

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

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Overview

Testing of specimens within the United States 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 dengue and chikungunya virus infections.

Current information and guidance for the U.S. Zika response is available on CDC’s Zika website:

<http://www.cdc.gov/zika/index.html>. Information specific to state and public health laboratory response:

<http://www.cdc.gov/zika/laboratories/index.html>.

Full testing algorithms are presented at the end of this document.

NOTE: Serum and urine are the primary diagnostic specimens for Zika virus infection.

Symptomatic individuals meeting epidemiological criteria:

Serum and urine collected from symptomatic patients < 14 days post onset of symptoms (DPO) should be tested by Zika virus real time reverse transcriptase-polymerase chain reaction (rRT-PCR). A positive

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>

Zika rRT-PCR result in either specimen is sufficient to diagnose Zika virus infection. If Zika virus rRT-PCR results are negative for both specimens, serum should be tested by antibody detection methods.

Serum that has been collected from patients presenting 2-12 weeks from onset of symptoms should be tested first by anti-Zika immunoglobulin (IgM) detection methods. Serum from symptomatic pregnant women should also be accompanied by a urine specimen.

- For non-pregnant symptomatic patients, anti-Zika IgM positive or equivocal result is followed by plaque reduction neutralization test (PRNT) directly.
- For symptomatic pregnant women, anti-Zika IgM positive or equivocal result is followed by rRT-PCR on both serum and urine. Some pregnant women have been reported to have detectable RNA present in serum and/or urine beyond the acute phase. If the rRT-PCR is negative, PRNT is necessary to confirm the presence of anti-Zika antibodies.

Asymptomatic pregnant women meeting epidemiological criteria for testing:

If serum and urine have been collected from a pregnant woman presenting within 2 weeks of her exposure or living in areas of ongoing transmission, serum and urine should be tested by rRT-PCR. If negative, a second serum specimen should be collected 2-12 weeks following return from travel and tested by antibody detection methods.

If serum from a pregnant woman first presenting 2-12 weeks following exposure is collected, the serum should be tested for anti-Zika IgM. If positive or equivocal, rRT-PCR should be performed on the serum and urine. If rRT-PCR is negative, PRNT should be performed for confirmation of IgM result.

A note on testing of other specimen types:

If testing of cerebrospinal fluid (CSF) from a symptomatic individual is requested for clinically indicated reasons (e.g., neurological symptoms), the specimen(s) must be submitted alongside a patient-matched serum specimen. A Zika virus rRT-PCR positive result from any specimen is indicative of Zika virus infection.

- Amniotic fluid testing by rRT-PCR may be considered following onset of symptoms in a pregnant woman; the optimal time to perform amniocentesis is unknown, however, amniocentesis performed ≥ 15 weeks of gestation is associated with lower rates of complications than those performed at earlier gestational ages, and early amniocentesis (≤ 14 weeks of gestation) is not recommended
- CSF should be tested by rRT-PCR if collected within the first week following onset or by antibody detection methods if collected after day 7 post onset of symptoms.

The patient-matched serum (and urine) submitted alongside these specimens should be tested according to the method(s) recommended for the time point post-onset indicated in the algorithms below.

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 a validated 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 or territorial public health department for testing of suspect specimens or referring specimens to CDC.

Within the 50 states, information about submitting specimens to CDC 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>

Specimen Type

Serum and urine are the preferred specimens for laboratory diagnosis of Zika virus infection.

Collection of specimens should be conducted using standard precautions for blood-borne pathogens. Guidance for collection storage and transport of body fluid specimens is provided at the following link: <http://www.cdc.gov/zika/laboratories/test-specimens-bodyfluids.html>.

Serum

Serum must be submitted for all patients to be tested for Zika virus infection. Serum may be tested by both antibody detection and molecular methods for Zika, dengue, and chikungunya (see algorithm on following pages for clarification). RNA from Zika, dengue, and chikungunya viruses is generally detectable in serum during the acute phase of infection). There is limited evidence suggesting some patients may have RNA detectable in serum and/or urine for a longer period of time (Bingham et al., 2016). Levels of anti-Zika IgM antibodies in infected individuals typically rise shortly after symptom onset and persist for 8-12 weeks.

NOTE: 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 collection and transport.

Urine

Urine should be tested alongside a patient-matched serum specimen by molecular methods for Zika virus only if the method has been validated for this specimen type. Antibody testing of urine is not recommended.

There is some limited evidence that Zika virus RNA may be detectable in urine for a longer period of time than in serum. Thus, CDC recommends that urine be collected alongside serum from symptomatic patients presenting up to 14 days post onset of symptoms and from asymptomatic pregnant women.

The patient-matched serum specimen should be tested using the recommended serum testing method(s) for the specimen collection time point post onset of symptoms.

NOTE: 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.

Other Specimen Types

Note: When testing of other specimen types is indicated, these specimens must be submitted alongside a patient-matched serum specimen.

CSF

CSF may be tested alongside a patient-matched serum specimen by both antibody detection and molecular methods for all three viruses.

Amniotic Fluid

Amniotic fluid may be tested alongside a patient-matched serum (and urine) specimens by molecular methods for Zika virus if the specimen type has been validated in the assay.

Molecular testing of amniotic fluid for the presence of Zika virus RNA can be requested following onset of symptoms in a pregnant woman as clinically indicated. The patient-matched serum and urine specimens should be tested using the recommended testing method(s) for the time point post onset of symptoms.

Tissue Specimens

Zika, dengue, and chikungunya virus testing on fixed and frozen tissue is available at CDC. Requests for testing should be coordinated through your state or local health department. Additional information about specimen collection and submission procedures is available on CDC's website:

<http://www.cdc.gov/zika/laboratories/test-specimens-tissues.html>.

Use of CDC Assays by Qualified Laboratories

The CDC Trioplex rRT-PCR and Zika MAC-ELISA (testing for anti-Zika IgM) are available to qualified laboratories in the United States. Eligible public health laboratories are those who have demonstrated proficiency with ELISA-based serological methods (for CDC Zika MAC-ELISA) or with rRT-PCR (for CDC Trioplex rRT-PCR) and who 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 who perform the CDC Zika MAC-ELISA and/or Trioplex rRT-PCR are first required to demonstrate proficiency with the assay(s) by successfully testing verification panels for each assay. Only labs that have been notified by CDC that they have successfully completed the verification testing are authorized to use the CDC assays for diagnostic testing.

Biological Safety

Please review CDC Biosafety Guidance for Transportation of Specimens and for Work with Zika Virus in the Laboratory: <http://www.cdc.gov/zika/laboratories/lab-safety.html>. See the Biosafety in Microbiological and Biomedical Laboratories (BMBL) for additional biosafety information about these viruses and laboratory biosafety practices: <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>.

Methods

Molecular testing

Detection of Zika virus RNA in serum and in urine collected from symptomatic individuals can be an effective aid to diagnose a recent Zika virus infection. CDC recommends the use of the FDA-authorized Triplex Real-time RT-PCR Assay (Triplex rRT-PCR) for detection of Zika, dengue, and chikungunya viral RNA in specimens collected from individuals suspected of Zika infection and meeting CDC clinical and epidemiological criteria.

Serum: Zika virus is usually detected in serum during the acute phase of infection. Due to the difficulty in many cases of precisely determining the onset date of symptoms, as well as the general low level of Zika viremia in serum, all rRT-PCR negative serum specimens should be tested by serological methods as well.

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. Beyond 14 days, urine testing may still be useful, but there are limited data to determine Zika virus RNA persistence in urine. CDC recommends that, for symptomatic individuals presenting up to 14 days after onset of symptoms, urine be collected alongside serum and be tested by rRT-PCR (MMWR, 2016).

If clinically indicated, CSF and amniotic fluid may be tested by rRT-PCR alongside a patient-matched serum specimen.

Available options for molecular testing include:

Analyte	Method(s)
Zika, chikungunya, and dengue RNA*	FDA-authorized kit (EUA): CDC Triplex rRT-PCR
Zika RNA only*	FDA-authorized kit (EUA): commercial laboratories Focus Diagnostics Zika Virus RNA Qualitative Real-Time RT-PCR altona RealStar Zika Virus RT-PCR Hologic Aptima Zika Virus Assay (transcription-mediated amplification TMA test)
Dengue RNA only	FDA-cleared kit: CDC DENV-1-4 Real-Time RT-PCR Assay

*FDA maintains a list of all Zika virus Emergency Use Authorizations on their website. Please refer to their website for the current list of the assays and the associated letters of authorization, fact sheets and product

labeling. Assay-specific information (e.g., acceptable specimen types, performance characteristics) is included in the labeling. <http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>

Co-infection is rare, but possible and should be considered in patients whose specimens generate positive results for more than one of these viruses. It is important to note that a positive result for one of these viruses does not preclude infection with the others.

Antibody detection methods

NOTE: If Zika rRT-PCR testing was conducted for a patient and any of the patient's specimens yielded positive results, the patient is positive for Zika infection. No antibody testing is required. Refer to algorithms at the end of this document.

Because of the potential for cross-reactivity, all serum specimens obtained from symptomatic individuals for whom serological testing is recommended should be tested with IgM antibody capture methods for the detection of anti-Zika IgM, and anti-dengue IgM. For those with exposure risk and a clinically compatible illness², anti-chikungunya IgM testing should also be performed.

Available options for clinical IgM testing:

Analyte	Method(s)
Anti-Zika IgM*	FDA-authorized kit (EUA): CDC Zika MAC-ELISA
Anti-Dengue IgM	FDA-cleared kit: 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)
Anti-Chikungunya IgM	Commercial laboratories with capability: Focus Diagnostics (http://www.focusdx.com/) ARUP Laboratories (http://www.aruplab.com/)

*FDA maintains a list of all Zika virus Emergency Use Authorizations on their website. Please refer to their website for the current list of the assays and the associated letters of authorization, fact sheets and product labeling. Assay-specific information (e.g., acceptable specimen types, performance characteristics) is included in the labeling. <http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>

Confirmation of antibody results

Confirmation of positive and equivocal IgM results is necessary and should be conducted according to the attached algorithm. If PRNT testing is indicated, that testing must be conducted by CDC or by a PRNT laboratory designated by CDC to do confirmatory PRNT testing for the Zika response.

Confirmation of anti-chikungunya IgM positive and equivocal results by PRNT:

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

Confirmation of anti-chikungunya virus IgM assay results should be conducted by PRNT using chikungunya virus.

- If no neutralization is observed (PRNT titer to chikungunya virus <10), the specimen is negative for evidence of recent chikungunya virus infection.
- If neutralization is observed (PRNT titer to chikungunya virus ≥ 10), the specimen is positive for evidence of recent chikungunya virus infection.

Confirmation of anti-Zika IgM positive and equivocal results and anti-dengue IgM positive and equivocal results by PRNT:

When rRT-PCR testing is not positive, anti-Zika IgM presumptive positive and equivocal results require confirmation by PRNT. It should be noted, however, that if a patient has a positive rRT-PCR result for the virus whose IgM assay gave a positive or equivocal result, no PRNT confirmation of the IgM result is necessary. A positive rRT-PCR result for a patient is a definitive indication of infection.

While no false negative results with the Zika MAC-ELISA have been detected during assay evaluation, there has been demonstrated cross-reactivity to anti-dengue virus IgM, and some false positive results in specimens with no detectable neutralizing flavivirus antibodies (by PRNT) upon confirmation.

PRNT may not be able to provide definitive determination of the specific virus causing the recent infection, particularly in individuals with a history of previous flavivirus infection.

Results of Zika/dengue PRNT testing should be interpreted alongside IgM assay results to come to an overall determination, as described in the following table reproduced from the CDC Interim Guidance for Interpretation of Zika Virus Antibody Results (Rabe et al., 2016). Please note that recommended time frame for testing of serum by rRT-PCR has changed since publication and has been updated in the table's footnote.

Interpretation of results of antibody testing for suspected Zika virus infection^{*,†,§,¶,} - United States, 2016**

Zika virus and dengue virus IgM ELISA	Zika virus PRNT	Dengue virus PRNT	Interpretation
Positive or equivocal (either assay)	≥10	<10	Recent Zika virus infection [†]
Positive or equivocal (either assay)	<10	≥10	Recent dengue virus infection [†]
Positive or equivocal (either assay)	≥10	≥10	Recent flavivirus infection; specific virus cannot be identified [†]
Any result (either or both assays)	<10	<10	No evidence of Zika virus or dengue virus infection
Inconclusive in one assay AND inconclusive or negative in the other	≥10	<10	Evidence of Zika virus infection; timing cannot be determined [§]
Inconclusive in one assay AND inconclusive or negative in the other	<10	≥10	Evidence of dengue virus infection; timing cannot be determined [§]
Inconclusive in one assay AND inconclusive or negative in the other	≥10	≥10	Evidence of flavivirus infection; specific virus and timing cannot be determined [§]
Positive for Zika virus AND negative for dengue virus	Not yet performed	Not yet performed	Presumptive recent Zika virus infection ^{†,§}
Positive for dengue virus AND negative for Zika virus	Not yet performed	Not yet performed	Presumptive recent dengue virus infection ^{†,§}
Positive for Zika virus AND positive for dengue virus	Not yet performed	Not yet performed	Presumptive recent flavivirus virus infection ^{†,§}
Equivocal (either or both assays)	Not yet performed	Not yet performed	Equivocal results [§]
Inconclusive (either or both assays)	Not yet performed	Not yet performed	Inconclusive results [§]
Negative for Zika virus AND negative for dengue virus	Not indicated	Not indicated	No evidence of recent Zika virus or dengue virus infection

*For persons with suspected Zika virus disease, Zika virus real-time reverse transcription–polymerase chain reaction (rRT-PCR) should be performed on serum and urine specimens collected <14 days after onset of symptoms.

[†]In the absence of rRT-PCR 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. In addition, equivocal and inconclusive results that are not resolved by retesting also should have PRNT titers performed to rule out a false-positive result.

Reporting

Each test result generated for each specimen should be reported as specified in the assay instructions for use. Results generated by methods used under FDA Emergency Use Authorization (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, patients and pregnant women 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 labs in combining results from multiple specimens/methods to make appropriate decisions about next testing steps.

Guidance documents are available to assist in applying lab 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 and chikungunya virus infections are all on the 2016 list of nationally notifiable conditions: <https://wwwn.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

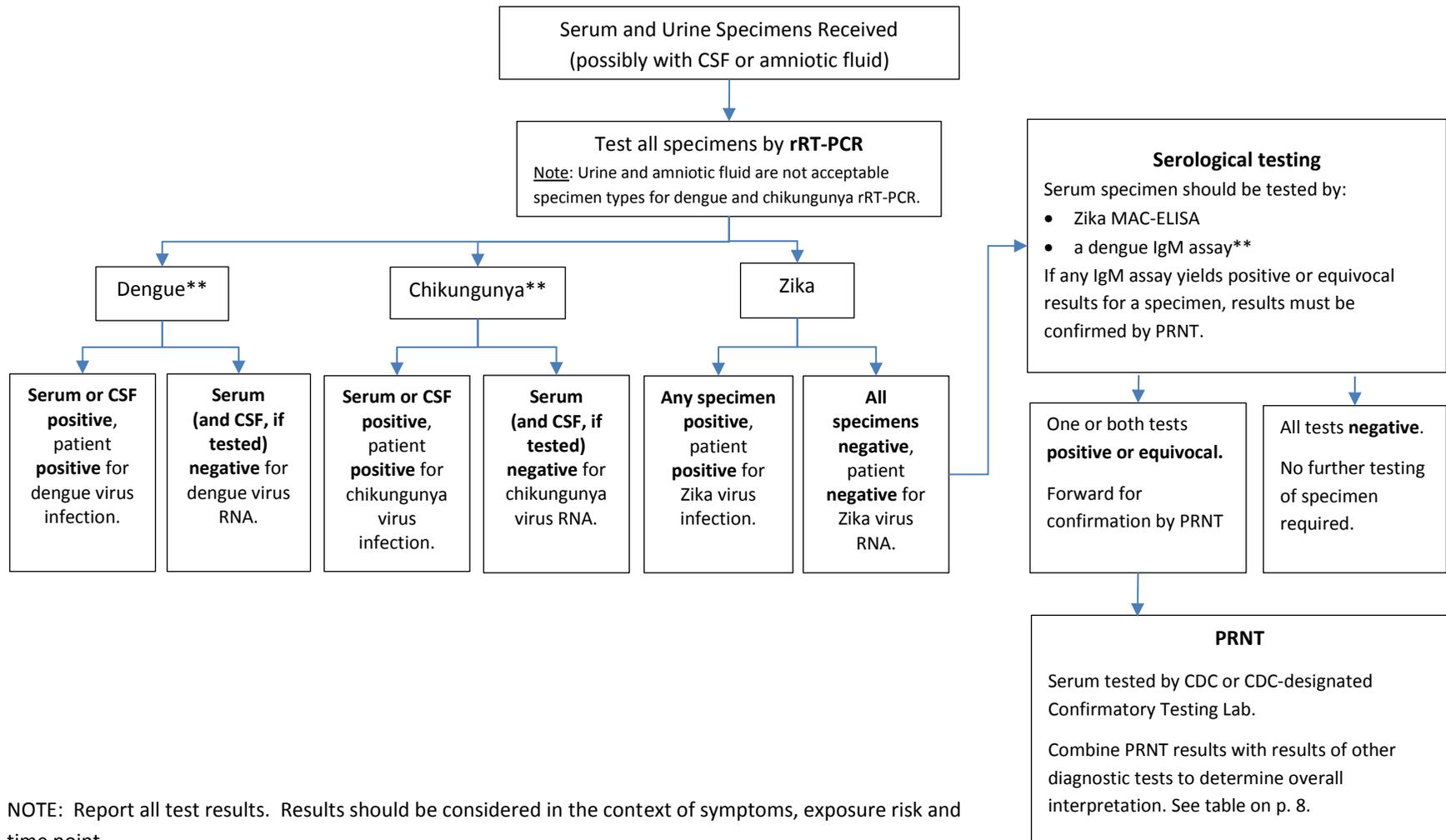
Bingham AM, Cone M, Mock V, et al. Comparison of Test Results for Zika Virus RNA in Urine, Serum, and Saliva Specimens from Persons with Travel-Associated Zika Virus Disease — Florida, 2016. MMWR Morb Mortal Wkly Rep 2016;65. DOI: <http://dx.doi.org/10.15585/mmwr.mm6518e2>.

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>.

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

Specimens Collected <14 days Following Symptom Onset



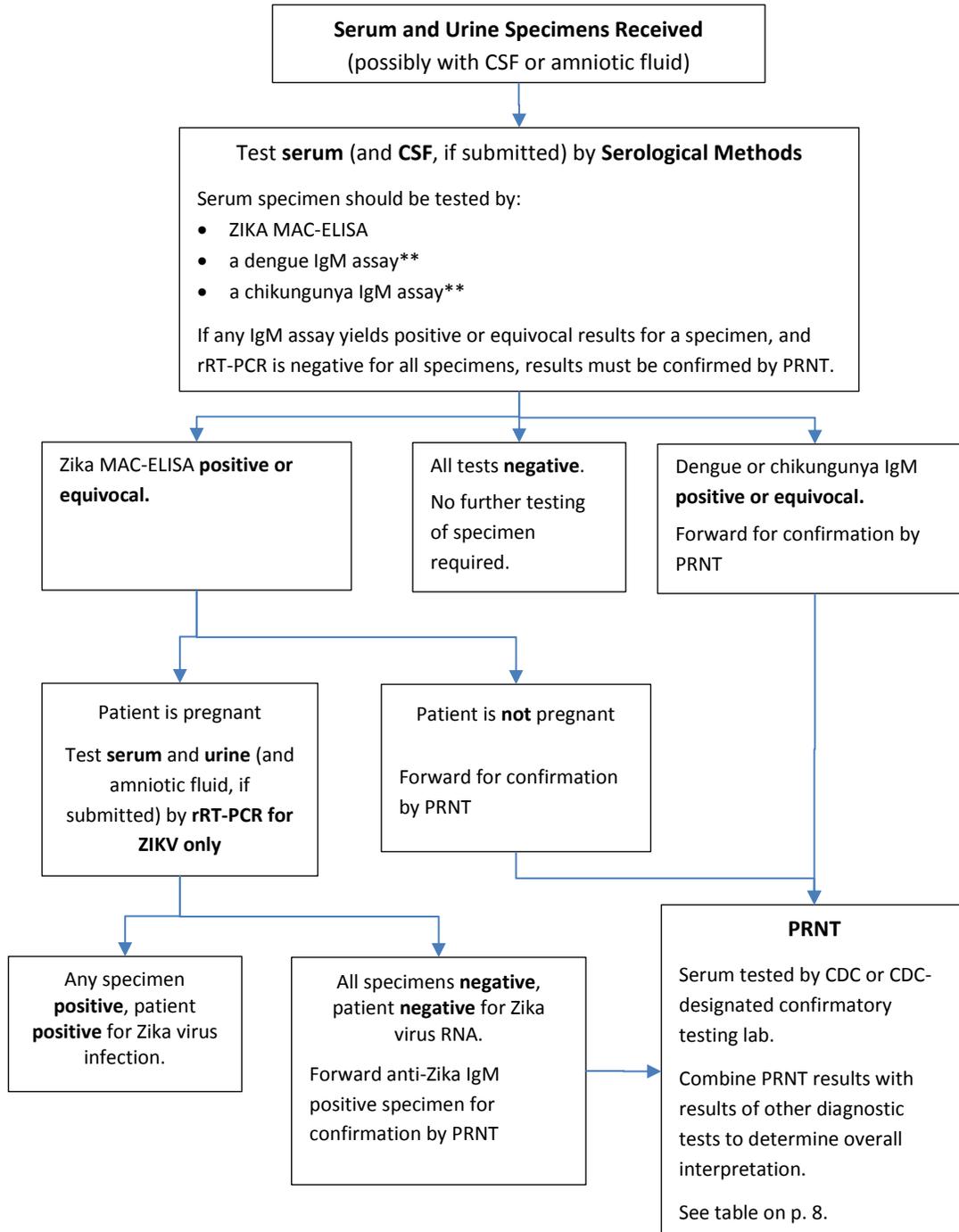
NOTE: Report all test results. Results should be considered in the context of symptoms, exposure risk and time point.

*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.

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

Specimens Collected \geq 14 Days Following Symptom Onset

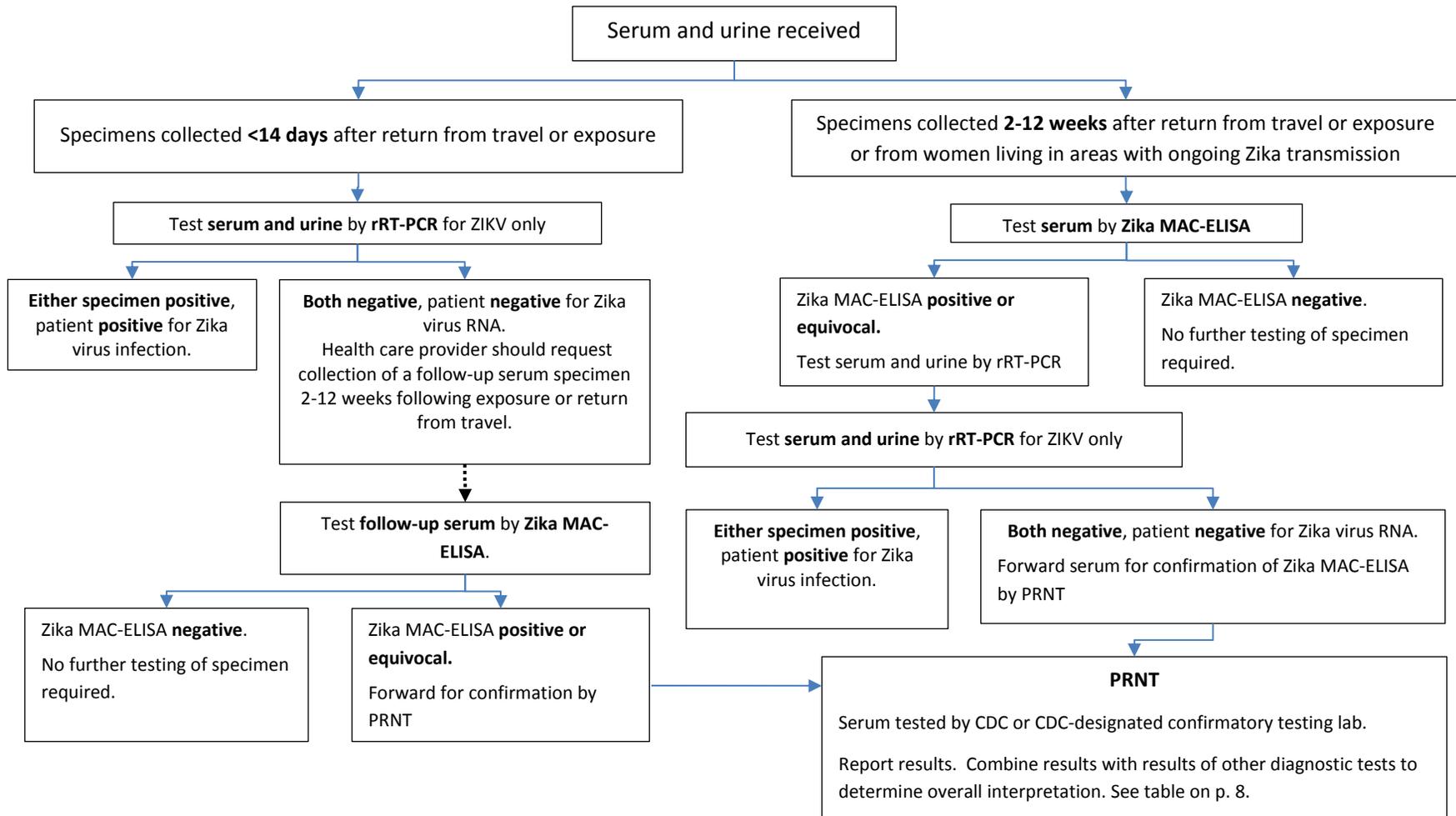


NOTE: Report all test results. Results should be considered in the context of symptoms, exposure risk and time point.

*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.

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.