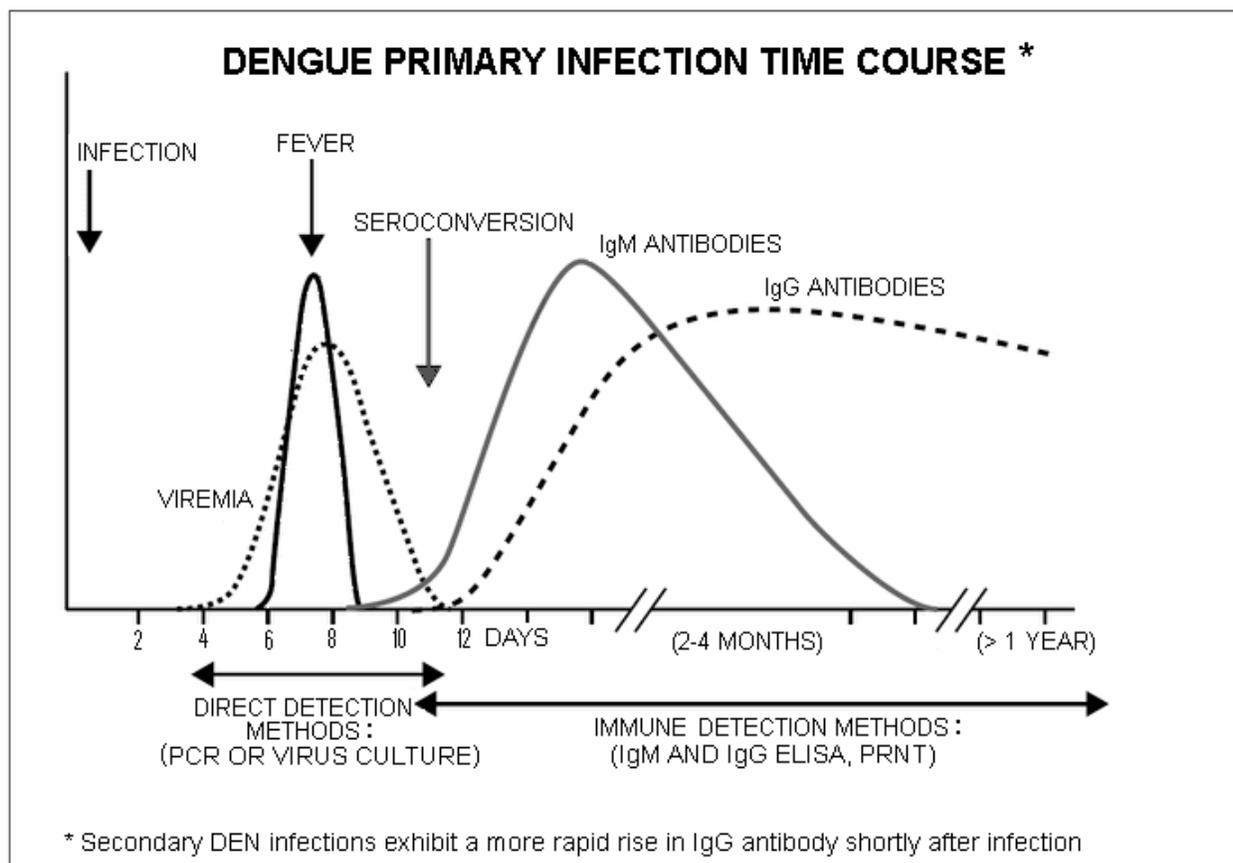




## Basics to Dengue and Chikungunya Diagnostics

### 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 <sup>1</sup>	Sample Storage Conditions	Turnaround Time
RT-PCR	Acute phase	500 µL serum	Freeze (-80°C)	4 days
IgM ELISA <sup>2</sup>	Day 4 to ~Day 90 post-infection	250 µL serum	Frozen or refrigerated	4 days

<sup>1</sup> Serum obtained from non-hemolyzed whole blood collected in a SST or red-top (clot) or gold top tube.

<sup>2</sup> 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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