Health Update

KITSAP PUBLIC HEALTH DISTRICT

June 7, 2016

Updated Zika virus testing interpretation guidance and new option for testing urine

Actions Requested

- **Be aware that diagnostic testing of urine specimens collected within 14 days of symptom onset is now available by RT-PCR.** Urine testing is available with pre-approval at CDC, and will soon be offered at some commercial labs.
- **Know that CDC has advised the following changes in the interpretation of test Zika virus testing results:**
  - A negative RT-PCR result on either urine or serum specimen collected at any time after illness onset *does not rule out* infection, and in these cases IgM antibody testing should be performed.
    - For serum specimens collected <7 days after onset of symptoms, the combination of a negative RT-PCR result and negative IgM antibody testing suggests that there was no recent infection.
  - For negative IgM antibody results on specimens collected early in illness (<7 days after onset) in the absence of RT-PCR testing, IgM testing should be repeated on a convalescent specimen.
  - A positive IgM antibody result by ELISA should be confirmed by PRNT, which is routinely done at CDC.
    - Patients who have positive PRNT results for more than one flavivirus should be clinically managed as if they are infected with each virus showing a positive titer. *(See below).*
- **Use attached *updated* screening form to help identify if Zika testing is warranted and the lab testing guidance to understand the timeframes and patients for which RT-PCR versus antibody testing is appropriate.** The lab testing guidance also provides help in the proper interpretation of negative results and the need for further testing.
- **Report any suspect Zika virus cases and request antibody testing by calling us.** Prior to collecting specimens, please call us with travel/exposure history and clinical information details ready to discuss.

For questions, please contact our Communicable Disease staff at 360-337-5235.

Background

The Centers for Disease Control and Prevention (CDC) recently issued a Health Update regarding diagnostic testing of urine and separately new interim guidance on the interpretation of antibody results *(see attachments)*. Given the potential for cross-reactivity between flaviviruses (e.g., Zika, dengue, West Nile, yellow fever viruses), positive IgM results by enzyme-linked immunosorbent assay (ELISA) should be confirmed by plaque-reduction neutralization testing (PRNT). Previously, a 4-fold difference between PRNT titers was used to differentiate between multiple flaviviruses to identify which virus was causing infection. However, because of the importance of clinical management and the potential that a 4-fold titer difference might not discriminate between cross-reacting antibodies, a more conservative approach to interpretation is now recommended. Patients who have positive PRNT results (i.e., ≥10) for multiple flaviviruses, regardless of the difference in titer, should be clinically managed as if they were infected with each. This is especially important because patients with dengue and Zika virus infections must be managed for both the potential hemorrhagic complications of dengue and the potential adverse pregnancy outcomes for Zika.

Resources

- CDC Zika virus website for healthcare providers: www.cdc.gov/zika/hc-providers/index.html
- Previous Zika virus health alerts from KPHD: www.kitsappublichealth.org/healthcare

Attachments:
(1) WA DOH / KPHD screening form, lab testing guidance, and CDC testing approval form (May 31, 2016)
(2) CDC MMWR: "Interim Guidance for Interpretation of Zika Virus Antibody Test Results" (May 30, 2016)
(3) CDC HAN Health Alert: "Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection" (May 25, 2016)

This is an official

HAN HEALTH UPDATE

Distributed via the CDC Health Alert Network
May 25, 2016, 14:15 EDT (2:15 PM EDT)
CDCHAN-00389

Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection

Summary
On May 13, 2016, the Centers for Disease Control and Prevention (CDC) issued interim guidance (http://www.cdc.gov/mmwr/volumes/65/wr/mm6518e1.htm) that recommends Zika virus rRT-PCR testing of urine collected less than 14 days after symptom onset, along with testing of patient-matched serum samples, for the diagnosis of suspected Zika virus infection (1). The purpose of this Health Alert Network (HAN) health update is to further disseminate information about the interim guidance to clinical and public health professionals.

Background
Zika virus is a mosquito-borne flavivirus. Zika virus infection during pregnancy can cause microcephaly and other severe fetal brain defects. Zika virus infection is also associated with Guillain-Barré syndrome. Transmission of Zika can occur through mosquito bite, from a pregnant woman to her fetus, through sexual contact with an infected male, and possibly through blood transfusion. The most common symptoms of Zika virus disease are fever, rash, joint pain, or conjunctivitis. Other common symptoms include muscle pain and headache. Evidence from case reports and experience from related flavivirus infections indicate that the incubation period for Zika is likely a few to 14 days.

Diagnostic testing for Zika virus infection can be accomplished using molecular and serologic methods. The U.S. Food and Drug Administration (FDA) has issued Emergency Use Authorizations (EUA) (http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm) for several diagnostic assays to detect Zika virus infection (2). The EUAs authorize real-time reverse transcription-polymerase chain reaction (rRT-PCR) assays to detect Zika virus RNA in specified clinical sample types, and an immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay (ELISA) to detect anti-Zika virus IgM antibodies in serum and cerebrospinal fluid. The CDC Trioplex rRT-PCR assay is authorized by FDA for Zika virus testing of urine and serum. Anti-Zika IgM antibodies develop during the first week of illness and persist for approximately 12 weeks following infection. However, extensive cross-reactivity can occur in flavivirus serological assays, and therefore additional tests, such as the plaque reduction neutralization test (PRNT), are necessary to distinguish Zika virus infection from other flavivirus infections.

Although Zika virus RNA is unlikely to be detected in serum after the first week of illness, recent data suggest that Zika virus RNA can persist in urine for at least two weeks post symptom onset (3). Given this information, on May 13, 2016, CDC issued interim guidance on rRT-PCR testing for Zika virus RNA in urine (1). CDC now recommends that, for persons with suspected Zika virus disease, Zika virus rRT-PCR should be performed on both urine and serum specimens collected within 7 days after onset of symptoms. Zika virus rRT-PCR also should be performed on urine specimens collected within 14 days after onset of symptoms. A positive rRT-PCR result in either specimen confirms Zika virus infection. However, a negative rRT-PCR in a serum or urine sample collected at any time point after illness onset does not exclude Zika virus infection, and in these cases IgM antibody testing should be performed on serum.

CDC recommendations for Zika virus testing of serum and other clinical specimens remain unchanged at this time. Please contact your state or local health department to facilitate testing.

Recommendations for Health Care Providers and Public Health Practitioners

- Collect urine samples within 14 days post symptom onset along with patient-matched serum samples for those who match CDC Zika virus clinical and/or epidemiological testing criteria for Zika virus infection.
- Perform Zika virus rRT-PCR testing on urine, in conjunction with testing of serum using the appropriate molecular or serologic assay, based on days post symptom onset.

Additional Considerations

- Further investigation is needed to determine the sensitivity and utility of Zika virus rRT-PCR on urine specimens collected ≥14 days after onset of symptoms: limited data in pregnant women suggest that viremia in serum might be prolonged in pregnancy (4, 5).

References

1. CDC. 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.

2. Food and Drug Administration. Emergency Use Authorizations. http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm

3. Comparison of Test Results for Zika Virus RNA in Urine, Serum, and Saliva Specimens from Persons with Travel-Associated Zika Virus Disease — Florida, 2016http://www.cdc.gov/mmwr/volumes/65/wr/mm6518e2.htm

4. Driggers RW, Ho CY, Korhonen EM, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. N Engl J Med. March 30, 2016. DOI: 10.1056/NEJMoa1601824

5. Bocanegra C. Zika virus infection in pregnant women in Barcelona, Spain. Clin Microbiol Infect. April 3, 2016. DOI: 10.1016/j.cmi.2016.03.025.

For More Information

- General information about Zika virus and disease: http://www.cdc.gov/zika/
- Zika virus information for clinicians: http://www.cdc.gov/zika/hc-providers/index.html
- Memorandum – Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories (not updated with urine guidance): http://www.cdc.gov/zika/state-labs/index.html
- Diagnostic testing: http://www.cdc.gov/zika/hc-providers/diagnostic.html
- Collection and submission of body fluids for Zika virus testing: http://www.cdc.gov/zika/hc-providers/body-fluids-collection-submission.html

The Centers for Disease Control and Prevention (CDC) protects people's health and safety by preventing and controlling diseases and injuries; enhances health decisions by providing credible information on critical health issues; and promotes healthy living through strong partnerships with local, national, and international organizations.

Categories of Health Alert Network messages:
Health Alert Requires immediate action or attention; highest level of importance
Health Advisory May not require immediate action; provides important information for a specific incident or situation
Health Update Unlikely to require immediate action; provides updated information regarding an incident or situation
HAN Info Service Does not require immediate action; provides general public health information

##This message was distributed to state and local health officers, state and local epidemiologists, state and local laboratory directors, public information officers, HAN coordinators, and clinician organizations##

Guidance for Providers Regarding Evaluation and Testing of Persons with Probable Exposure to Zika virus

May 31, 2016

Criteria for testing: person must meet any one of the following criteria:

** Check the CDC web site for current risk areas: (http://www.cdc.gov/zika/geo/)*

#	Criteria	Yes	No
1	**Any person** (male or female) **with illness consistent with Zika virus disease, including at least two of: acute onset of fever, maculopapular rash, arthralgia, or conjunctivitis,** occurring EITHER: a) during or within 2 weeks of last travel date to a risk area*; **OR** b) within 2 weeks of unprotected sex with a man who has tested positive for Zika virus *or* who traveled to a risk area* and had symptoms of Zika virus disease during his travel or within 2 weeks of his return → Ideally obtain serum within the first week of illness and urine within 2 weeks of illness		
2	**Symptomatic pregnant women with at least 1 symptom** (acute onset of fever, maculopapular rash, arthralgia, or conjunctivitis) within 2 weeks after unprotected sex with a man with possible Zika virus exposure in the past 6 months → Ideally obtain serum within the first week of illness and urine within 2 weeks of illness		
3	**Asymptomatic pregnant women** who EITHER: a) traveled while pregnant to a risk area*; **OR** b) had unprotected sex with a man who has tested positive for Zika virus or who traveled to a risk area* and showed Zika symptoms during travel or within 2 weeks of his return; **OR** c) had possible exposure (sexual^ or travel) in the 8 weeks before conception (6 wks before LMP) → Collect serum 2-12 weeks after exposure *^Testing is not currently recommended if both partners are asymptomatic.* *NOTE: If fetal ultrasounds detect microcephaly or intracranial calcifications, pregnant women who originally tested negative for Zika virus infection or who were not tested following travel should be retested.*		
4	**Woman experiencing fetal loss** with possible exposure to Zika during pregnancy if not previously tested. → Contact Kitsap Public Health District for specimen collection and submission instructions		
5	**Infants** born to women with possible exposure to Zika during pregnancy with EITHER: a) maternal positive or inconclusive test result for Zika virus; **OR** b) infant microcephaly,[¥] intracranial calcifications, or other brain or eye abnormalities consistent with congenital Zika virus infection; **OR** c) acute symptoms of Zika disease (see #1 above) in the infant within 2 weeks of birth and maternal exposure occurred within 2 weeks of delivery → Collect maternal serum if not previously tested and as many of the following as applicable and available: amniotic fluid, fixed and frozen placenta and umbilical cord tissue, umbilical cord serum or infant serum within 2 days of birth. [¥] *For possible congenital Zika, microcephaly is defined as occipitofrontal circumference <3rd percentile, based on standard charts for sex, age, and gestational age at birth. If circumference is ≥3rd percentile but notably disproportionate to body length, or if CNS deficits exist, further evaluation for Zika infection might be considered.*		

NOTE: Our Communicable Disease staff at Kitsap Public Health District (KPHD) are available for consultation as needed. Call us at (360)-337-5235.

➔ **See page 2 for Laboratory Testing guidance**

Laboratory Testing for Zika Virus

- Patients with Zika virus disease symptoms should generally also be evaluated for dengue and/or chikungunya because of strong cross-reactivity and clinical similarity. Consider ordering these tests simultaneously via commercial lab. If dengue infection is possible, advise the patient to avoid aspirin and NSAIDs.
- **Limited Zika virus testing (RT-PCR) is now being offered commercially, with more extensive testing available at CDC.**
- **Timeframes and patients for which RT-PCR versus IgM antibody testing is appropriate:**

Patient Symptomatic	Specimen Collection Timing	Test	Comments	Pre-Approval Required
Yes	• Serum specimen within 7 days of illness onset • Urine specimen within 14 days of illness onset	RT-PCR	• Positive RT-PCR results from serum or urine are indicative of current infection. • Negative RT-PCR result on a serum or urine specimen collected at any time *does not rule out* infection. A specimen should be obtained for ELISA IgM testing at CDC (*with approval*). • *Not* indicated for asymptomatic individuals. Negative result on an asymptomatic person *does not rule out* infection.	No; commercially available *(CDC testing still requires pre-approval)*
Yes	Serum specimen ≥7 days after illness onset	IgM ELISA and PRNT	IgM antibodies against Zika virus, dengue virus, and other flaviviruses have strong cross-reactivity.	Yes; offered at CDC
No	2-12 weeks after exposure	IgM ELISA and PRNT	Interpretation is complex in asymptomatic persons. While a negative IgM obtained 2-12 weeks after exposure would suggest a recent infection did not occur, it does not definitively rule out infection.	Yes; offered at CDC

- **For CDC testing: Submissions must still be pre-approved.**
  - In Kitsap County, please call KPHD at (360)-337-5235 to request testing prior to collecting specimens.
  - Serum (0.25 mL minimum, 2 mL preferred) spun down in a red or tiger top (serum separator) tube and kept cold or frozen to -70°C.
  - Urine (>1 mL) in a sterile container with a tight fitting screw cap and kept cold or frozen to -70°C.
  - For perinatal cases collect maternal serum **and** as many of the following as applicable and available: amniotic fluid, fixed placenta and umbilical cord tissue, frozen placental tissue and umbilical cord tissue, umbilical cord serum or infant serum (>0.25 mL) within 2 days of birth. For still births, contact KPHD.
  - All specimens require two patient identifiers, both on the specimen label and the submission form
  - **Specimen submission form**: www.doh.wa.gov/Portals/1/Documents/5230/302-017-SerVirHIV.pdf
  - Ship appropriate specimen(s) using Category B labels and packaging in an insulated container with ice packs or on dry ice, with completed submission form to WA PHL (address on form).
  - **The following intake form (page 3) MUST be completed and submitted to KPHD for approval prior to specimen submission.** Be sure to complete all fields. Missing details will result in specimen rejection. Fax completed form to our KPHD Communicable Disease confidential fax at (360)-337-5241.

Date:_____

Zika Virus Intake Form

PATIENT

Last name:_____ First name:_____

DOB: _____ Sex: ☐ Male ☐ Female County: _____

Patient Address:_____ Phone Number:_____

SUBMIT BY

Physician / Hospital / Lab / Clinic name:

_____

Contact name:_____Phone:_____

SPECIMEN

Date of Specimen Collection (*if asymptomatic pregnant woman, must be 2-12 weeks after travel*):_____

Shipping date:_____Specimen Source: ☐ Serum ☐ Urine ☐ Amniotic Fluid ☐ CSF ☐ Fixed tissue

☐ Frozen tissue ☐ Other:_____

EPIDEMIOLOGY

Date of Symptom Onset**:**_____ OR ☐ Asymptomatic

Symptoms *(check all) if patient is not pregnant, must have 2*: ☐ **Fever** ☐ **Rash** ☐ **Conjunctivitis** ☐ **Arthralgia**

☐ Guillain-Barré Syndrome ☐ Other: _____

Patient pregnant? ☐No ☐Yes, # weeks gestation currently:_____ OR estimated delivery date: _____

Fetal/infant anomalies: ☐ None ☐ Unk ☐ Microcephaly ☐ Intracranial calcifications

☐ Other: _____

Flavivirus Vaccination			
	N	Unk	If Yes/date
Yellow Fever			
Japanese Enceph.			
Tick-borne Enceph.			

Past Arboviral Infection			
	N	Unk	If Yes/Date
Yellow fever			
Japanese encephalitis			
Tick-borne enceph.			
St. Louis encephalitis			
West Nile virus			
Dengue			
Chikungunya			

Commercial Labs Ordered					
	N	Unk	If Yes/DOC	Lab	Results
CHIK PCR					
CHIK IgM/IgG					
Deng PCR					
Deng IgM/IgG					

TRAVEL HISTORY

Patient traveled to an area with Zika transmission within 14 days prior to symptom onset or within 12 weeks if asymptomatic?

□ Unk □ No □ Yes, countries/cities and dates of travel:

Infant with maternal history of exposure during pregnancy?

□ N/A □ unk □ No □ Yes, countries/cities and dates of travel:

OR dates of last sexual exposure:

Unprotected sex within 14 days prior to symptom onset or within past 12 weeks if asymptomatic with male sexual partner who: □ Tested positive for Zika virus

□ Had exposure (travel or sexual) in past 6 months AND had symptoms of disease within 2 weeks of last exposure

□ Had exposure (travel or sexual) in past 6 months AND no symptoms of disease

Date of male sexual partner symptom onset:_____AND countries and dates of travel OR dates of sexual exposure:

NOTES

Notes:

CALL KITSAP PUBLIC HEALTH AT (360) 337-5235. FAX COMPLETED FORM TO (360) 337-5241.

Centers for Disease Control and Prevention
MMWR
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Interim Guidance for Interpretation of Zika Virus Antibody Test Results

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Zika virus is a single-stranded RNA virus in the genus *Flavivirus* and is closely related to dengue, West Nile, Japanese encephalitis, and yellow fever viruses (*1*,*2*). Among flaviviruses, Zika and dengue virus share similar symptoms of infection, transmission cycles, and geographic distribution. Diagnostic testing for Zika virus infection can be accomplished using both molecular and serologic methods. For persons with suspected Zika virus disease, a positive real-time reverse transcription–polymerase chain reaction (rRT-PCR) result confirms Zika virus infection, but a negative rRT-PCR result does not exclude infection (*3*–*7*). In these cases, immunoglobulin (Ig) M and neutralizing antibody testing can identify additional recent Zika virus infections (*6*,*7*). However, Zika virus antibody test results can be difficult to interpret because of cross-reactivity with other flaviviruses, which can preclude identification of the specific infecting virus, especially when the person previously was infected with or vaccinated against a related flavivirus (*8*). This is important because the results of Zika and dengue virus testing will guide clinical management. Pregnant women with laboratory evidence of Zika virus infection should be evaluated and managed for possible adverse pregnancy outcomes and be reported to the U.S. Zika Pregnancy Registry or the Puerto Rico Zika Active Pregnancy Surveillance System for clinical follow-up (*9*,*10*). All patients with clinically suspected dengue should have proper management to reduce the risk for hemorrhage and shock (*11*). If serologic testing indicates recent flavivirus infection that could be caused by either Zika or dengue virus, patients should be clinically managed for both infections because they might have been infected with either virus.

Zika Virus Infection and Immune Response

Most Zika virus infections are asymptomatic (*12*). Viremia is expected to occur from several days before illness onset until a week after illness onset (*6*,*13*,*14*). Zika virus–specific IgM antibodies develop during the first week of illness (*5*,*6*). Data on duration of IgM antibody persistence following Zika virus infection are limited. However, IgM antibodies against West Nile virus, a closely related flavivirus, have been detected in asymptomatic, infected blood donors for at least 3 months after their viremic donation, and almost half of tested patients with West Nile virus neuroinvasive disease had detectable serum IgM antibodies >1 year after illness onset (*15*,*16*). Neutralizing antibodies to Zika virus develop shortly after IgM antibodies and consist primarily of IgG antibodies. Neutralizing antibodies are expected to persist for many years after flavivirus infections and are believed to confer prolonged, possibly lifelong, immunity (*17*–*19*). In persons previously infected with a flavivirus or vaccinated against yellow fever, Japanese encephalitis, or tick-borne encephalitis, subsequent exposure to a related flavivirus can result in a rapid and brisk rise in neutralizing antibodies against multiple flaviviruses (*20*). In addition, the neutralizing antibody titer against a flavivirus to which the person previously was exposed might be higher than the titer against the virus with which they were most recently infected (*20*). For example, a person who was previously infected with dengue virus or who received yellow fever vaccine might respond with high levels of neutralizing antibodies against those viruses when later infected with Zika or West Nile viruses. When performing serologic testing, the presence of these neutralizing antibodies against multiple flaviviruses can preclude conclusive determination of which flavivirus was responsible for the recent infection.

Zika Virus Antibody Testing

An enzyme-linked immunosorbent assay (ELISA) can be used to detect anti-Zika virus IgM antibodies in serum or cerebrospinal fluid; however, the Zika virus IgM ELISA can provide false-positive results because of cross-reacting IgM antibodies against related flaviviruses or nonspecific reactivity. The plaque

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Centers for Disease Control and Prevention

reduction neutralization test (PRNT) measures virus-specific neutralizing antibody titers and should be performed against various related flaviviruses to rule out false-positive ELISA results. In primary flavivirus infections (i.e., the first time a person is infected with a flavivirus), PRNT also can be used to identify the infecting virus. Usually, this is determined with a neutralizing antibody titer ≥4-fold higher than titers against cross-reacting flaviviruses. Based on earlier flavivirus research and limited preliminary data specific to Zika virus, the historical use of a 4-fold higher titer by PRNT might not discriminate between anti-Zika virus antibodies and cross-reacting antibodies in all persons who have been previously infected with or vaccinated against a related flavivirus (i.e., secondary flavivirus infection) (*20*,*21*). Because of the importance of appropriate clinical management of Zika and dengue virus infections, and the risk for adverse pregnancy outcomes in women infected with Zika virus during pregnancy, a conservative approach to the interpretation of antibody test results is now recommended to reduce the possibility of missing the diagnosis of either infection (*9*,*11*).

CDC Zika Virus Diagnostic Tests

The Food and Drug Administration (FDA) has issued an Emergency Use Authorization for the CDC Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) for antibody testing (*3*). This assay has been introduced and is being used in qualified public health and Department of Defense laboratories in the United States. The Zika MAC-ELISA is used for the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid collected from persons meeting the clinical and epidemiologic criteria for suspected Zika virus disease (*3*,*22*). Results are reported as positive (termed "presumptive positive" to denote the need to perform a confirmatory PRNT), equivocal, negative, or inconclusive (i.e., results uninterpretable because of high background optical density). To resolve false-positive results that might be caused by cross-reactivity or nonspecific reactivity, presumptive positive results should be confirmed with PRNT against Zika, dengue, and other flaviviruses to which the person might have been exposed (*3*,*23*). In addition, equivocal and inconclusive results that are not resolved by retesting also should have PRNT performed to rule out a false-positive result.

Interpretation of Zika Virus Testing Results

For persons with suspected Zika virus disease, a positive rRT-PCR result confirms Zika virus infection, and no antibody testing is indicated (*3*,*4*,*7*). However, because of the decline in the level of viremia over time and possible inaccuracy in reporting of dates of illness onset, a negative rRT-PCR result does not exclude Zika virus infection. Therefore, serum IgM antibody testing for Zika and dengue virus infections should be performed if rRT-PCR is negative. For serum specimens collected <7 days after onset of symptoms, the combination of a negative rRT-PCR result and negative IgM antibody testing suggests that there was no recent infection. However, a negative IgM antibody test, in the absence of rRT-PCR testing, might reflect specimen collection before development of detectable antibodies and does not rule out infection with the viruses for which testing was performed. For specimens collected from 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.

Summary

What is already known about this topic?

Zika virus is a mosquito-borne flavivirus closely related to dengue, West Nile, Japanese encephalitis, and yellow fever viruses. Diagnostic testing for Zika virus infection can be accomplished using both molecular and serologic methods. However, results of Zika virus antibody testing can be difficult to interpret because of cross-reactivity with related flaviviruses, which can preclude identification of the specific infecting virus, especially when the person previously was infected with or vaccinated against a related flavivirus.

What is added by this report?

For persons with suspected Zika virus disease, a positive real-time reverse transcription–polymerase chain reaction (rRT-PCR) result confirms Zika virus infection, but a negative result does not exclude infection. In these cases, antibody testing can identify additional recent Zika virus infections. If immunoglobulin (Ig) M test results are positive, equivocal, or inconclusive, performing a plaque reduction neutralization test (PRNT) is needed to confirm the diagnosis. However, recent evidence suggests that a 4-fold higher titer by PRNT might not discriminate between anti-Zika virus antibodies and cross-reacting antibodies in all persons who have been previously infected with or vaccinated against a related flavivirus. Thus, a more conservative approach to interpreting PRNT results is now recommended to reduce the possibility of missing the diagnosis of either Zika or dengue virus infection.

What are the implications for public health practice?

All patients with clinically suspected dengue should receive appropriate management to reduce the risk for hemorrhagic medical complications. Pregnant women with laboratory evidence of a recent Zika virus infection or flavivirus infection should be evaluated and managed for possible adverse pregnancy outcomes and reported to the appropriate Zika virus pregnancy registry. Health care providers should consult with state or local public health authorities for assistance in interpreting test results.

TABLE. 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
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
Any result (either or both assays)	<10	<10	No evidence of Zika virus or dengue virus infection
Positive for Zika virus AND negative for dengue virus	Not yet performed		Presumptive recent Zika virus infection
Positive for dengue virus AND negative for Zika virus	Not yet performed		Presumptive recent dengue virus infection
Positive for Zika virus AND positive for dengue virus	Not yet performed		Presumptive recent flavivirus virus infection
Equivocal (either or both assays)	Not yet performed		Equivocal results
Inconclusive in one assay AND inconclusive or negative in the other	Not yet performed		Inconclusive results
Negative for Zika virus AND negative for dengue virus	Not indicated		No evidence of recent Zika virus or dengue virus infection

Abbreviations: ELISA = enzyme-linked immunosorbent assay; IgM = immunoglobulin M antibodies; PRNT = plaque reduction neutralization test.

* For persons with suspected Zika virus disease, Zika virus real-time reverse transcription–polymerase chain reaction (rRT-PCR) should be performed on serum specimens collected <7 days after onset of symptoms, and on urine specimens collect <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.

If either the Zika or dengue virus IgM antibody testing yields positive, equivocal, or inconclusive results, PRNTs against Zika and dengue viruses (or other flaviviruses endemic to the region where exposure occurred) should be performed. A PRNT using a 90% cutoff value with a titer ≥10 (the typical starting serum dilution used to establish the presence of virus-specific neutralizing antibodies) against Zika virus, together with negative PRNTs (i.e., <10) against other flaviviruses is confirmatory for recent infection with Zika virus (Table). A PRNT titer ≥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. A negative PRNT against Zika virus in a specimen that is collected >7 days after illness onset rules out Zika virus infection. For specimens collected <7 days after onset of symptoms, the combination of a negative rRT-PCR and a PRNT titer <10 suggests that there was no infection with Zika virus. However, in the absence of rRT-PCR testing, a PRNT titer <10 might reflect specimen collection before development of detectable neutralizing antibodies and does not rule out infection with the viruses for which testing was conducted. Without confirmatory PRNTs, it is not possible to determine whether a presumptive positive IgM antibody result against Zika virus reflects recent flavivirus infection or a false-positive result.

For asymptomatic pregnant women residing in an area with local Zika virus transmission, IgM testing should be performed upon initiation of prenatal care, mid-second trimester, and if any fetal abnormalities are detected during ultrasound evaluation (*9*). For asymptomatic pregnant women with a history of travel to areas where ongoing Zika virus transmission is occurring, Zika virus antibody testing should be performed on specimens collected 2–12 weeks post travel (*9*). Results are interpreted as for symptomatic persons. If a serum specimen was collected >12 weeks after travel, although IgM might still be present, it is possible that antibody levels have dropped below the detectable limit. Performing routine PRNTs for women in this group is not recommended because any result other than a PRNT titer <10 for Zika virus could represent infection with or vaccination against a flavivirus at any time in the past and does not provide specific evidence of Zika virus exposure during pregnancy.

Management of Persons with Suspected Zika or Dengue Virus Infection

All patients with clinically suspected dengue virus infection should receive appropriate management to reduce the risk for hemorrhagic complications (*11*). Symptomatic and asymptomatic pregnant women with serologic or molecular evidence of recent Zika virus infection should be evaluated and managed for possible adverse pregnancy outcomes and reported to the U.S. Zika Pregnancy Registry or the Puerto Rico Zika Active Pregnancy Surveillance System (*9*,*10*). Among

persons for whom serologic testing is unable to determine the most recent infecting flavivirus, an epidemiologic link to a laboratory-confirmed case of dengue or Zika virus disease can be considered in determining the most likely infecting virus (*22*). In addition, data on the epidemiology of viruses known to be circulating at the location of exposure and clinical features of these viral infections should be considered. If serologic testing is inconclusive or there is evidence of recent infection with either Zika or dengue virus, patients should be clinically managed for both infections because they might have been infected with either virus. Health care providers with questions about test result interpretation should consult with state or local public health authorities for assistance.

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References

1. Faye O, Freire CCM, Iamarino A, et al. Molecular evolution of Zika virus during its emergence in the 20th century. PLoS Negl Trop Dis 2014;8:e2636. http://dx.doi.org/10.1371/journal.pntd.0002636
2. Hayes EB. Zika virus outside Africa. Emerg Infect Dis 2009;15:1347–50. http://dx.doi.org/10.3201/eid1509.090442
3. Food and Drug Administration. Zika virus emergency use authorization. Silver Spring, MD: US Department of Health and Human Services, Food and Drug Administration; 2016. http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm
4. CDC. Interim guidance for Zika virus testing of urine—United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:474. http://dx.doi.org/10.15585/mmwr.mm6518e1
5. 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;65:475–8. http://dx.doi.org/10.15585/mmwr.mm6518e2
6. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 2008;14:1232–9. http://dx.doi.org/10.3201/eid1408.080287
7. CDC. Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US public health laboratories. http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf
8. Calisher CH, Karabatsos N, Dalrymple JM, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989;70:37–43. http://dx.doi.org/10.1099/0022-1317-70-1-37
9. Petersen EE, Polen KND, Meaney-Delman D, et al. Update: interim guidelines for health care providers caring for pregnant women and women of reproductive age with possible Zika virus exposure—United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:315–22. http://dx.doi.org/10.15585/mmwr.mm6512e2
10. Simeone RM, Shapiro-Mendoza CK, Meaney-Delman D. Possible Zika virus infection among pregnant women—United States and territories, May 2016. MMWR Morb Mortal Wkly Rep 2016;65:514–9.
11. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control, 2009. Geneva, Switzerland: World Health Organization; 2009. http://apps.who.int/iris/bitstream/10665/44188/1/9789241547871_eng.pdf
12. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–43. http://dx.doi.org/10.1056/NEJMoa0805715
13. Musso D, Nhan T, Robin E, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill 2014;19:20761. http://dx.doi.org/10.2807/1560-7917.ES2014.19.14.20761
14. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. J Clin Virol 2015;68:53–5. http://dx.doi.org/10.1016/j.jcv.2015.04.021
15. Prince HE, Tobler LH, Yeh C, Gefter N, Custer B, Busch MP. Persistence of West Nile virus–specific antibodies in viremic blood donors. Clin Vaccine Immunol 2007;14:1228–30. http://dx.doi.org/10.1128/CVI.00233-07
16. Roehrig JT, Nash D, Maldin B, et al. Persistence of virus-reactive serum immunoglobulin M antibody in confirmed West Nile virus encephalitis cases. Emerg Infect Dis 2003;9:376–9. http://dx.doi.org/10.3201/eid0903.020531
17. Whitehead SS, Blaney JE, Durbin AP, Murphy BR. Prospects for a dengue virus vaccine. Nat Rev Microbiol 2007;5:518–28. http://dx.doi.org/10.1038/nrmicro1690
18. Busch MP, Kleinman SH, Tobler LH, et al. Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis 2008;198:984–93. http://dx.doi.org/10.1086/591467
19. Poland JD, Calisher CH, Monath TP, Downs WG, Murphy K. Persistence of neutralizing antibody 30–35 years after immunization with 17D yellow fever vaccine. Bull World Health Organ 1981;59:895–900.
20. Halstead SB, Rojanasuphot S, Sangkawibha N. Original antigenic sin in dengue. Am J Trop Med Hyg 1983;32:154–6.
21. Johnson BW, Kosoy O, Martin DA, et al. West Nile virus infection and serologic response among persons previously vaccinated against yellow fever and Japanese encephalitis viruses. Vector Borne Zoonotic Dis 2005;5:137–45.
22. Council of State and Territorial Epidemiologists. Zika virus disease and congenital Zika virus infection interim case definition and addition to the Nationally Notifiable Diseases list. Atlanta, GA: Council of State and Territorial Epidemiologists; 2016. https://www.cste2.org/docs/Zika_Virus_Disease_and_Congenital_Zika_Virus_Infection_Interim.pdf
23. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. J Clin Microbiol 2000;38:1823–6.

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