Zika Virus Testing
What Local Health Departments Need to Know

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February 10, 2016
Objectives

At the conclusion of this webinar, local health departments (LHDs) will be able to:

• Describe the epidemiology, clinical manifestations, and reporting of Zika virus
• Articulate NYSDOH’s recommendations for Zika virus testing
• Discuss diagnostic testing for Zika virus infection available through NYSDOH’s Wadsworth Center and interpretation of test results
• Understand the steps needed to pre-authorize individuals for Zika testing via CDMS
Zika Virus

- Single stranded RNA Virus
- Genus *Flavivirus*, Family *Flaviviridae*
- Closely related to dengue, yellow fever, Japanese encephalitis and West Nile viruses
- Transmitted to humans primarily by *Aedes* species mosquitoes
Zika Virus Vectors

Aedes Mosquitoes

• *Aedes* species mosquitoes
  – *Ae aegypti*: more efficient vectors for humans
  – *Ae albopictus*: found in some parts of NYS
• Also transmit dengue and chikungunya viruses
• Lay eggs in domestic water-holding containers
• Live in and around households
• Aggressive and primarily daytime biters, but can also bite at night
Aedes aegypti and Aedes albopictus Mosquitoes: Geographic Distribution in the United States
Laboratory experiments have shown that *Aedes albopictus* can transmit Zika virus from infected animals to uninfected animals. Its current involvement in transmission in the Americas is not known. *Aedes albopictus* has been implicated in transmission of other flavivirus outbreaks, such as a recent Dengue outbreak in Hawaii.
Modes of Transmission

- Mosquito Bite
  - From infected to uninfected humans and primates by bite of a mosquito
- Maternal-fetal
  - Intrauterine
  - Perinatal
- Other
  - Sexual
  - Blood transfusion
- Theoretical
  - Organ or tissue transplantation
  - Breast milk
Sexual transmission

CDC

- Sexual transmission of Zika virus is possible, and is of particular concern during pregnancy.
- Men who reside in or have traveled to an area of active Zika virus transmission who have a pregnant partner should abstain or consistently and correctly use condoms during sex for the duration of the pregnancy.
- Pregnant women should discuss their male partner’s potential exposures to mosquitoes and history of Zika-like illness with their health care provider.
- Men who reside in or have traveled to an area of active Zika virus transmission who are concerned about sexual transmission of Zika virus might consider abstaining from sexual activity or using condoms consistently and correctly during sex.

Public Health England

- A small number of cases of sexual transmission of Zika virus have been reported, and in a limited number of cases, the virus has been shown to be present in semen, although it is not yet known how long this can persist. The risk of sexual transmission of Zika virus is thought to be very low.
- If a female partner is at risk of getting pregnant, or is already pregnant, condom use is advised for a male traveler:
  - for 28 days after his return from an active Zika transmission area if he has not had any symptoms compatible with Zika virus infection
  - for 6 months following recovery if a clinical illness compatible with Zika virus infection or laboratory confirmed Zika virus infection was reported.
Zika Virus: Countries and Territories with Active Zika Virus Transmission

As of February 3, 2016
Zika Virus Epidemiology

- Infection rate: 73% (95%CI 68-77)
- Symptomatic attack rate among infected: 18% (95%CI 10-27)
- All age groups affected
- Adults more likely to present for medical care
- No severe disease, hospitalizations, or deaths

Note: Rates based on serosurvey on Yap Island, 2007 (population 7,391)
Zika Virus Clinical Disease Course and Outcomes

• Clinical illness usually mild, lasting for several days to a week

• Characteristic clinical findings are acute onset of fever with maculopapular rash, arthralgia, or conjunctivitis.
  • Other commonly reported symptoms include myalgia and headache
  • Severe disease requiring hospitalization uncommon

• Fatalities are rare, typically only with comorbidities

• Guillain-Barré syndrome reported in patients following suspected Zika virus infection
  • Relationship to Zika virus infection is not known, but under active investigation
Reporting Zika Virus Disease Cases

- As an arboviral disease, Zika virus disease is a nationally notifiable disease
  - Hospitals and providers must report suspected cases of Zika virus to the LHD where the patient resides (10NYCRR 2.10)
- Case definition: Arboviral diseases, neuroinvasive and non-neuroinvasive
- Timely reporting allows NYSDOH and local health departments to assess and reduce the risk of local transmission or mitigate further spread
Differential Diagnosis for Zika Virus Disease in Returning Travelers

- Dengue
- Chikungunya
- Leptospirosis
- Malaria
- Rickettsia
- Parvovirus
- Group A streptococcus
- Rubella
- Measles
- Adenovirus
- Enterovirus
Diagnostic Testing for Zika Virus

• No commercially-available diagnostic tests at present
  • CDC is working with commercial laboratories to address this issue
• Testing is available at NYSDOH’s Wadsworth Center
• CDC is working to expand laboratory diagnostic testing to other state and large urban area public health laboratories
There are a number of diagnostic tests for Zika virus infection, including:

- PCR assay to detect viral RNA in serum and urine
- Serological assays to detect either IgM or IgG in serum collected ≥4 days after illness onset.
  - Detect both Zika-specific and cross-reactive antibody
- Plaque reduction neutralization test (PRNT) to detect a ≥4-fold rise in Zika virus-specific neutralizing antibodies in paired sera
NYSDOH Zika Testing Process

• The process for Zika virus testing is for all healthcare providers and facilities in New York State (including those based in New York City).

• This process applies to all patients except neonates with microcephaly or intracranial calcifications, born to women who traveled to an area with active Zika virus transmission while pregnant.
  – Healthcare providers and facilities caring for these neonates should directly contact NYSDOH at 1-888-364-4723 between 9AM and 6PM weekdays for consultation and facilitation of testing.
  – If the patient resides in another state, the NYS healthcare provider/or facility caring for the patient should directly contact NYSDOH at 1-888-364-4723 between 9AM and 6PM weekdays.

• No cost for laboratory testing at the NYSDOH Wadsworth Center.
NYSDOH Zika Testing Procedures

• Healthcare provider identifies a patient presenting for care who meets the following criteria:
  – Pregnant woman who traveled to an area with Zika virus transmission while pregnant (see http://www.cdc.gov/zika/geo/index.html) OR
  – Non-pregnant woman or man who develops (or developed) compatible symptoms during or within 4 weeks of travel to an area with Zika virus transmission OR
  – A person who traveled to an area with active Zika virus transmission and who presents with Guillain-Barré syndrome
NYSDOH Zika Testing Procedures: LHD Role

- Verify that the patient resides in your county
  - If not, please give the provider contact information for the appropriate LHD
- Verify that the patient meets testing criteria
  - Providers whose patients who do not meet testing criteria should be advised as such
  - If a provider or LHD wish to discuss a specific case that does not meet criteria (e.g. pregnant sex partner of zika-positive male traveler), contact your regional epidemiologist, who will consult with BCDC
- If testing is approved by the LHD, the healthcare provider must provide the patient with a written order/prescription for Zika virus testing that specifies serum and urine for polymerase chain reaction (PCR) and serum for serology
- The order/prescription must clearly indicate the name, complete address and date of birth of the patient and the NYS license number of the provider
NYSDOH Zika Testing Procedures: LHD Role

• Assist the provider (and patient, if available) with completing the NYSDOH Zika virus testing questionnaire on CDMS
  – https://health.ny.gov/go2clinic/63
  – CDMS will be updated as needed

• Be certain that the provider and patient understand that specimen collection can be performed at ANY authorized specimen collection site, regardless of where the patient resides or where the provider practices

• A list of authorized specimen collection sites and telephone numbers is available at https://commerce.health.state.ny.us/hcsportal/docs/Source/hpn/preparedness/zika/ZikaCollectionSites.pdf and should be offered to the provider during the call, so that the patient can choose the site most convenient for them
NYSDOH Zika Testing Procedures: LHD Role

- If the patient provides an email address for use in CDMS, the authorization ticket will be emailed to them
  - Specimen collection sites will accept a printed ticket or an image of the ticket on a smartphone
  - If they do not have an email address, staff should use a LHD email address for retrieval of the authorization ticket and fax the ticket to the patient, provider or directly to the specimen collection site
NYSDOH Zika Testing Procedures: LHD Role

• Be certain that the provider and patient understand that patients who present for testing without a written order from a NYS licensed healthcare provider and the CMDS authorization ticket will be turned away.

• LHDs should immediately notify their regional epidemiologist of the testing request.

• All providers, including NYC based providers/facilities caring for patients who reside outside of NYC, must follow this process and obtain LHD approval.
CDMS Demonstration

NYS DOH Events

NYSDOH Zika Virus Testing Campaign

Zika Virus testing will only be approved for those who provide BOTH: NYSDOH Authorization for Zika Virus Testing Form Prescription sheet from your health care provider LINKS:
NYSDOH Zika Virus Information Page
CDC Zika Virus Information Page

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<tr>
<td>Where</td>
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Specimen Collection: Information for LHDs

• Serum:
  – At least six milliliters (ml) of blood in a serum tube (red top, serum separator tube, tiger top, speckle top, gold top). These tubes contain clot activator, so that serum can be readily obtained.
  – Do NOT use blood tubes that contain anti-coagulants such as green top, yellow top or purple top. Centrifuge the blood tube, transfer the serum to a separate labeled tube (at least 3 ml serum required) and discard the clot. Seal the serum tubes with parafilm.

• Urine:
  – Collect urine in a sterile cup with a minimum volume of 3 ml and a maximum volume of 20 ml. Close the lid tightly and seal with parafilm. Specimens that leak will not be tested.
Specimen Collection: Information for LHDs

• Label the specimens: Failure to properly label a specimen will result in rejection and the specimen will not be tested.

• Specimens must be labeled with:
  – Patient’s First and Last Names
  – Patient’s Date of Birth
  – Date and Time of Collection

• All information on the specimen label must exactly match the information on Wadsworth’s Infectious Diseases Requisition (IDR) form (described below), including the spelling of the patient’s first and last names.
Specimen Handling: Information for LHDs

- It is best that blood specimens be centrifuged, separated and frozen immediately.
- Specimens that are not directly centrifuged should be immediately refrigerated and must be centrifuged and the serum frozen within six hours.
- Specimens must be on cold packs when transported from the refrigerator to the freezer during this 6 hour time period.
- Both the urine and serum need to be frozen at -70 to -80 degrees Celsius. Freezing preserves the integrity of the RNA in the samples.
Specimens

**Serum**
Collect whole blood in red top tubes
minimum 6ml

Centrifuge and remove serum
minimum 3ml

Freeze serum and urine -70 to -80°C

**Urine**
Minimum 3ml
Specimen Transport for Non-NYC Residents

- Wadsworth’s Infectious Diseases Requisition (IDR) form, which is available at http://www.wadsworth.org/sites/default/files/WebDoc/1065760803/infectious_diseases_requisition_DOH_4463.pdf must be completed in full and accompany each specimen being submitted on a non-NYC resident. If present, symptoms should be clearly noted on the IDR.
  - Follow shipping regulations for UN 3373 Biological Substance, Category B and UN 1875, Class 9 for dry ice.
  - Specimens must be shipped on dry ice to the Wadsworth Center, David Axelrod Institute, 120 New Scotland Avenue, Albany, NY 12208. Label the outside of the package with storage conditions (-70 to -80C).
Patient name and address

Physician name and address

Specimen details

Test request

Travel history – location and dates

? Pregnant ? trimester

Clinical symptoms
Packaging, Shipping, and Certification

IATA
DOT
Certification
NYSDOH Zika Testing Results

• Results of Zika virus testing will be made available to LHDs via ECLRS. LHDs should check ECLRS at least once a day for Zika virus testing results and fax those results to the ordering provider on same day.
  – ECLRS results should be moved into CDESS (as with any other disease), but CDESS Zika supplemental does not need to be completed until a positive Zika result.
• LHD staff can access public health consultation for assistance with interpretation of results by calling the NYSDOH Zika Information Line at: 1-888-364-4723 between 9AM and 6PM weekdays.
NYSDOH Arboviral\(^1\) Testing
Algorithm for individuals returning from areas with active Zika virus activity

\(^1\)Arboviral testing includes analysis for evidence of infection with chikungunya as well as several flaviviruses such as Dengue, Zika, and West Nile Virus
\(^2\)Testing of initial specimens may take 14-21 days to complete
\(^3\)RT-PCR will be performed on specimens collected within 1 month of symptom onset or within 6 weeks of travel
\(^4\)Serology will be performed on specimens collected between the day of the patient’s return from travel and 9 months following return
\(^5\)The Zika IgM ELISA will soon be available for use at Wadsworth Center
\(^6\)Convalescent specimens should be collected 3 weeks after the acute specimen is collected
\(^7\)Plaque reduction neutralization testing
\(^8\)PREGNANT WOMEN ONLY: single specimen PRNT will be performed on the original specimen received however collection of a convalescent specimen is still required

Algorithm as of February 10, 2016
Zika virus serology (IgM) can be positive due to antibodies against related flaviviruses (e.g., dengue and yellow fever viruses)

Neutralizing antibody testing may discriminate between cross-reacting antibodies in individuals who have never had a flavivirus infection before

Difficult to distinguish infecting virus in people previously infected with or vaccinated against a related flavivirus

Health care providers should work with NYSDOH and local health departments to ensure test results are interpreted correctly
Polymerase Chain Reaction (PCR)

- PCR will be performed for chikungunya, dengue, and Zika virus on all travelers returning from areas with Zika virus activity. PCR tests for genetic material of viruses and generally detects infection within one week of onset of symptoms.
- If any of these are positive (DETECTED), results indicate current infection with the virus identified.
- Positive results are confirmatory.
- If results are negative (NOT DETECTED), await serology results.
The West Nile MACELISA (IgM antibody capture enzyme-linked immunosorbent assay) should be considered a SCREENING TEST for flavivirus exposure (dengue, Zika, West Nile, etc.). By itself, it is NOT confirmatory!

If results are Nonreactive, results suggest that there was no evidence of flavivirus exposure however if current infection is suspected, a convalescent serum specimen should be collected approximately 3 weeks later to completely rule out infection. Paired acute and convalescent serum will be tested by plaque reduction neutralization testing (PRNT).

If results are Reactive, a convalescent serum specimen should be collected approximately 3 weeks later. Paired acute and convalescent serum will be tested by PRNT.

FOR PREGNANT WOMEN ONLY: single specimen PRNT will be performed on the original specimen received however collection of a convalescent specimen is still required.
Serology

Test: Arbovirus Microsphere Immunofluorescence Assay

Specimen Source: Serum specimen
Request Status: Final

These tests should be considered SCREENING TESTS for flavivirus exposure. By themselves, they are NOT confirmatory!

If results are Nonreactive, results suggest that there was no evidence of flavivirus exposure however if current infection is suspected, a convalescent serum specimen should be collected approximately 3 weeks later to completely rule out infection. Paired acute and convalescent serum will be tested by PRNT.

If results are Reactive, a convalescent serum specimen should be collected approximately 3 weeks later. Paired acute and convalescent serum will be tested by PRNT.

FOR PREGNANT WOMEN ONLY: single specimen PRNT will be performed on the original specimen received however collection of a convalescent specimen is still required.

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Serology

Test: Arbovirus Immunofluorescence Assay
Specimen Source: Serum specimen
Request Status: Final
Relevant Clinical Data: ONSET 01/13/2016;

These tests should be considered SCREENING TESTS for flavivirus exposure (St. Louis Encephalitis, dengue, Zika, West Nile, etc.), alphavirus exposure (Venezuelan Equine Encephalitis, chikungunya), and bunyavirus exposure (California serogroup viruses). By themselves, they are NOT confirmatory!

- If results are negative (<16), results suggest that there was no evidence of arbovirus exposure however if current infection is suspected, a convalescent serum specimen should be collected approximately 3 weeks later to completely rule out infection. Paired acute and convalescent serum will be tested by PRNT.

- If results are positive (>=16), a convalescent serum specimen should be collected approximately 3 weeks later. Paired acute and convalescent serum will be tested by PRNT.

- FOR PREGNANT WOMEN ONLY: single specimen PRNT will be performed on the original specimen received however collection of a convalescent specimen is still required.
Serology

This test should be considered a supportive laboratory result for chikungunya virus.

If results are negative, results suggest that there was no evidence of chikungunya exposure however if current infection is suspected, a convalescent serum specimen should be collected approximately 3 weeks later to completely rule out infection. Paired acute and convalescent serum will be tested by PRNT.

If results are positive, a convalescent serum specimen should be collected approximately 3 weeks later. Paired acute and convalescent serum will be tested by PRNT.

The Chikungunya ELISA tests are cross-reactive with other alphavirus infections. These tests could be positive when a patient is infected with Venezuelan Equine Encephalitis (VEE) virus.
If the ‘Arbovirus PRNT Interpretation’ field states ‘Results suggest no evidence of infection with any of the viruses listed’ AND testing was performed on BOTH acute and convalescent specimens, there is no evidence of infection with any of the viruses listed.

If PRNT results are negative on a SINGLE SPECIMEN ONLY and current/recent infection is suspected, a convalescent specimen must be obtained (drawn at least 3 weeks after acute). The paired specimens will be tested by PRNT to rule out current/recent infection.

If the ‘Arbovirus PRNT Interpretation’ field states ‘current/recent infection’ with one of the viruses listed, the result is confirmatory for current/recent infection with the virus listed.

If the ‘Arbovirus PRNT Interpretation’ field states ‘infection at an undetermined time’ with one of the viruses listed, health care providers are encouraged to consult with their Local Health Department to discuss results and interpretation. Additional specimens/testing may be required.

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Zika Resources for LHDs

• CDC’s Zika Virus Information Page

• NYSDOH’s Zika Virus Information Page

• CIDRAP’s Zika Virus Clearinghouse
  – http://www.cidrap.umn.edu/infectious-disease-topics/zika#literature

• PAHO’s Zika Virus Information Page
Questions?