



Test Information and Instructions for Chikungunya, Dengue and Zika Diagnostics

Please visit the NIDDL website to ensure you have the most up to date version of this information: <https://www.med.navy.mil/Naval-Medical-Research-Command/Fleet-Support/Naval-Infectious-Diseases-Diagnostic-Laboratory/>

- **Real-Time Reverse Transcriptase (RT) Polymerase Chain Reaction (PCR)/Molecular:** Used to detect the presence of the virus RNA during the acute viremia phase of infection (see figure: 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 chikungunya, dengue and 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.

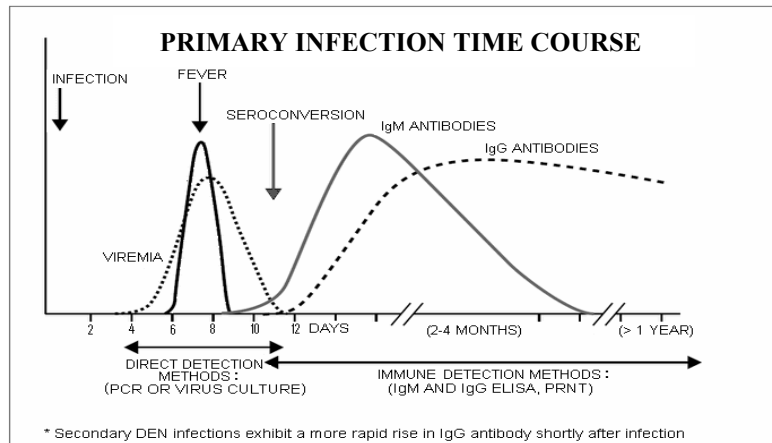
Please note: The ELISA test is currently unable to distinguish between various classes of Flavivirus, which may result in a false-positive. All positive ELISA results will be reported out as a presumptive positive, and they will need further confirmation via a plaque reduction neutralization test.

- **Plaque Reduction Neutralization Test (PRNT):** This test is more specific than ELISA and used to quantify the titer of neutralizing antibody for a virus. Lab results may take up to three weeks.

Tests Available for the Laboratory Diagnosis of Chikungunya, Dengue and Zika Infection at the NIDDL

Test	Diagnostic Window	Sample Required	Sample Storage Condition	Turnaround Time
RT-PCR/ Molecular ^{1,2}	Acute/ Less than 14 days after onset	1 mL serum or other sample type	Frozen (-80°C)	4 days
IgM ELISA ¹	Day 5 ~Day 90 post-infection	250 µL serum	Frozen or refrigerated	4 days
PRNT ^{1,2,3}	Day 7 to several months	500 µL serum	Frozen or refrigerated	1-3 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. Only Zika PRNT is available for order and is still being verified for clinical use, so results should be interpreted with caution and in context with clinical history and other test results.



Sample Collection and Storage Procedure

- Serum: Collect blood samples in two separate red gel separator tubes (aka 'tiger-topped' tubes or SST). Following collection, gently invert collection tubes five times. Allow blood to clot for a minimum of 30 minutes in a vertical position. Centrifuge at 1100 -1300xg for 10 minutes. If possible, aseptically pipette off serum into separate aliquots (one for each test procedure) and put into sterile screw capped vials secure with thermoplastic, self-sealing lab film.
 - ELISA samples: store in a refrigerator or frozen, ship with ice pack or dry ice.
 - RT-PCR samples: store frozen (preferably at -80°C) and ship with dry ice.No more than one freeze-thaw cycle is recommended, as additional cycles may result in RNA degradation and lower PCR efficacy.

Do not freeze blood samples that contain red blood cells. Serum must first be separated and then frozen.

- Urine and other samples: Please transfer sample to sterile screw capped tubes, secure with thermoplastic, self-sealing lab film. Store frozen and ship with dry ice.

Zika samples should be submitted with the **Zika Test Request Form** and **Interim Zika Virus Testing Criteria** which can be found at the address below: <https://www.med.navy.mil/Naval-Medical-Research-Command/Fleet-Support/Naval-Infectious-Diseases-Diagnostic-Laboratory/>

Point of Contact for any questions about testing or shipping:

Naval Infectious Diseases Diagnostic Laboratory (NIDDL)

Phone: 301-319-7150;

Email: usn.detrick.nmrc.list.didd-dsd-niddl@health.mil

Ship samples to:

Naval Infectious Diseases Diagnostic Laboratory (NIDDL)

Naval Medical Research Command

503 Robert Grant Avenue, Room 3N60

Silver Spring MD, 20910