Zika Diagnostic Tools, Testing Algorithms, and Interpretation Guidance

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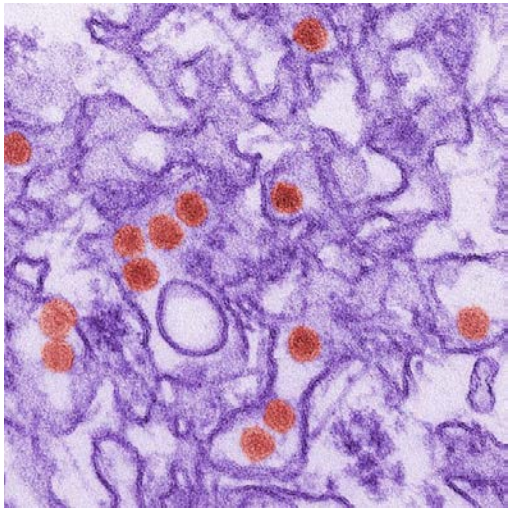
OVERVIEW

- Zika Virology and History
- Zika Diagnostics
- Diagnostic Testing Algorithms
- Test Interpretation Guidance
- Q&As

Zika Virology and History

Zika Virus

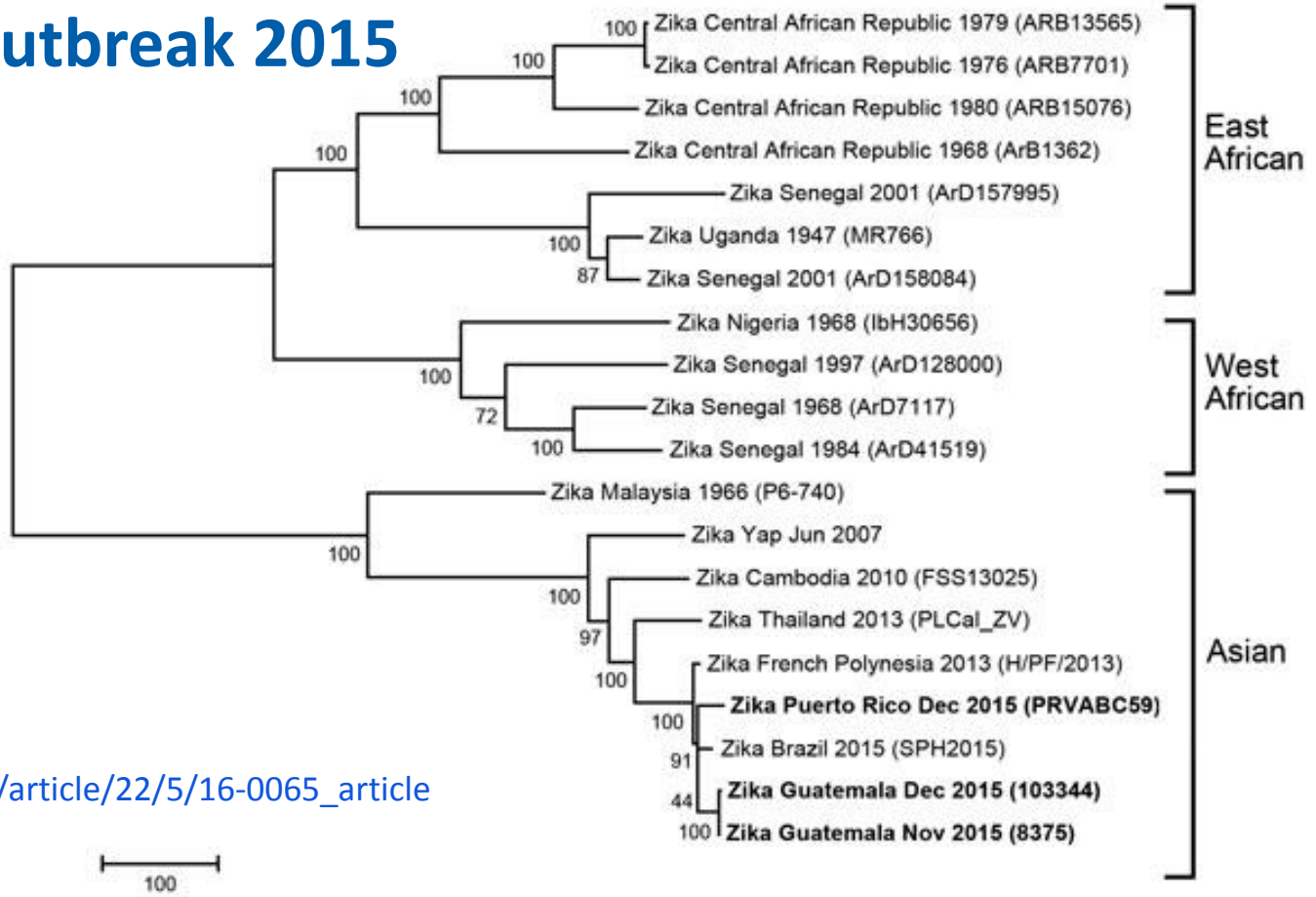
- Discovered in the Zika forest, Uganda 1947
- Flavivirus, single-stranded RNA
- Mosquito-borne (*Aedes aegypti* and *Aedes albopictus*)
- Symptoms may include fever, rash, conjunctivitis, and myalgia
- Most infections are asymptomatic
- Virus is detectable in serum, urine, saliva, and semen
- Recent outbreaks include Yap Island, Federated States of Micronesia 2007, and French Polynesia 2013-2014



Zika Virus Outbreak 2015: Western Hemisphere

- In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infection in Brazil
- Reports of Guillain-Barré syndrome and pregnant women giving birth to babies with birth defects and poor pregnancy outcomes

Zika Virus Outbreak 2015



Lanciotti et al., EID 2016:
http://wwwnc.cdc.gov/eid/article/22/5/16-0065_article

Zika Virus Epidemiology Update

Zika virus disease cases reported to ArboNET, 2015-2016 as of June 12, 2016

- **US**
  - 691 travel associated cases
    - 206 pregnant women
    - 2 cases of GBS
    - 11 cases sexual transmission
- **US Territories**
  - 1,305 cases (~99% local transmission)
    - 166 pregnant women
    - 7 cases of GBS

Zika Diagnostics

CDC Trioplex rRT-PCR Assay

- Emergency Use Authorization issued on March 17, 2016
- Detects Zika, dengue, and chikungunya in serum and cerebrospinal fluid
- Detects Zika in urine and amniotic fluid
- Testing is limited to qualified laboratories designated by CDC

Trioplex rRT-PCR Assay: Amendments to EUA

- External lysis protocol added (May 31, 2016)
- Increase sample input volume to increase sensitivity – data collection ongoing
- Include additional automated extraction platforms to increase throughput – plan being finalized with FDA

Focus Diagnostics Zika Virus RNA Qualitative Real-Time RT-PCR

- Emergency Use Authorization issued on April 28, 2016
- Testing is limited to Quest Diagnostics laboratories
- Acceptable specimen: Serum
- Detects Zika only

Altona RealStar® Zika Virus RT-PCR

- Emergency Use Authorization issued on May 13, 2016
- Testing is limited to CLIA labs certified to perform high complexity tests
- Acceptable specimens: Serum and urine
- Detects Zika virus only

CDC Zika MAC-ELISA

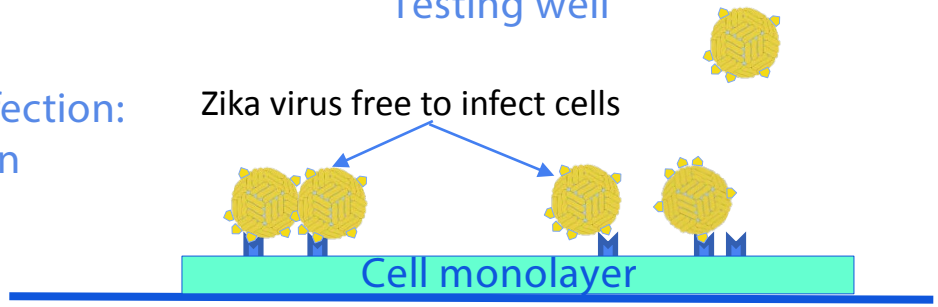
- Emergency Use Authorization issued on February 26, 2016
- Testing is limited to qualified laboratories designated by CDC
- Presumptive positive, equivocal or repeated inconclusive results require confirmatory testing
- Acceptable specimens: Serum and cerebrospinal fluid

Confirmatory Antibody Testing: Plaque-Reduction Neutralization Test (PRNT)

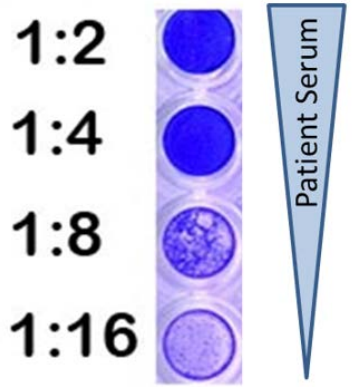
- **Anti-Zika antibodies present in a specimen will neutralize virus**

Testing well

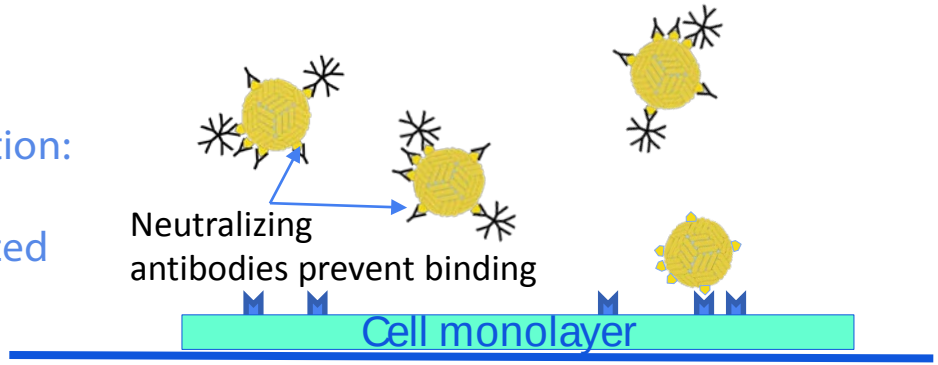
Patient without Zika infection:
Plaque formation



Example results using infected patient's serum



Patient with Zika infection:
Plaque reduction
since virus is neutralized



CDC Zika MAC-ELISA: Amendments to EUA

- Integrate Zika virus-like particles (VLPs) into the Zika MAC-ELISA assay to speed manufacturing of antigen – data under review by FDA
  - Can be easily grown in large amounts and are non-infectious
- Add automated robotic system to the Zika MAC-ELISA – initial data collected

Laboratory Response Network Testing Capacity in the US

- **CDC Trioplex rRT-PCR**
  - 102 labs have received reagents (all states plus DC and PR)
  - 74 have completed verification panel (39 states plus DC and PR)
- **CDC Zika MAC-ELISA**
  - 52 labs have received reagents – 42 states, DC, and PR
  - 38 laboratories have completed verification panel (32 states plus DC)

Surge Capacity for Zika Diagnostic Testing in the US

- Meeting with states with high risk for local transmission and developing capacity to meet potential testing demand
- Encouraging all labs to consider maximum capacity even if the state is not considered to be high risk
- Identifying challenges with shipping samples across state lines and reporting results
- Awarding $40 million to ELC grantees in August for Zika virus surveillance, laboratory diagnostic, and communication activities

Diagnostic Testing Algorithms

Collection of Urine from Symptomatic Patients

- Urine samples <14 days should be collected and tested by rRT-PCR along with patient-matched serum
- MMWR describing detection of Zika virus RNA in urine 5/10/2016
- CDC Health Alert Network (HAN) Health Advisory Regarding Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection on 5/25/2016

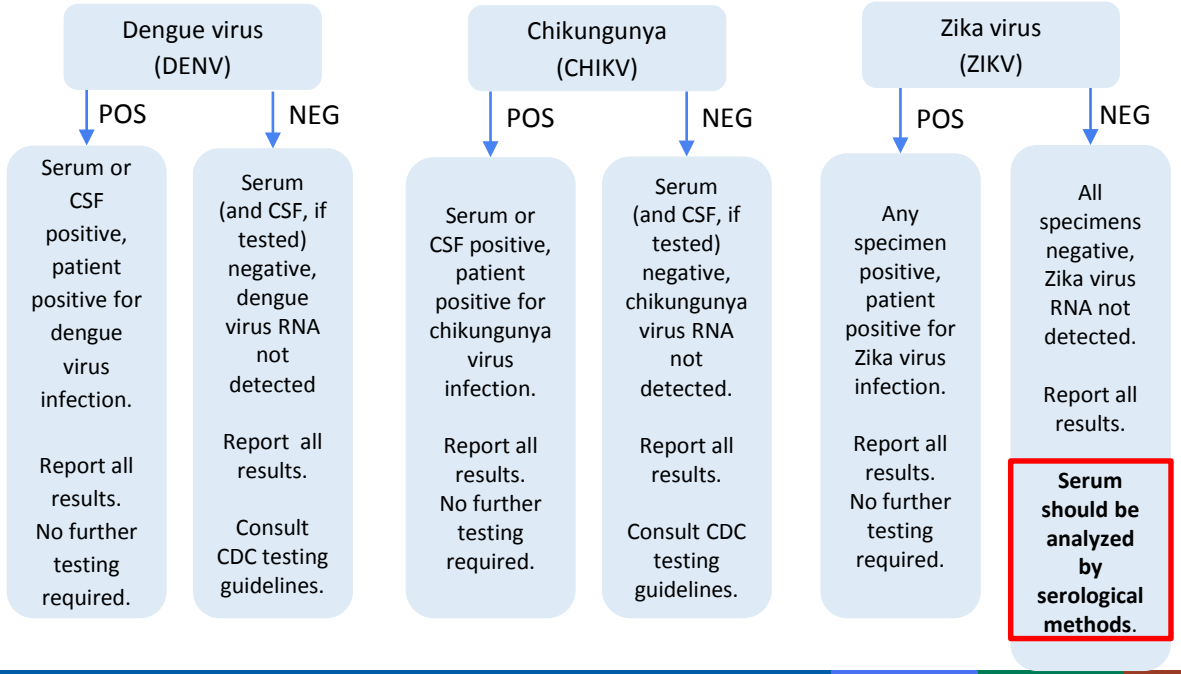
Algorithm for Testing Symptomatic Patients:

Serum collected < 7 days and urine collected < 14 days after symptom onset

(CSF or amniotic fluid for some tests)

Test all specimens by **Trioplex rRT-PCR**

Note: Urine and amniotic fluid testing are authorized only for ZIKV.



Algorithm for Testing Symptomatic Patients:

Serum and/or CSF collected >7 days after symptom onset

Serological testing

Serum and/or CSF specimen should be tested by:

- Zika MAC-ELISA
- a dengue IgM assay
- a chikungunya IgM assay

POS

Any test **presumptive positive, equivocal or inconclusive.**

Report results.

Forward for confirmation by PRNT

NEG

All tests **negative**, no evidence of recent virus infection.

Report results.

No further testing of specimen required.

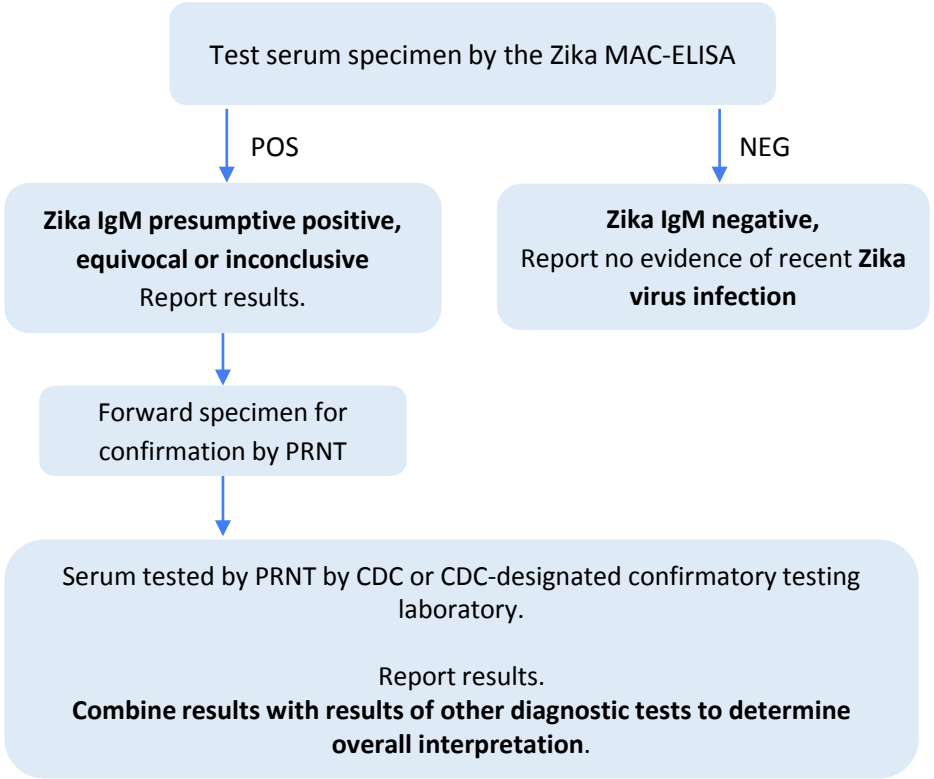
Serum tested by PRNT by CDC or CDC-designated confirmatory testing laboratory.

Report results.

Combine results with results of other diagnostic tests to determine overall interpretation.

Algorithm for Testing Asymptomatic Pregnant Women:

Women residing in an active Zika transmission area or 2-12 weeks after travel to an active Zika virus transmission area or sexual contact with a person confirmed to have Zika virus infection.



Reporting

- **Zika virus disease is a nationally notifiable condition**
  - ArboNET – National surveillance system for arboviral diseases

- **U.S. Pregnancy Registries**
  - ZAPSS – Zika Active Pregnancy Surveillance System (Puerto Rico)
  - USZPR – Zika Pregnancy Registry (US patients)

Test Interpretation Guidance

Interim Guidance: Interpretation of Zika Virus Antibody Test Results

- 4-fold difference in PRNT titer may not differentiate between anti-Zika virus antibodies and cross-reacting antibodies in all persons who have been previously infected with or vaccinated against a related flavivirus
- PRNT result interpretations have been updated to reduce the possibility of missing the diagnosis of either Zika or dengue virus infection
- Pregnant women with a Zika or flavivirus infection interpretation should be evaluated and managed for adverse pregnancy outcomes and be reported to the appropriate registry.

Interim Guidance: Interpretation of Zika Virus Antibody 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.

Interim Guidance: Interpretation of Zika Virus Antibody Test Results

- A PRNT using a 90% cutoff value with a titer ≥10 against Zika virus, together with negative PRNTs (i.e., <10) against other flaviviruses is confirmatory for infection with Zika virus
- A PRNT titer ≥10 for both Zika and dengue virus (or another flavivirus) provides evidence of an infection with a flavivirus but precludes identification of the specific infecting virus
- 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

Questions?

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov