AAP ZIKA ECHO
(EXTENSION FOR COMMUNITY HEALTHCARE OUTCOMES)
HOUSEKEEPING ITEMS

• For educational and quality improvement purposes, this ECHO session will be recorded.
• Project ECHO® collects participation data for each ECHO session. This data allows Project ECHO to measure, analyze, and report on the ECHO movement’s reach. Data is used in reports, on maps and visualizations, for research, for communications and surveys, for data quality assurance activities, and for decision-making related to new initiatives.
• To protect patient privacy, please do not provide any (PHI) protected health information.
• Please mute your microphone when not speaking. If you have video capability, please enable it.
• There is a chat function in Zoom that may be used to send messages to the group. For IT help, please chat to the AAP Admin and we will assist you.
ACKNOWLEDGMENTS

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Diagnostics and Testing for Zika Virus Infection

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DISCLOSURES

- I have no financial disclosures.
Laboratory Testing to Detect Zika Infection According to the Body’s Response

Symptom Onset

Symptoms Resolve

Viremia

IgM Antibodies

RT-PCR

IgM ELISA

Source: Courtesy - Emilio Dirlikov, PhD, CDC Dengue Branch
PLAQUE-REDUCTION NEUTRALIZATION TEST (PRNT)

- Measures neutralizing antibodies
- Determines level of protective antibodies towards flaviviruses
- Still may be inconclusive
- May help determine a false positive IgM
TESTING FOR PREGNANT WOMEN

- Always ask about travel history and symptoms
  - Symptoms: fever, rash, conjunctivitis, joint pain, headache, muscle pain

- Possible exposure to Zika virus that might warrant testing includes:
  - Recent travel to or residence in an area with risk of Zika (during pregnancy or the periconceptional period [the 6 weeks before last menstrual period or 8 weeks before conception]), or
  - Sex (vaginal, anal, or oral sex) or sharing sex toys without a condom during pregnancy with a person who traveled to or lives in an area with risk of Zika

# Pregnant Women: When to Test

<table>
<thead>
<tr>
<th>If patient...</th>
<th>Testing recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was exposed to Zika <strong>AND</strong> has symptoms of Zika virus infection or a history or symptoms at any time during her pregnancy</td>
<td>Concurrent RNA nucleic acid test (NAT) testing and Zika virus IgM testing ASAP or through 12 weeks after symptom onset.</td>
</tr>
<tr>
<td>Lives in or frequently travels to an area with risk of Zika <strong>but does not have symptoms</strong> of Zika virus infection.</td>
<td>Offer RNA NAT testing 3x during pregnancy.</td>
</tr>
<tr>
<td>Traveled to or had sex without a condom with a partner who lived in or traveled to an area with risk of Zika <strong>but does not have symptoms</strong> of Zika virus infection</td>
<td>Testing not routinely recommended. Consider testing using a shared decision-making model and the jurisdiction’s recommendations.</td>
</tr>
<tr>
<td>Was exposed to Zika <strong>AND</strong> had birth defects potentially associated with Zika detected on a prenatal ultrasound</td>
<td>Concurrent RNA NAT testing and Zika virus IgM testing. If amniocentesis is being done for clinical care, healthcare providers should also test the amniotic fluid for Zika RNA NAT. Consider testing of placental and fetal tissues if results of maternal Zika virus testing are not definitive.</td>
</tr>
</tbody>
</table>

TESTING RECOMMENDATIONS FOR CONGENITAL ZIKA VIRUS INFECTION

• Testing is recommended for:
  – Infants with clinical findings consistent with CZS and
  – Infants without clinical findings consistent with CZS who were born to mothers with lab evidence of possible Zika virus exposure during pregnancy

• Concurrent Zika virus RNA nucleic acid testing (NAT) of serum and urine and Zika virus IgM testing of serum should be performed within a few days after birth, if possible
# Interpreting Test Results for Congenital Zika Virus Infection

## Infant test result (serum, urine or cerebrospinal fluid)

<table>
<thead>
<tr>
<th>NAT</th>
<th>IgM</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Any result</td>
<td>Confirmed congenital Zika virus infection(1)</td>
</tr>
<tr>
<td>Negative</td>
<td>Nonnegative*</td>
<td>Probable congenital Zika virus infection(2)(4)</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Congenital Zika virus infection unlikely(3)(4)</td>
</tr>
</tbody>
</table>

*Nonnegative serology terminology varies by assay and might include “positive,” “equivocal,” “presumptive positive,” or “possible positive”

- (1) Distinguishing between congenital and postnatal infection is difficult in infants who live in areas with ongoing Zika virus transmission and who are not tested soon after birth. If timing of infection cannot be determined, evaluate infants as if they have congenital Zika virus infection.
- (2) If Zika virus plaque reduction neutralization test is negative, this suggests infant’s IgM test is a false positive.
- (3) Congenital Zika virus infection is unlikely if specimens are collected within first few days after birth and clinical evaluation is normal, but providers should remain alert for any new findings.
- (4) Lab results should be interpreted in context of timing of infection during pregnancy, maternal serology results, clinical findings consistent with CZS, and any confirmatory testing with plaque reduction neutralization testing.
Testing Recommendations for Postnatal Zika Virus Infection

- Guidance for testing and clinical management of infants and children with postnatal Zika virus infection is in line with recommendations for adults
  - Zika virus PCR and serologic testing is recommended during the first 2 weeks after symptom onset to diagnose postnatal Zika virus disease.
  - Serologic testing is recommended 2-12 weeks after symptom onset
## Time in Days Between Symptom Onset and Loss of Zika RNA Detection — Preliminary Report

<table>
<thead>
<tr>
<th>Body Fluid</th>
<th>Median (95% CI)</th>
<th>95th Percentile (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>14 (11–17)</td>
<td>54 (43–64)</td>
</tr>
<tr>
<td>Urine</td>
<td>8 (6–10)</td>
<td>39 (31–47)</td>
</tr>
<tr>
<td>Semen</td>
<td>34 (28–41)</td>
<td>81 (64–98)</td>
</tr>
</tbody>
</table>

CURRENT FDA EMERGENCY USE AUTHORIZATION

APPROVED DIAGNOSTIC TESTS FOR ZIKA VIRUS INFECTION

https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika
Molecular Tests for Zika Virus Infection

Trioplex Real-time (RT-PCR) Assay
RNA NAT (nucleic acid testing) tests for Zika virus, dengue virus and chikungunya virus RNA in serum, urine, amniotic fluid, as well as whole blood and cerebrospinal fluid (CSF).

- Serum/Urine - ≤14 days post onset symptoms

LIMITATIONS – MOLECULAR TESTS

• Virus is only detectable in acute phase of symptomatic patients
• Unknown how long virus is detectable in asymptomatic patients
• Risk of false negatives
SEROLOGIC TEST FOR ZIKA VIRUS

Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA)

Qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid

- Turns positive ~4 days and declines after 12 weeks

LIMITATIONS – SEROLOGIC TESTS

Results may be hard to interpret due to

- Cross-reaction with other flaviviruses
- Possible nonspecific reactivity
- Cannot distinguish between past and recent infections

Therefore....

Presumed *positive, equivocal, or inconclusive* tests must be forwarded (to the CDC or PRNT Reference Center) for confirmation by plaque-reduction neutralization testing (PRNT).
PLAQUE-REDUCTION NEUTRALIZATION TEST (PRNT)

• Measures neutralizing antibodies
• Determines level of protective antibodies towards flaviviruses
• Still may be inconclusive
• May help determine a false positive IgM
## PRNT Interpretation (Simplified)

<table>
<thead>
<tr>
<th>Zika PRNT</th>
<th>Dengue PRNT</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥10</td>
<td>&lt;10</td>
<td>Recent Zika virus infection</td>
</tr>
<tr>
<td>&lt;10</td>
<td>≥10</td>
<td>Recent dengue virus infection</td>
</tr>
<tr>
<td>≥10</td>
<td>≥10</td>
<td>Recent flavivirus infection, virus cannot be identified</td>
</tr>
<tr>
<td>&lt;10</td>
<td>&lt;10</td>
<td>No evidence of either; likely false positive IgM</td>
</tr>
</tbody>
</table>
TESTING COMPLICATIONS

• Imperfection with tests as they currently exist
• Effect of lab calibration on results
• False positives/negatives increase as infection prevalence goes down
• “Original Antigenic Sin” phenomena with previous dengue infection
• Cross reactivity with other flaviviruses
• Delays in receiving the results
CDC Websites to Reference

Collecting and Submitting Specimens at Time of Birth for Zika Virus Testing

Diagnostic Tests for Zika Virus

Understanding Zika Virus Test Results