

Zika Test Information and Instructions

Please visit the NIDDL website to ensure you have the most up to date version of this information:
<http://www.med.navy.mil/sites/nmrc/NMRC/Pages/NIDDL.aspx>

- Real time Reverse Transcriptase (RT) Polymerase Chain Reaction (PCR): Used to detect the presence of the virus RNA during the acute viremia phase of infection (see figure below, Primary Infection Time Course). In blood samples this assay is very sensitive and specific within the first five days of infection. In semen and urine samples, Zika virus can be detected after the first five days of infection. Lab results are usually available four days after NIDDL has received the sample.
- The Enzyme-Linked Immunosorbent Assay (ELISA) Immunoglobulin M (IgM) Antibody Detection: The first immunoglobulin to appear in response to Zika virus is IgM. IgM is usually detected after the first five days of infection. IgM levels peak at two weeks and can become undetectable after 2-3 months. Lab results from the ELISA test are usually available after four days.

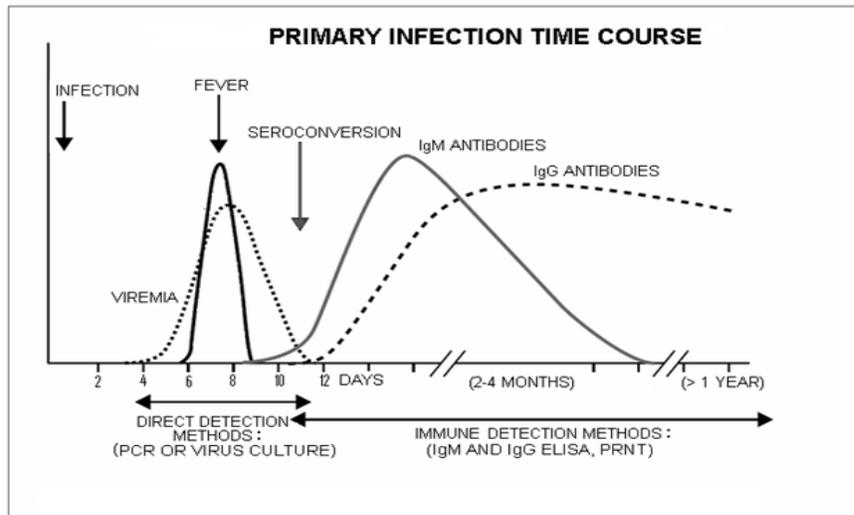
Please note: The ELISA test is currently unable to distinguish between various classes of Flavivirus and certain vaccinations, which may result in a false-positive for Zika virus. All positive ELISA results will be reported out as a presumptive positive for Zika, and they will need further confirmation via a plaque reduction neutralization test (PRNT).

- Plaque Reduction Neutralization Test (PRNT): This test is used to quantify the titer of neutralizing antibody for a virus. The PRNT assay is able to distinguish between false-positive and true-positive results for Zika virus. Lab results may take up to two weeks.

Tests Available for the Laboratory Diagnosis of Zika Infection at the NIDDL

Test	Diagnostic Window	Sample Required	Sample Storage Conditions	Turnaround Time
RT-PCR ^{1,2}	Acute phase day 0-5	500 µL serum or other sample type	Freeze (-80°C)	4 days
IgM ELISA ^{1,4}	Day 5 to ~Day 90 post-infection	250 µL serum	Frozen or refrigerated	4 days
PRNT ^{1,2,3,4}	Day 7 to several months	250 µL serum	Frozen or refrigerated	1-2 weeks

1. Serum obtained from non-hemolyzed whole blood collected in a SST or red-top (clot).
2. Other specimen types that can be run for Zika are urine, semen, saliva, or amniotic fluid.
3. All positive ELISA testing will be followed with a PRNT for confirmation.
4. Currently reported out as research use only as these tests have not yet been validated for clinical use.



Sample Collection & Storage Procedures

Collect patient blood samples in two separate **red gel separator** tubes (aka ‘tiger- topped’ tubes or SST).

1. Following collection, gently invert collection tubes five times.
2. Allow blood to clot for a minimum of 30 minutes in a vertical position.
3. Centrifuge at 1100 -1300G for 10 minutes.
4. If possible, aseptically pipette off serum into separate aliquots (one for each test procedure) and put into cryovials.
5. If ELISA only is desired, the serum sample should be stored in a refrigerator or on ice (2-8°C).
6. If RT-PCR is desired, it should be aliquoted and stored frozen (preferably at -80°C). No more than one freeze-thaw cycle is recommended, as additional cycles may result in RNA degradation and lower PCR efficacy.
7. Do not freeze blood samples that contain red blood cells. Serum must first be separated and then frozen. If you cannot separate the serum, then use EDTA and send on ice packs or cold.
8. For urine, semen, and saliva samples: Please freeze and ship samples frozen in collection device.

All samples should be submitted with the Zika Test Request Form which can be found at the address below:

<http://www.med.navy.mil/sites/nmrc/NMRC/Pages/NIDDL.aspx>

Point of Contact for any questions about testing or shipping:

LCDR Todd Myers, PhD, HCLD (ABB), MB (ASCP), USPHS, Clinical Laboratory Director

Todd.e.myers.mil@mail.mil or 301-319-3113

Susan Widjaja, Laboratory Manager

Susana.widjaja.ctr@mail.mil or 301-319-9507

Ship samples to:

Susan Widjaja, Room 3W20
 Naval Medical Research Center
 503 Robert Grant Avenue
 Silver Spring MD, 20910

Interim Zika Virus Testing Criteria*

Please check the indication for testing: If all responses are “No”, then testing is not indicated.

1. Symptomatic adult travelers:

YES NO Does the adult patient have acute onset of fever, maculopapular rash, arthralgia, **or** conjunctivitis, **AND** does the patient have a travel history within the past 14 days to a Zika transmission area? For the latest list of transmission areas please consult: <http://www.cdc.gov/zika/geo/active-countries.html>

2. Symptomatic pediatric travelers (<18 years old):

YES NO Does the pediatric patient have 1) acute onset of fever **AND** maculopapular rash, arthralgia, **or** conjunctivitis, **AND** 2) does the patient have a travel history within the past 14 days to a Zika transmission area? For the latest list of transmission areas please consult: <http://www.cdc.gov/zika/geo/active-countries.html>

3. Asymptomatic pregnant women:

YES NO Has the patient during any trimester:

1. Lived in or traveled to a Zika transmission area,

OR

2. Received blood products or organs that may have originated in a Zika transmission area,

OR

3. Had condomless sex (vaginal, anal, or fellatio) with a male partner who has either:

a. Been diagnosed with Zika,

OR

b. Traveled to an area of ongoing Zika virus transmission **AND** had symptoms of Zika virus disease during travel or within 14 days of return?

4. Symptomatic non-travelers who have condomless sex with men:

YES NO Does the patient have symptoms according to criteria in questions 1 or 2 above, and have they engaged in any condomless sex (vaginal, anal, or fellatio) with a male partner who has been diagnosed Zika and/or both traveled to an area of ongoing Zika virus transmission **AND** had symptoms of Zika virus disease during travel or within 14 days of return? **Note:** the CDC does not recommend testing asymptomatic men or asymptomatic non-pregnant women who have traveled to Zika affected areas.

5. Neonates:

YES NO Is the patient a neonate with microcephaly or intracranial calcifications whose mother traveled to or resided in an area with Zika virus transmission during pregnancy?

YES NO Is the patient a neonate born to a mother with positive or inconclusive test results for Zika virus infection?

YES NO Is the patient a neonate in the first 14 days of life with two or more of the following symptoms: fever, rash, conjunctivitis, or arthralgia **AND** meets one of the following criteria:

1. Whose mother traveled to or resided in an affected area within 2 weeks of delivery,

OR

2. Whose mother had condomless sex (vaginal, anal, or fellatio) with a male partner who has been diagnosed with Zika,

OR

3. Whose mother had condomless sex (vaginal, anal, or fellatio) with a male partner who has **both** traveled to an area of ongoing Zika virus transmission **and** had symptoms of Zika virus disease during travel or within 2 weeks of return? (The CDC notes: Arthralgia can be difficult to detect in infants and young children and can manifest as irritability, walking with a limp (for ambulatory children), difficulty moving or refusing to move an extremity, pain on palpation, or pain with active or passive movement of the affected joint.)

***Note: This is interim testing criteria until a similar CDC tool is created.**

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