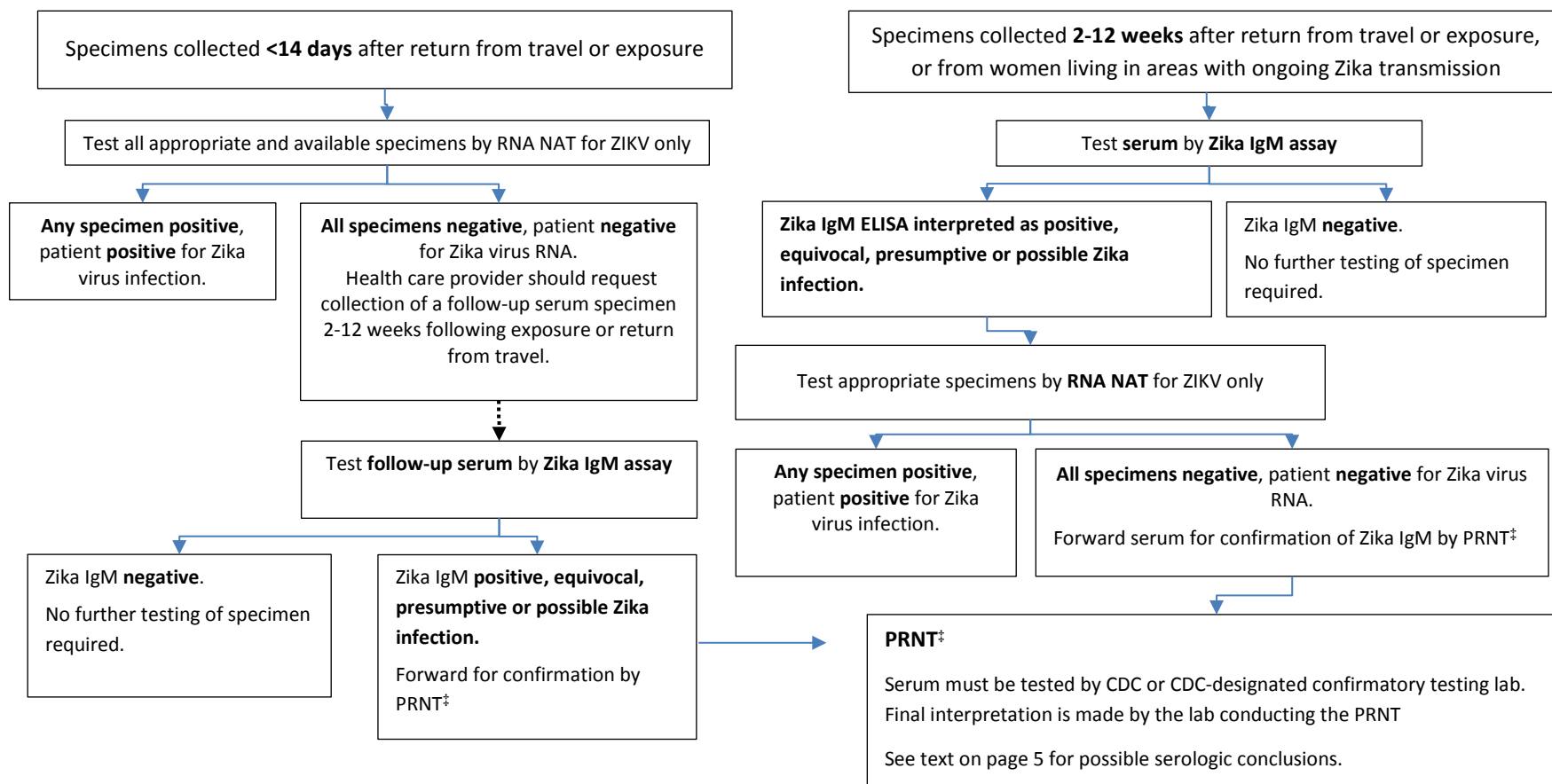
*Pregnant and non-pregnant symptomatic individuals

**Note antibody cross-reactivity to other flaviviruses complicates interpretation of the current anti-Zika IgM tests. Dengue IgM testing should be conducted for symptomatic pregnant women, individuals with a potential dengue exposure and when a presumptive other flavivirus result is obtained. See text on page 3-5 for additional information.

***Note if tests for Zika and Dengue IgM are not reactive, anti-chikungunya IgM testing should be performed for persons with chikungunya exposure risk and a clinically compatible illness.

‡PRNT confirmation is not currently routinely recommended for Puerto Rico. See page 5 for more information.

2016 Zika Response: Algorithm for U.S. Testing of Asymptomatic Pregnant Women Meeting Epidemiologic Criteria



NOTE: Report all test results to the appropriate Health Authorities. Results should be considered in the context of exposure risk and time point of specimen collection.

See this link for additional information for assessing epidemiologic risk: <http://www.cdc.gov/zika/geo/index.html>

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