Annexure-IV

Protocol for Sample Collection, Transportation and Testing

1. Overview of the Zika virus investigation/diagnosis process

Several methods can be used for diagnosis, such as viral nucleic acid detection, virus isolation and serological testing. Diagnosis by serology can be difficult as the virus can cross-react with other flaviviruses. Thus, viral nucleic acid detection remains the preferred method for diagnosis.

2. Clinical Specimens

i Type of sample : serum

ii Volume of sample: About 500 µl or available quantity of serum should be sent. Higher volume is preferred.

3. Storage of Specimen

i. Keep refrigerated (2-8 °C) if it is to be processed (or sent to a reference laboratory) within 48 hours.

ii. Keep frozen (-10 to -20 °C) if it is to be processed after the first 48 hours or within 7 days.

iii. Keep frozen (-70 °C) if it is to be processed after a week. The sample can be preserved for extended periods.

4. Guidelines for specimen Collection

- A BSL-2 containment level is required to handle suspected samples.
- Consider all specimens as potentially hazardous/ infectious.
- Handle all specimens with gloves in a secure manner.
- Place each specimen into a separate container labeled with the patient's name and identification number, the collection site, the date of collection and the time of the collection.
- Do not contaminate the outside of the specimen container.
- Do not handle laboratory requisition forms with gloves.

5. Information to be sent to the Apex laboratories

a) Every sample should be accompanied by appropriate data form

b) **NOTE:** Following details are must in the data form:

1. **Date of onset** of symptoms

2. **Date of specimen** collection
3. Any **pertinent travel history** (3 months prior to the date of symptom onset)
4. If female patient, details of LMP, pregnancy, if any

6. Packaging

a) Samples to be sent on ice. (+2 to +8 degree °C)
b) The original samples should be packed, labeled and marked, and documented as **Category B.**
c) Standard triple packing for Category B to be followed.
d) Sender should provide prior intimation about shipment of samples to RCVRDL.

Note: In case you have any logistic problem or suggestion, kindly contact NIV, Pune

7. Sample registration

The sample registration will be done in LIMS, which is the prerequisite for further processing. **Sample handling –**
The sample aliquoting and maintenance team will be headed by senior staff. Three aliquots of each sample will be prepared. In case of samples with less volume it will be processed for diagnostic PCR,with minimum of 200 ul back up for referecnce. In general proforma for aliquoting will be as follows

Sr. no.	Name	Sample sent from	NIV ID	Aliquot 1	Aliquot 2	Aliquot 3	ELISA 20ul	PCR 140ul	Isolation 200ul	Remark

After aliquoting the empty vials as well as gloves should be packed in biohazard bags, secured and loaded to autoclave for decontamination. The note of “Do not open” should be mentioned on the autoclave by aliquoting team.

8. Processing of sample:

The clinical samples which are negative for Dengue and Chikungunya should be screened for ZIKV diagnosis.

9. Diagnostic RT-PCR

Nucleic acid detection by reverse transcriptase-polymerase chain reaction targeting the non-structural protein 5 genomic region is the primary means of diagnosis. Standard RT-PCR and quantitative RT-PCR provide a rapid, specific and sensitive method for ZIKV early detection.
Viral RNA has been detected in serum up to day 10 after the onset of symptoms. ZIKV RNA also has been detected in urine or saliva samples. However, and since more studies

are needed, it is **recommended that the serum sample be taken during the first 5 days after the onset of symptoms.**

The RT-PCR test available with NIV is standardized from published primers (Reference: Balm MN, Lee CK, Lee HK, et al. A diagnostic polymerase Chain reaction assay for Zika virus. J Med Virol 2012; 84: 1501-5).

9. Detection of IgM antibodies to Zika virus by diagnostic ELISA.

Convalescent phase (> 5 days):
Serology by testing IgM antibodies in blood. This is not the main stay of diagnosis as cross reactivity with other flaviviruses is very high.
Plaque Reduction Neutralization Test (PRNT): this is a confirmatory diagnosis.

10. Documentation:

Readings and interpretations are to be maintained in a file with test number and date for easy reference.

11. Virus Isolation:

Viral isolation is not regarded as a diagnostic tool and is recommended only for supplemental research studies in public health surveillance.