Naval Infectious Diseases Diagnostic Laboratory
503 Robert Grant Avenue, Silver Spring, MD 20910

Test Request Form

Tests Requested (Check all that apply) see "Basic Guide" for instructions on sample collection/storage.

- ☐ Chikungunya and Dengue PCR (Red top or EDTA tube)
- ☐ Lyme ELISA (SST or red top tube)
- ☐ Rickettsia PCR (EDTA tube, biopsy)
- ☐ MERS-CoV PCR (Red top, stool, sputum, nasopharyngeal swab, lower respiratory aspirate/wash)
- ☐ H7N9 PCR (Sputum; Swab: nasal, throat or nasopharyngeal; Aspirate/wash: respiratory tract)
- ☐ FLU A/B PCR (Swab: nasal, throat or nasopharyngeal swab)

Type of Specimen(s) (Ensure patient ID is on label; no additional preservative)

- ☐ Whole blood/serum/plasma (No. of tubes_____) Please do not freeze whole blood.
- ☐ Biopsy (No. of tubes_____) (i.e. eschar, rash, etc.)
- ☐ Swab/aspirate/wash (Organ: _____, No. of tubes_____)

Patient Identification	Contact Information
Patient Stamp must include Full Name*, FMP*/ SSN*, DOB* Ship Date: _____ Sample Storage (circle) Frozen / Refrig Sample Shipping (circle) Dry Ice / Cold pack / Ambient Specimen Draw Date/Time*: _____ *Required Onset date: _____	Physician Name*_____ Clinic/Center* _____ Address* _____ _____ _____ Telephone Number_____ Fax Number _____ E-mail _____

Ship To:
Susana Widjaja
Room 3W20
Naval Infectious Diseases Diagnostic Laboratory
503 Robert Grant Avenue, Silver Spring MD, 20910

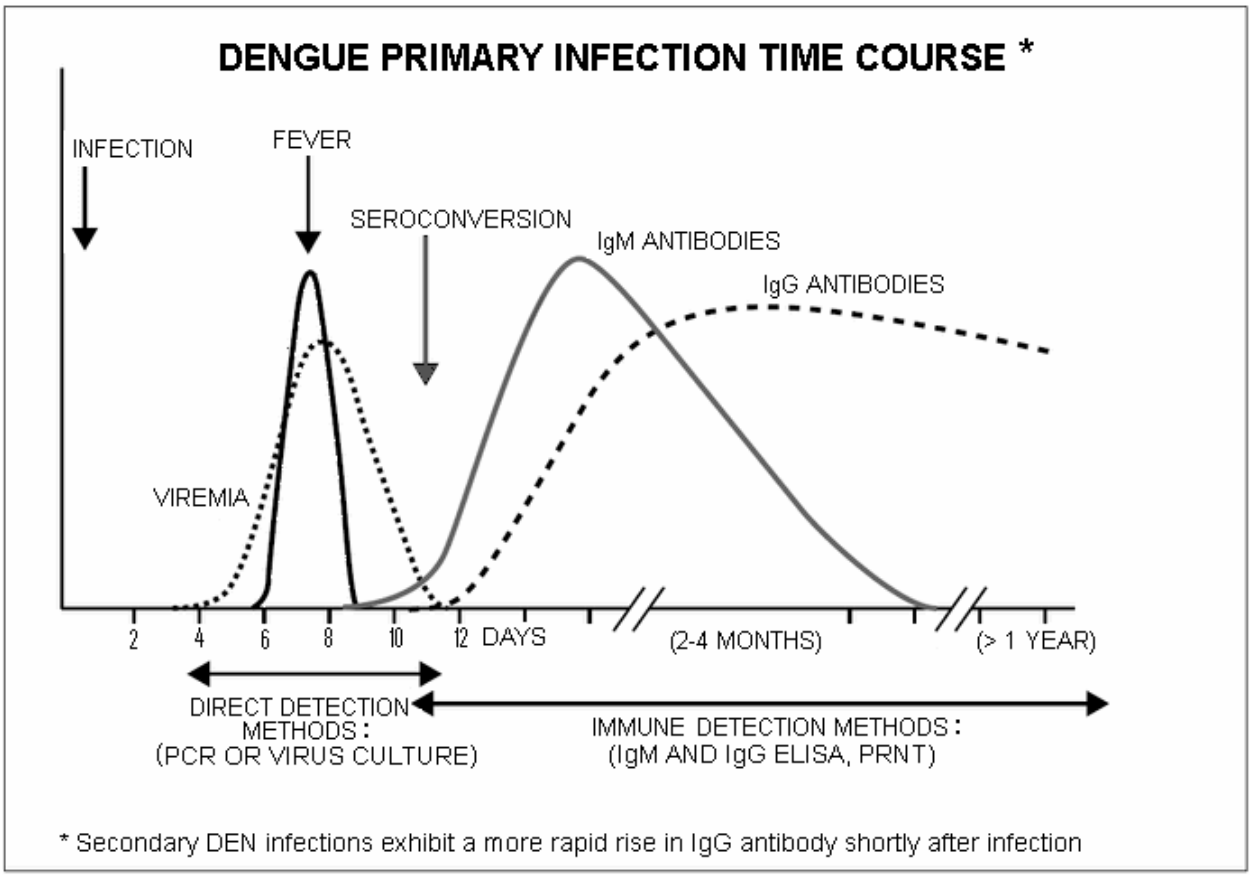
Processing Lab (For internal Use only)		
NIDDL No.	Date Received	Quantity/Type Received/Initials

Basics to Dengue and Chikungunya Diagnostics

Dengue

- Most readily available diagnostic tests: RT-PCR to detect the presence of dengue virus RNA. RT-PCR assays can provide a same-day laboratory diagnosis of dengue infection in primary and secondary patient samples and have a sensitivity similar to that of viral isolation in cell culture.

- ELISA IgM antibody detection. In primary infections, IgM to dengue virus is the first immunoglobulin to appear, by the end of the 3-5 day period of fever typical of dengue infection; levels peak at 2 weeks, and are undetectable by 2-3 mos. The kinetics is different in secondary infections (and more complicated).



Adapted from Robert Putnak Ph.D. Viral Diseases, WRAIR 2010.

Chikungunya

- Rapid diagnostic test: RT-PCR to detect the presence of Chikungunya virus RNA. Viral RNA can be detected between 4 to 12 days post infection.

- ELISA IgM antibody detection. IgM antibody can be detected 4-5 days after infection to several weeks to several months.

Tests available for the Laboratory Diagnosis of Dengue (DEN) virus and Chikungunya virus infection at the NIDDL

Test	Diagnostic Window	Sample Required[1]	Sample Storage Conditions	Turnaround Time
RT-PCR	Acute phase	500 µL serum	Freeze (-80°C)	4 days
IgM ELISA[2]	Day 4 to ~Day 90 post-infection	250 µL serum	Frozen or refrigerated	4 days

[1] Serum obtained from non-hemolyzed whole blood collected in a SST or red-top (clot) or gold top tube.

[2] May not be detectable in cases of secondary DEN infection.

Requirements and Procedures

Preferable: patient blood sample 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 -1300 G for 10 minutes.
4. If possible, aseptically pipette off serum into separate aliquots (one for each test procedure) and put into cryovials.
5. Store in refrigerator or on ice (2-8°C) if ELISA is desired.
6. If RT-PCR is desired the serum sample should be aliquoted and stored at -80°C. If frozen, no more than one freeze-thaw is recommended as additional freeze-thaws may result in RNA degradation and lower PCR efficacy.

Basics to Rickettsial pathogen Diagnostics

Rickettsia:

- Are obligate intracellular bacteria, thus, they don't "grow" on artificial media like agar plates. They have to be isolated from the blood/tissue specimens in lab animals, embryonated chicken eggs or tissue culture.
- Serology is used to confirm diagnosis, and is often retrospective
- Antibodies to specific rickettsial antigens are detected using indirect immunofluorescence and ELISA (doesn't discriminate between the spotted fever group (SFG) rickettsioses though)
- SFG= *R. rickettsii, conorii, slovaca, africae, etc*; typhus group is distinct (*R. typhi*)

Tests available for the detection of Rickettsial pathogens at the NIDDL

Serology IgM

Specimens: serum or plasma

Time to result: Four days after specimens received

Molecular (standard, quantitative real-time PCR)

Specimens:

Whole blood (6 ml)(prefer without heparin as anticoagulant, do not freeze)

Biopsy specimens from eschars or rash (former are preferable as rickettsiae are more abundant; both specimens are preferable to whole blood)

Time to result; two days after specimen receipt

Requirements and Procedures

Depending on patient's presentation, aim to submit both **whole blood (purple topped-tube, do not freeze)** and/or biopsy specimens from rash or eschar.

1. Label tube of blood as appropriate.
2. Obtain diagnostic biopsy by using a 2.5-6.0 mm skin punch and bisect.
3. Place specimen in a sterile piece of gauze (2 x 2 or 4 x 4) moistened with sterile non-bacteriostatic saline and place in sterile transport cup (urine cup or 50 cc test tube, etc). Keep refrigerated (4°C) or freeze (-80°C). Label appropriately

Point of Contact for any questions about testing:

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