

Algorithm for Zika virus diagnosis, National Institute of Virology, Pune

Introduction

Zika virus (ZIKV) is an emerging mosquito-borne pathogen first described in 1952(1), after being isolated from a sentinel rhesus macaque monkey in 1947 and a pool of *Aedes africanus* mosquitoes in 1948 from the Zika forest in Uganda. Since it was first reported, only a small number of cases had been described in Africa and Asia until 2007 when there was a large outbreak on Yap Island in the Federated States of Micronesia.(2,3) In October 2013, ZIKV was detected in French Polynesia affecting ~10% of the total population.(4) In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infections in Brazil. (CDC) Currently, outbreaks are occurring in many countries, including Columbia, Venezuela, Paraguay, EL Salvador etc. As of 28th January 2016, twenty three countries in the Americas have reported cases (WHO). Due to widespread international travel, there is risk of spread of outbreak across the world. Cases are being reported among travellers from other continents including Europe.

ZIKV is an approximately 11-kb single-stranded, positive sense ribonucleic acid (RNA) virus from the *Flaviviridae* family, most closely related to the Spondweni virus.(Thailand) Two major lineages, African and Asian, have been identified through phylogenetic analyses. (2, 6, 7) Transmission occurs via mosquito vectors from the *Aedes* genus of the Culicidae family, the same mosquito that transmits dengue, chikungunya and yellow fever. .Non vector transmission including potential sexual transmission and through monkey bite are also reported. Mother to child transmission during pregnancy or during delivery is also a potential route of transmission.

The incubation period of ZIKV disease is not clear, but is likely to be a few days. About 1 in 5 people infected with Zika are symptomatic. Symptoms typically begin 2 to 7 days after being bitten by an infected mosquito. The symptoms are similar to other arbovirus infections such as dengue, and include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are usually mild and last for 2-7 days (WHO). ZIKV infection in pregnant women has been reported to result in microcephaly, with Brazil reporting a surge in such cases.

Zika virus is diagnosed using PCR (polymerase chain reaction) and virus isolation from blood samples. Diagnosis by serology can be difficult as the virus can cross-react with other flaviviruses such as Dengue, West Nile and Yellow fever (WHO).

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Need of Diagnosis and Training

With increase in global travel, India is one of the countries to which ZIKV may spread. Preparedness for dealing with this agent is therefore vital. India harbours the vector mosquito, *Aedes aegyptii* and *Aedes albopictus* and once infection is transmitted, there is a possibility of establishment of disease. With several VRDLs established across the country, it would be feasible to train the staff and help to establish diagnostic facility for ZIKV, with NIV being the nodal apex centre. This would also help in early identification of suspected ZIKV infection and help to adopt suitable measures for prevention and control of ZIKV.

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.**

I. Clinical Specimens

- a. Type of sample : serum
- b. Volume of sample: About 500 µl or available quantity of serum should be sent. Higher volume is preferred and appreciated.

Guidelines for specimen Collection

1. A BSL-2 containment level is required to handle suspected samples.
2. Consider all specimens as POTENTIALLY HAZARDOUS / INFECTIOUS.
3. Handle all specimens with gloves in a secure manner.
4. 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.
5. Do not contaminate the outside of the specimen container.
6. Do not handle laboratory requisition forms with gloves.

II. Storage of Specimen

- Keep refrigerated (2-8 °C) if it is to be processed (or sent to a reference laboratory) within 48 hours.
- Keep frozen (-10 to -20 °C) if it is to be processed after the first 48 hours or within 7 days.
- Keep frozen (-70 °C) if it is to be processed after a week. The sample can be preserved for extended periods.

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Transport of Zika virus infected patient samples (*Annexure-I*)

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

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 RC-VRDL.

Note: In case you have any logistic problem or suggestion, kindly contact Apex laboratory.

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 reference. 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
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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.

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Processing of sample:

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

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).

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.

Documentation:

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

Virus Isolation:

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

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References

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Annexure I

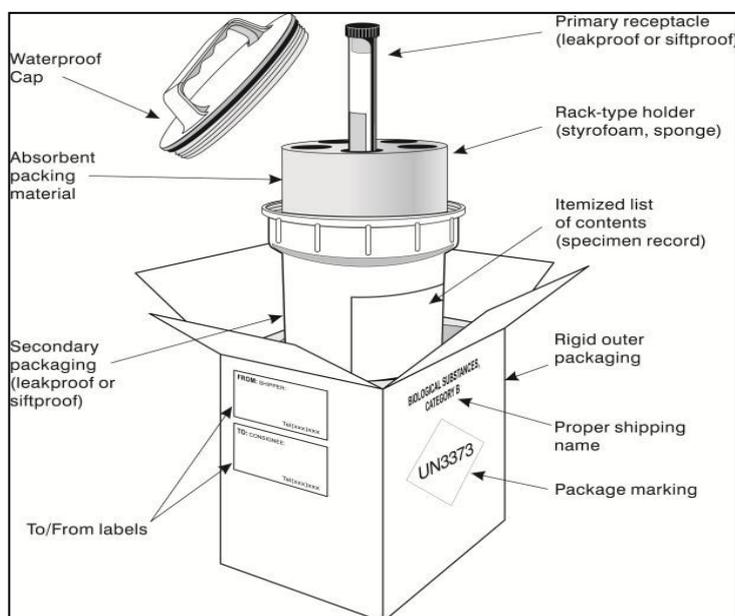
Sample packing and transport

It is to be done as per *WHO Guidance on regulations for the Transport of Infectious Substances 2013–2014*

Packaging, labeling and documentation requirements for infectious substances in Category B

Packaging

The triple packaging system is applicable.



Example of the triple packaging system for the packing and labeling of Category B infectious substances (WHO Guidance on regulations for the Transport of Infectious Substances 2013–2014)

Fig 1 Basic triple packaging system

This system of packaging shall be used for all infectious substances. It consists of three layers as follows.

- Primary receptacle. A primary watertight, leak-proof receptacle containing the specimen. Screw capped vials to be used. Vials should be labeled with the sample number and test required

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The receptacle is packaged with enough absorbent material (paper napkins/old newspaper) around it to absorb all fluid in case of breakage or leakage.

- Secondary packaging. A second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material shall be used to absorb all fluid in case of breakage or leakage.

- Outer packaging. Secondary packagings are placed in outer shipping packagings with suitable cushioning material. Outer packagings protect their contents from outside influences, such as physical damage, while in transit. The smallest overall external dimension shall be 10 x 10 cm.

Refrigerants

Refrigerants are to be used to stabilize the specimens during transit.

- Ice, ice pads shall be placed outside the secondary receptacle or in an outer packaging or Wet ice shall be placed in a leak-proof container; the outer packaging shall also be leak-proof.
- The secondary receptacle shall be secured within the outer package to maintain the original orientation of the inner packages after the refrigerant has melted.

Labeling

Each package shall display the following information:

- the sender's, name, address and telephone number
- Whom to contact in case of emergency with telephone number
- the receiver's name, address and telephone number
- the proper shipping name ("BIOLOGICAL SUBSTANCE, CATEGORY B") adjacent to the diamond-shaped mark with the UN number 3373
- Temperature storage requirements (optional).

Fig2 UN Numbering:



Minimum dimension: the width of the line forming the square shall be at least 2 mm, and the letters and numbers shall be at least 6 mm high. For air transport, each side of the square shall have a length of at least 50 mm

Colour: none specified, provided the mark is displayed on the external surface of the outer packaging on a background of contrasting colour and that it is clearly visible and legible

The words "BIOLOGICAL SUBSTANCE, CATEGORY B" in letters at least 6 mm high shall be displayed adjacent to the mark.

Documentation Required

The following shipping documents are required.

- To be prepared and signed by the sender: A packing list/proforma invoice that includes the sender's and the receiver's address, the number of packages, detail of contents, weight, value (Note: the statement "no commercial value" shall appear if the items are supplied free of charge)

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- To be prepared by the sender or the shipper's agent: An air waybill for air transport or equivalent documents for road, rail and sea journeys.

Responsibilities

The sender, carrier and the receiver have specific responsibilities in ensuring successful transportation.

Sender

- Makes advance arrangements/notifies the receiver about the transport
- Makes advance arrangements with the carrier to ensure:
 - that the shipment will be accepted for appropriate transport
 - that the shipment (direct transport if possible) is undertaken by the most direct routing
- Prepares necessary documentation, including permits, dispatch and shipping documents
- Notifies the receiver of transportation arrangements once these have been made, well in advance of the expected arrival time

The carrier

- Assists the sender in arranging the most direct routing and then confirms the routing
- Maintains and archives the documentation for shipment and transport.

Receiver

- Arranges for the most timely and efficient collection on arrival
- Should acknowledge receipt to the sender

Note: Shipments should not be dispatched until advance arrangements have been made between the sender, carrier and receiver.