AMIDA CARE
ABOVE AND BEYOND FOR YOU

Zika Diagnostic Laboratory Updates

Dear Provider,

As you may already be aware, several specimens tested at commercial labs for Zika IgM were positive, but showed no evidence of Zika virus infection upon repeat testing at the New York State Wadsworth Center laboratory.

The New York City Department of Health and Mental Hygiene (NYC DOHMH) issued a Health Advisory (number 2) on January 30, 2017, on the subject of Zika Diagnostic Laboratory updates. Some of the highlights include:

- A specimen with a positive Zika IgM result must be confirmed by plaque reduction neutralization testing at Wadsworth Center.
- Do NOT base decisions about managing a pregnant woman on positive IgM testing alone.
- Use venipuncture only, not heel sticks, to collect blood specimens from infants to reduce risk for hemolysis.

The full text of the advisory is available below.

A second attachment is an algorithm, "Zika Testing Guidance for Providers," which was part of the same NYC DOHMH Health Advisory on January 30, 2017. Providers are encouraged to call the Health Department at 1-866-692-3641 for assistance. To follow up on laboratory reports, please call the Public Health Laboratories (PHL) at 1-212-447-2864.

Amida Care appreciates your efforts to keep our members and the city safe and healthy.

Sincerely,

Jerome Ernst, MD
Chief Medical Officer

NEW YORK CITY DEPARTMENT OF
HEALTH AND MENTAL HYGIENE
Mary T. Bassett, MD, MPH
Commissioner

2016 DOHMH Advisory #2: Zika Diagnostic Laboratory Updates

- Several Zika IgM positive specimens tested at commercial labs show no evidence of Zika virus infection following repeat testing at the New York State Wadsworth Center laboratory.
- Zika IgM specimens with a positive test result must be confirmed by plaque reduction neutralization testing at Wadsworth Center.
- Do not base decisions about managing pregnant women on positive IgM testing alone.
- Use venipuncture only, not heel sticks, to collect blood specimens from infants to reduce risk for hemolysis.

January 30, 2017

Dear Colleagues,

The New York City Health Department has found that 43% of patients with a positive* Zika IgM test performed at a commercial laboratory had no evidence of Zika infection on confirmatory testing by Wadsworth Center (WC) at the New York State Department of Health.

Commercial laboratories are required to send specimens to WC for any patient with any positive Zika IgM test result. For these specimens, WC repeats the Zika IgM test and also performs two additional tests: microsphere immunoassay (MIA) for IgM, IgG and IgA antibodies and the plaque reduction neutralization test (PRNT). Among 74 patients who were Zika IgM positive at a commercial laboratory and for whom confirmatory testing was completed at WC, 32 (43%) had no laboratory evidence of Zika virus infection. Twenty were negative on repeat IgM testing and had no detectable antibodies to both Zika and dengue viruses on PRNT. Another 12 were negative on repeat IgM and had no detectable antibody to Zika on PRNT; dengue virus antibodies were detected on PRNT, suggesting dengue infection at an undetermined time. The reason for the discrepant results is unknown at this time, though it may be that commercial lab assays are detecting cross reactive antibodies for other Flaviviruses.

Providers should not base decisions about managing pregnant women on positive IgM testing alone. Providers should wait for additional test results from WC or another reference laboratory, such as CDC, before making any decisions regarding a pregnant woman. All commercial laboratories testing NYC residents are required to forward all specimens with a non-negative Zika IgM result to WC for both repeat IgM and PRNT. It may take up to 4 weeks for WC to report final results back to the commercial laboratory that submitted the specimen. Interpretation of the final panel of results can be challenging. Providers are encouraged to call the Health Department at 1-866-692-3641 for assistance.

The 32 specimens with Zika positive IgM test results includes all of the following; ‘presumptive positive’ (18), ‘possible positive’ (2), ‘positive’ (4), ‘equivocal’ (8). Test result interpretations reported by commercial labs will vary depending on which assay is used to test for evidence of Zika IgM antibodies.

Table 1. Zika IgM Test Interpretations and Results that May be Reported by Commercial Labs

Zika IgM Test Performed	Result reported	Additional Zika testing needed?	Commercial lab will automatically forward specimen to Wadsworth Center for repeat Zika IgM and PRNT?
MAC ELISA Assay	Positive	Yes	Yes
	Equivocal	Yes	Yes
	Negative	No	No
InBios Assay	Presumptive Zika Positive	Yes	Yes
	Possible Zika Positive	Yes	Yes
	Negative	No	No
	Presumptive Other Flavivirus Positive	No	Yes

Specimens from Infants

Providers are also reminded that specimens need to be collected correctly from infants. Of the over 200 serum specimens collected from NYC infants and submitted for Zika IgM testing at PHL, about half have had visible hemolysis. Hemolysis can interfere with the test assay. For Zika IgM testing of infants, please collect a minimum of 3 mL whole blood through venipuncture to reduce the likelihood of hemolysis. Serum should be centrifuged and frozen as soon as possible after collection to maintain integrity of the specimen and optimize the chance of attaining an interpretable result.

For more information about Zika virus testing in NYC including guidance on which tests to order, where to obtain testing and interpretation of test results, please see (www.nyc.gov/zika/provider).

For questions about testing, or to discuss a case, please call **1-866-692-3641**. To follow up on laboratory reports, please call the PHL at **1-212-447-2864**.

We appreciate your continued diligence and cooperation as NYC responds to Zika virus.

Sincerely,

Jay K. Varma, MD

Deputy Commissioner, Division of Disease Control

New York City Department of Health and Mental Hygiene

ZIKA TESTING GUIDANCE FOR PROVIDERS

- Deciding which tests to order should be based on the patient's pregnancy status and date of illness onset or, if asymptomatic, the last date of potential exposure (e.g., last date in an area with active transmission of Zika virus or last unprotected sexual encounter with a partner who traveled to an area with active transmission of Zika virus).
- Zika IgM specimens with a positive test result must be confirmed by plaque reduction neutralization testing at Wadsworth Center, do not base decisions about managing pregnant women on positive IgM testing alone.
- For guidance on which tests to order, where to obtain testing and interpretation of test results, please see (www.nyc.gov/zika/provider).

1/27/2017

Infant with with findings concerning for congenital Zika infection?
Contact the NYC Health Department by calling the Provider Access Line at 866-692-3641

Pregnant Patient?

YES → **Pregnant Patient With Symptoms?**

YES → Specimen collection within 1 week after symptom onset date[a] — **TESTING OPTION A**

YES → Specimen collection between 1 and 12 weeks after symptom onset date[b] — **TESTING OPTION B**

YES → Specimen collection more than 12 weeks after symptom onset date[b] — **TESTING OPTION C**

NO → Specimen collection within 12 weeks after last potential exposure*[,a,b] — **TESTING OPTION D**

NO → Specimen collection more than 12 weeks after last potential exposure*[,b] — **TESTING OPTION E**

NO → **Non-Pregnant Patient With Symptoms**

Specimen collection within 1 week after symptom onset date[a] — **TESTING OPTION A**

Specimen collection between 1 and 4 weeks after symptom onset date[b] — **TESTING OPTION B**

Specimen collection more than 4 weeks after symptom onset date[b] — **TESTING OPTION C**

TESTING OPTION A
1. Zika virus RNA detection serum and urine (NAAT)[f]
2. Zika virus IgM (serology) followed by PRNT if positive[e]
3. Dengue virus RNA detection serum (NAAT)[c,f]
4. Dengue virus IgM/IgG (serology)[d]
5. Chikungunya virus RNA detection serum (NAAT)[c,f]
6. Chikungunya virus IgM/IgG (serology)[c]

TESTING OPTION B
1. Zika virus RNA detection serum and urine (NAAT)[f]
2. Zika virus IgM (serology) followed by PRNT if positive[e]
3. Dengue virus IgM/IgG (serology)[d]
4. Chikungunya virus IgM/IgG (serology)[c]

TESTING OPTION C
1. Zika virus IgM (serology) followed by PRNT if positive[e]
2. Dengue virus IgM/IgG (serology)[d]
3. Chikungunya virus IgM/IgG (serology)[c]

TESTING OPTION D
1. Zika virus RNA detection serum and urine (NAAT)[f]
2. Zika virus IgM (serology) followed by PRNT if positive[e]
3. Dengue virus IgM/IgG (serology)[d]

TESTING OPTION E
1. Zika virus IgM (serology) followed by PRNT if positive[e]
2. Dengue virus IgM/IgG (serology)[d]

* Exposure is defined as travel to an area with active transmission of Zika virus or unprotected sexual encounter with a partner who traveled to an area with active transmission of Zika virus, but may also include a blood or organ transfusion.

[a] Patients for whom specimens were collected fewer than 8 days after symptom onset (if symptomatic) or 3 weeks after last potential exposure (if asymptomatic) may not have mounted a detectable IgM response. If all tests are negative, consider repeating the IgM to rule out infection.

[b] The expected duration of Zika IgM antibodies in serum is 12 weeks following infection, but in a small proportion of persons with a previous dengue or other flavivirus infection, there may be a muted IgM response (i.e., duration of antibodies is shorter and/or the titers diminished). For pregnant women with negative IgM but whose fetus or infant has microcephaly or another concerning abnormality, please call the NYC Health Department at 866-692-3641 to discuss the case and to pursue additional testing.

[c] Only indicated for symptomatic infections where mosquito transmission is suspected. Sexual transmission not considered a primary route of transmission for dengue or chikungunya.

[d] Dengue serologic testing may be helpful for interpreting complicated serologic results for those persons with a recent Zika infection but who have a history of previous dengue infection, or, those persons for whom dengue is the cause of the acute infection.

[e] Repeat Zika IgM testing should be performed for persons with a positive or equivocal Zika IgM and a negative NAAT result, so that paired specimens (the initial and repeat serum specimens) can be forwarded to the New York State Wadsworth Center Laboratory for plaque reduction neutralization testing (PRNT).

[f] Zika virus RNA is typically detected in serum for up to 7 days and in urine up to 14 days following infection; however, viral persistence in serum may be prolonged in pregnant women. Zika virus RNA is detected through the use of nucleic acid amplification testing (NAAT) such as real time reverse transcriptase-polymerase chain reaction (rRT-PCR), and transcription-mediated amplification (TMA) testing.