



2016 DOHMH Advisory #5 (UPDATED): Diagnostic Testing for Zika Virus and Interpretation of Results

Please share with your colleagues in Obstetrics/Gynecology, Maternal/Fetal Medicine, Internal Medicine, Family Medicine, Emergency Medicine, Urgent Care, Pediatrics, Neonatology, Infectious Disease, and Neurology:

- **As of April 28, 2016, the Public Health Laboratory will no longer routinely test specimens submitted for Zika testing from symptomatic patients for dengue and chikungunya viruses by reverse transcriptase polymerase chain reaction (RT-PCR). Clinicians should request RT-PCR testing for dengue and chikungunya viruses through a commercial laboratory.**
- **Diagnosis of Zika virus infection often includes testing by both RT-PCR and serology.**
- **Some patients will require a follow-up (convalescent) specimen for serologic testing to verify Zika virus infection or determine which flavivirus caused the infection.**
- **Call the PAL to arrange all Zika virus testing at 1-866-692-3641.**

April 28, 2016

Dear Colleagues,

We are writing to provide information on interpretation of Zika virus testing and to emphasize the need for convalescent serologic testing for most patients.

Tests for Zika virus

Reverse transcriptase polymerase chain reaction (RT-PCR) testing is the primary diagnostic test. It is very specific for Zika virus, but viral RNA levels decrease over time and may already be undetectable in serum by 7 days after onset of symptoms. Interpretation of results from serology testing is often difficult if the patient has a history of previous flavivirus infection (including West Nile virus, dengue virus, Saint Louis encephalitis virus, Powassan virus, Japanese encephalitis virus, and yellow fever virus) or vaccination against yellow fever virus. Even after serological testing, it may not be possible to determine whether Zika virus was the cause of a recent flavivirus infection.

Previously, the Public Health Laboratory routinely ran dengue and chikungunya RT-PCR tests on specimens submitted for Zika RT-PCR testing from symptomatic patients. As of April 28, 2016, the Public Health Laboratory will no longer test specimens submitted for Zika testing for dengue or chikungunya viruses by RT-PCR, regardless of the clinical scenario. Clinicians should request RT-PCR testing for dengue and chikungunya viruses through a commercial laboratory for patients who are symptomatic and have an appropriate travel history.

- 1) **RT-PCR** performed on serum or urine detects acute infection. Sensitivity is highest when specimens are collected within 7 days of illness onset. The Health Department Public Health Laboratory will only perform RT-PCR for the following patients:
 - a. Symptomatic patients whose specimens were collected within 4 weeks of illness onset
 - i. Clinicians should send specimens for dengue and chikungunya RT-PCR testing to a commercial clinical laboratory.

- b. Asymptomatic pregnant patients whose specimens were collected within 6 weeks of the last date of being in a Zika-affected area or within 6 weeks of their last potential exposure (e.g., suspected sexual transmission).
- 2) **Serology** will be performed using two types of tests on serum from all patients to screen for flavivirus infection. The two screening tests are:
- a. IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC ELISA)
 - i. Performed for all patients.
 - ii. Screens for acute infection with flaviviruses and is not specific for Zika virus (although it is also called the “CDC Zika assay”).
 - iii. Detects IgM antibodies.
 - b. Arbovirus Microsphere Immunofluorescence Assay (MIA)
 - i. Performed for all patients.
 - ii. Screens for acute and previous infection with flaviviruses and is not specific for Zika virus.
 - iii. Detects IgM, IgG, and IgA antibodies.
- 3) **Plaque reduction neutralization test (PRNT)**
- a. Performed only for patients with a reactive or equivocal MAC ELISA and/or reactive MIA.
 - b. Requires paired sera: the acute (initial) and convalescent (follow-up) specimens collected 3 weeks after the acute specimen was collected but within 12 weeks of exposure.
 - c. PRNT may help determine the flavivirus to which the patient was exposed by comparing the titer of neutralizing antibodies in acute and convalescent specimens to a panel of flaviviruses.
 - d. Results take longer than MAC ELISA and MIA, because PRNT requires substantially more labor and time to perform.

Collecting Convalescent Specimens

Convalescent (follow-up) specimens will be indicated for some patients and may determine more definitively the presence or absence of recent Zika virus infection. Collect a follow-up serum specimen three weeks after the acute (initial) serum specimen, **even if you have not yet received results regarding the initial specimen.** Once initial serum and RT-PCR results are reported, serologic testing on the convalescent specimen will be conducted for the following patients:

- Patients with non-reactive acute serology results whose initial specimen may have been collected before they mounted a detectable immune response. This includes:
 - Symptomatic patients whose initial specimens were collected within 8 days of symptom onset.
 - Asymptomatic pregnant patients whose initial specimens were collected within 3 weeks of being in a Zika affected area or their last potential exposure (e.g., suspected sexual transmission).
- Patients with reactive or equivocal serology (MAC ELISA and/ or MIA)

Reporting Test Results

The NYC Health Department will immediately report all Zika positive results by phone to the ordering provider, specifically positive RT-PCR or positive serology confirmed by PRNT. For specimens submitted to the NYC Health Department Public Health Laboratory, Zika test results will be mailed to the provider or facility listed as the submitter. Some facilities may receive some results via secure fax if a secure fax number is provided. Each test result (RT-PCR on serum, RT-PCR on urine, MAC ELISA, MIA, and, if performed, PRNT) is forwarded to the provider once available; thus, results of each test may be sent to the ordering provider separately. While RT-PCR results may be reported out within one week of specimen receipt, serology results may take up to three weeks or more from the date of specimen receipt.

For assistance in interpreting your patient's results, please see the attached *Quick Guide to Interpretation of Zika Test Results*. If you have questions about the test results, please call the NYC Provider Access Line at **1-866-692-3641**.

Sincerely,

Jay Z. Varma, MD

Deputy Commissioner

Division of Disease Control, New York City Health Department

Quick Guide to Interpretation of Zika Test Results

Zika RT PCR			
	Interpretation*	Next Steps	
Not Detected	No evidence of recent infection with Zika virus. Perform serologic testing.	Refer to serologic results for further patient assessment.	
DETECTED	Evidence of recent Zika virus infection.	No additional testing required. Results are confirmatory for Zika virus infection.	
ZIKA SEROLOGY			
MAC ELISA	MIA	Interpretation	Next steps
PRESUMPTIVE POSITIVE	REACTIVE	Screening tests suggest recent exposure to a flavivirus but are unable to determine which flavivirus. Tests are not specific for Zika virus. A presumptive positive and reactive result may be due to another flavivirus.	Additional testing required. -Submit convalescent (follow-up) specimen collected 3 weeks after acute (initial) specimen. Paired acute and convalescent sera will be tested by PRNT. PRNT may help determine the flavivirus to which the patient was exposed. -If this patient also had a previous flavivirus infection, it may be impossible to determine if the patient was acutely infected with Zika virus versus another flavivirus.
PRESUMPTIVE POSITIVE	Non-Reactive	Screening tests suggest recent exposure to a flavivirus but are unable to determine which flavivirus. Tests are not specific for Zika virus. A presumptive positive and reactive result may be due to another flavivirus.	Additional testing required. -Submit convalescent (follow-up) specimen collected 3 weeks after acute (initial) specimen. Paired acute and convalescent sera will be tested by PRNT. PRNT may help determine the flavivirus to which the patient was exposed.
Negative	REACTIVE	Screening tests suggest exposure to a flavivirus at an undetermined time.	Additional testing may be required for; - Asymptomatic pregnant patient - if specimen was collected less than 3 weeks after last day of travel or last exposure, or - Symptomatic patient - if specimen was collected <8 days after onset of illness In these patients, the specimen may have been collected before the patient mounted a detectable immune response. Submit a follow-up specimen collected 3 weeks after initial specimen. Otherwise, if specimen collected 8 or more days after symptom onset or 3 weeks after travel or last exposure, additional testing may still be required. To help determine if the MIA is positive due to Zika virus, determine the amount of time that has elapsed between when the specimen was collected in relation to the exposure or onset of illness. If the specimen was collected less than 10 weeks from first day of arrival in affected country (or exposure if sexual transmission) OR first day of onset of illness, then results suggest “No evidence of infection with Zika.” If the specimen was collected more than 10 weeks from first day of arrival in affected country (or exposure if sexual transmission) OR first day of onset, then further testing via PRNT is required to determine which flavivirus (Zika or another virus) the individual was exposed to and whether exposure was past or present. Collect a convalescent specimen approximately 3 weeks after the original (acute) specimen was collected. Paired acute and

			convalescent sera will be tested by PRNT. Note, previous exposure to a flavivirus other than Zika may make results difficult to interpret.
Negative	Non-Reactive	No evidence of current or previous flavivirus infection if either: -Asymptomatic pregnant woman whose specimen was collected more than 3 weeks after last date of travel or last exposure, or -Symptomatic patient whose specimen was collected 8 or more days after the onset of illness.	Additional testing may be required for; - Asymptomatic pregnant patient - if specimen was collected less than 3 weeks after last day of travel or last exposure, or - Symptomatic patient - if specimen was collected <8 days after onset of illness In these patients, the specimen may have been collected before the patient mounted a detectable immune response. Submit a follow-up specimen collected 3 weeks after initial specimen. Otherwise, if specimen collected 8 or more days after symptom onset or 3 weeks after travel or last exposure, consider negative and NO additional testing is required.

MAC ELISA= IgM Antibody Capture Enzyme Linked Immunosorbent Assay;

MIA= Arbovirus Microsphere Immunofluorescence Assay

PRNT= Plaque reduction neutralization test

*For equivocal or indeterminate PCR results, collect additional serum or urine if it can be obtained within 4 weeks of onset of illness or 6 weeks of last exposure to repeat PCR testing. Otherwise, await serology results for further patient assessment.