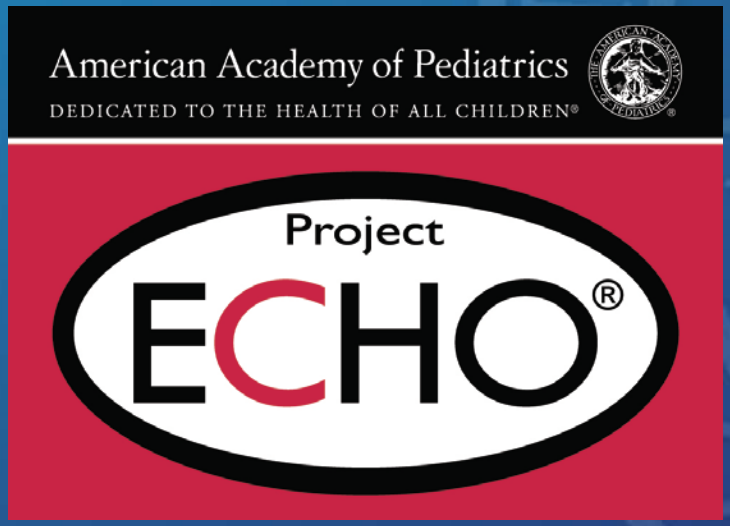
AAP ZIKA ECHO

(EXTENSION FOR COMMUNITY HEALTHCARE OUTCOMES)



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- 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.
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- 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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Presented May 7 & 15, 2018

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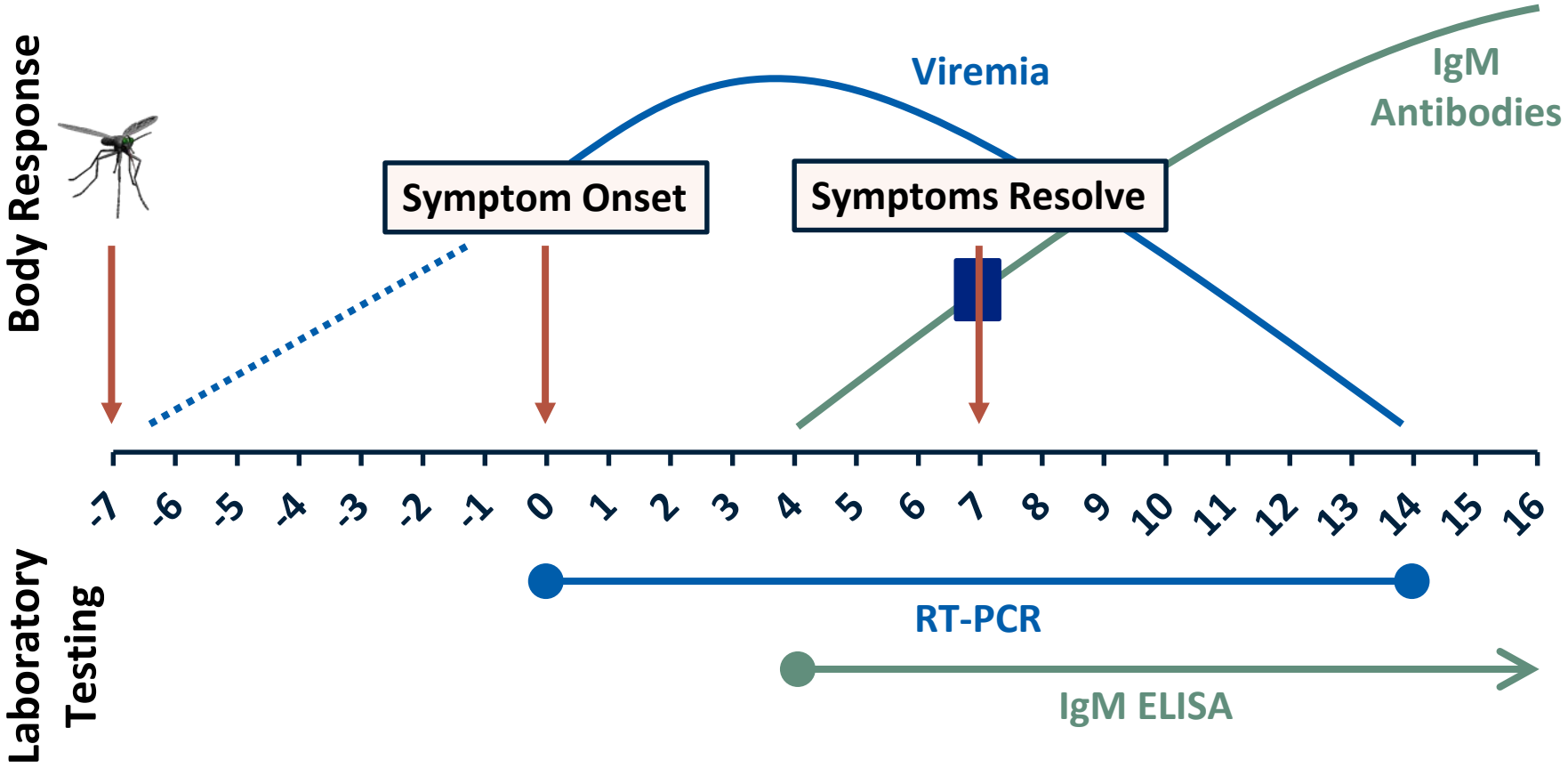
DISCLOSURES

- I have no financial disclosures.

Laboratory Testing to Detect Zika Infection According to the Body's Response



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

https://www.cdc.gov/pregnancy/zika/testing-follow-up/testing-and-diagnosis.html

CDC's Response to **Zika**

UPDATED INTERIM PREGNANCY GUIDANCE: SYMPTOMATIC PREGNANT WOMEN WITH POSSIBLE ZIKA VIRUS EXPOSURE

Accessible Version: https://www.cdc.gov/zika/laboratories/lab-guidance.html

Testing Recommendations and Interpretation of Results for Healthcare Providers

ASK PREGNANT WOMEN ABOUT

Travel to or residence in any areas with risk for Zika virus transmission *before* and *during* the current pregnancy[1,2] • **Possible sexual exposure** *before* and *during* the current pregnancy
A diagnosis of laboratory-confirmed Zika virus infection before current pregnancy[3] • Symptoms of Zika virus disease during current pregnancy (e.g., fever, rash, conjunctivitis, arthralgia)
If no symptoms reported, refer to asymptomatic algorithm.

Before testing, discuss testing limitations and potential risks for misinterpretations of test results.

WHOM to test?

Pregnant women reporting possible exposure during current pregnancy and symptoms of Zika virus disease[4]

WHEN to test?

Test as soon as possible; through **12** weeks after symptom onset

WHICH tests?

Zika virus NAT (serum and urine) *AND* **Zika virus IgM serology** (serum)[5,6]

RESULTS and ADDITIONAL tests

- **Positive Zika virus NAT**[7] (If Zika IgM negative, see footnote.[8])
- **Negative Zika virus NAT** *AND* **non-negative Zika virus IgM**[9]
  - Plaque reduction neutralization test (**PRNT**)[10]
    - **Zika virus PRNT ≥10** *AND* **dengue virus PRNT <10**
    - **Zika virus PRNT ≥10** *AND* **dengue virus PRNT ≥10**
    - **Zika virus PRNT <10**
- **Negative Zika virus NAT** *AND* **negative Zika virus IgM**

INTERPRETATION

- ***ACUTE ZIKA VIRUS INFECTION***
- ***ZIKA VIRUS INFECTION, TIMING OF INFECTION CANNOT BE DETERMINED***
  *For pregnant women without Zika virus exposure before the current pregnancy, **a positive IgM result represents recent Zika virus infection.***
- ***FLAVIVIRUS INFECTION, SPECIFIC VIRUS AND TIMING OF INFECTON CANNOT BE DETERMINED***
  *For pregnant women without Zika virus exposure before the current pregnancy, **a positive IgM result represents recent unspecified flavivirus infection.***
- ***NO EVIDENCE OF ZIKA VIRUS INFECTION***

Abbreviations: IgM = immunoglobulin M; NAT = nucleic acid test; PRNT = plaque reduction neutralization test

1 Ask about type and duration of Zika virus exposure before and during the current pregnancy. Exposure before the current pregnancy might limit interpretation of Zika virus IgM antibody results; pretest counseling can help inform testing decisions. Some patients may choose not to receive Zika virus IgM testing.

2 Possible Zika virus exposure includes travel to or residence in an area with risk for Zika virus transmission (https://wwwnc.cdc.gov/travel/page/zika-travel-information) during pregnancy or the periconceptional period (8 weeks before conception [6 weeks before the last menstrual period]), or sex without a condom during pregnancy or the periconceptional period, with a partner who traveled to, or resides in an area with risk for Zika virus transmission.

3 Zika virus testing is not routinely recommended for pregnant women with a previous diagnosis of laboratory-confirmed Zika virus infection by either NAT or serology (positive/equivocal Zika virus or dengue virus IgM and Zika virus PRNT ≥10 and dengue virus PRNT <10 results).

4 This algorithm also applies to pregnant women with possible Zika virus exposure who have a fetus with prenatal ultrasound findings consistent with congenital Zika syndrome.

5 The duration of detectable ZIKA virus in pregnant women following infection is not known. Preliminary data suggest NAT may remain positive for several weeks after symptom onset in some pregnant women. Zika virus IgM antibodies are most likely to be detected within 12 weeks after infection however IgM antibodies might be detected for months after infection, limiting the ability to determine whether infection occurred before or during the current pregnancy.

6 Dengue virus IgM antibody testing is recommended for symptomatic pregnant women. For laboratory interpretation in the presence of dengue virus IgM results, refer to https://www.cdc.gov/dengue/clinicallab/laboratory.html

7 Despite the high specificity of NAT, false positive NAT results have been reported. **If both serum and urine specimens are NAT-positive, regardless of IgM antibody results, results should be interpreted as evidence of acute Zika virus infection. If either serum or urine specimen is NAT positive in conjunction with a positive Zika virus IgM (see Table 1), results should be interpreted as evidence of acute Zika virus infection.**

8 If NAT is only positive on serum or urine and IgM antibody testing is negative, repeat testing on the original NAT positive specimen. **If repeat NAT is positive, results should be interpreted as evidence of acute Zika virus infection.** If repeat NAT testing is negative, results are indeterminate and healthcare providers should repeat Zika virus IgM antibody testing on a serum specimen collected ≥ 2 weeks after symptom onset. **If subsequent IgM antibody test is positive, interpret as evidence of acute Zika virus infection** but if negative, interpret as no evidence of Zika virus infection.

9 Non-negative results include positive, equivocal, presumptive positive, or possible positive. These are examples of assay interpretations that might accompany test results; non-negative serology terminology varies by assay. For explanation of a specific interpretation, refer to the instructions for use for the specific assay performed. Information on each assay can be found at https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika, under the "Labeling" for the specific assay.

10 Currently, PRNT confirmation is not routinely recommended for individuals living in Puerto Rico. For laboratory interpretation in the absence of PRNT testing, refer to Table 1.

Note: For the purposes of this guidance, recent possible Zika virus exposure or Zika virus/flavivirus infection is defined as a possible exposure or infection during the current pregnancy or periconceptional period.

https://www.cdc.gov/mmwr/volumes/66/wr/mm6629e1.htm?s_cid=mm6629e1_w

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CS267383-A September 15, 2017

Pregnant Women: When to Test

If patient…	Testing recommendation
Was exposed to Zika **AND** has symptoms of Zika virus infection or a history or symptoms at any time during her pregnancy	Concurrent RNA nucleic acid test (NAT) testing and Zika virus IgM testing ASAP or through 12 weeks after symptom onset.
Lives in or frequently travels to an area with risk of Zika **but does not have symptoms** of Zika virus infection.	Offer RNA NAT testing 3x during pregnancy.
Traveled to or had sex without a condom with a partner who lived in or traveled to an area with risk of Zika **but does not have symptoms** of Zika virus infection	Testing not routinely recommended. Consider testing using a shared decision-making model and the jurisdiction's recommendations.
Was exposed to Zika **AND** had birth defects potentially associated with Zika detected on a prenatal ultrasound	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.

https://www.cdc.gov/pregnancy/zika/testing-follow-up/testing-and-diagnosis.html

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)

NAT	IgM	Interpretation
Positive	Any result	Confirmed congenital Zika virus infection(1)
Negative	Nonnegative*	Probable congenital Zika virus infection(2)(4)
Negative	Negative	Congenital Zika virus infection unlikely(3)(4)

*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

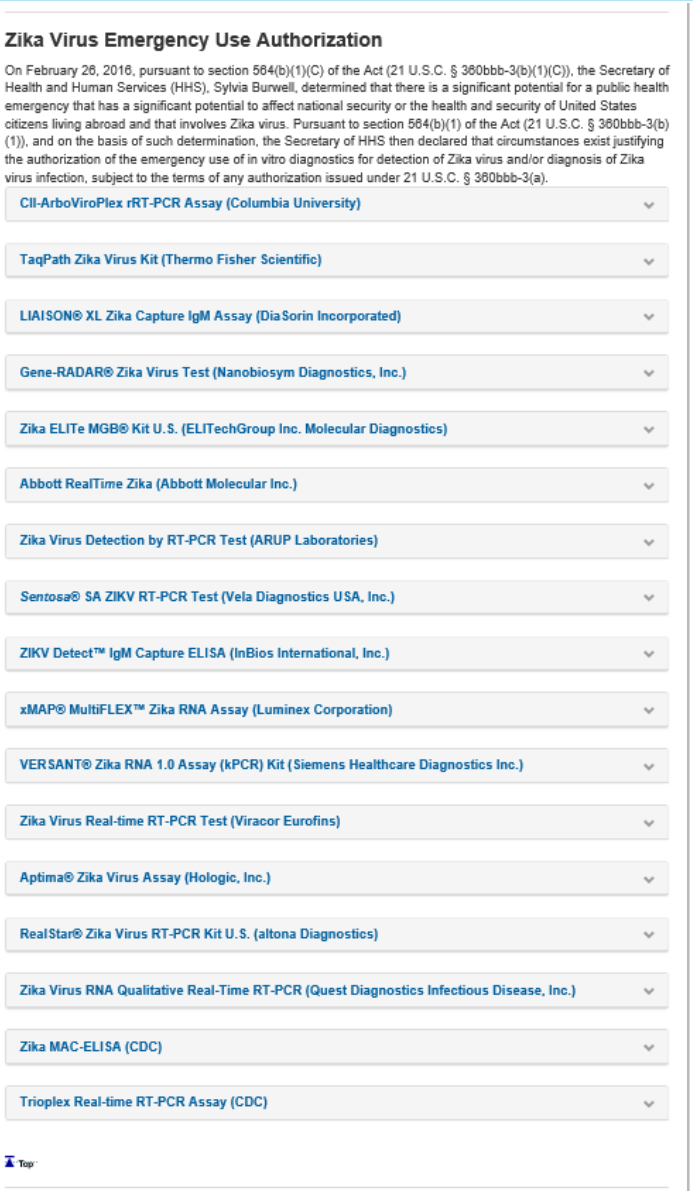
- Guidance for testing and clinical management of infants and children with postnatal Zika virus infection is in line with recommendations for adults
  - Zika virus PCR and serologic testing is recommended during the first 2 weeks after symptom onset to diagnose postnatal Zika virus disease.
  - Serologic testing is recommended 2-12 weeks after symptom onset

Time in Days Between Symptom Onset and Loss of Zika RNA Detection — Preliminary Report

	Median (95% CI)	95th Percentile (95% CI)
Serum	14 (11–17)	54 (43–64)
Urine	8 (6–10)	39 (31–47)
Semen	34 (28–41)	81 (64–98)

Source: Paz-Bailey G, et al. Persistence of Zika Virus in Body Fluids — Preliminary Report, May 15, 2015–December 15, 2016. NEJM 2017

Current FDA Emergency Use Authorization Approved Diagnostic Tests for Zika Virus Infection



Zika Virus Emergency Use Authorization

On February 26, 2016, pursuant to section 564(b)(1)(C) of the Act (21 U.S.C. § 360bbb-3(b)(1)(C)), the Secretary of Health and Human Services (HHS), Sylvia Burwell, determined that there is a significant potential for a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad and that involves Zika virus. Pursuant to section 564(b)(1) of the Act (21 U.S.C. § 360bbb-3(b)(1)), and on the basis of such determination, the Secretary of HHS then declared that circumstances exist justifying the authorization of the emergency use of in vitro diagnostics for detection of Zika virus and/or diagnosis of Zika virus infection, subject to the terms of any authorization issued under 21 U.S.C. § 360bbb-3(a).

- CII-ArboViroPlex rRT-PCR Assay (Columbia University)
- TaqPath Zika Virus Kit (Thermo Fisher Scientific)
- LIAISON® XL Zika Capture IgM Assay (DiaSorin Incorporated)
- Gene-RADAR® Zika Virus Test (Nanobiosym Diagnostics, Inc.)
- Zika ELITe MGB® Kit U.S. (ELITechGroup Inc. Molecular Diagnostics)
- Abbott RealTime Zika (Abbott Molecular Inc.)
- Zika Virus Detection by RT-PCR Test (ARUP Laboratories)
- *Sentosa*® SA ZIKV RT-PCR Test (Vela Diagnostics USA, Inc.)
- ZIKV Detect™ IgM Capture ELISA (InBios International, Inc.)
- xMAP® MultiFLEX™ Zika RNA Assay (Luminex Corporation)
- VERSANT® Zika RNA 1.0 Assay (kPCR) Kit (Siemens Healthcare Diagnostics Inc.)
- Zika Virus Real-time RT-PCR Test (Viracor Eurofins)
- Aptima® Zika Virus Assay (Hologic, Inc.)
- RealStar® Zika Virus RT-PCR Kit U.S. (altona Diagnostics)
- Zika Virus RNA Qualitative Real-Time RT-PCR (Quest Diagnostics Infectious Disease, Inc.)
- Zika MAC-ELISA (CDC)
- Trioplex Real-time RT-PCR Assay (CDC)

Top

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

Source: http://mediven.com.my/index.php/catalog/genoamp-trioplex-real-time-rt-pcr-zikadenchiku-kit/

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

https://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM488044.pdf

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)

Zika PRNT	Dengue PRNT	Interpretation
≥10	<10	Recent Zika virus infection
<10	≥10	Recent dengue virus infection
≥10	≥10	Recent flavivirus infection, virus cannot be identified
<10	<10	No evidence of either; likely false positive IgM

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

https://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html

Diagnostic Tests for Zika Virus

https://www.cdc.gov/zika/hc-providers/types-of-tests.html

Understanding Zika Virus Test Results

https://www.cdc.gov/zika/hc-providers/testresults.html