Interim Guidelines for Zika / CMV / Rubella Diagnosis in Suspected / Proven Microcephaly Cases

SAMPLE COLLECTION

Infants with Microcephaly/Intracranial calcifications/ Newborn babies with birth defect <2 Months

1. CSF specimen = 3ml CSF in a tube at 2-8ºc storage
2. Infant serum = 2-3ml at 2-6ºc
3. Infant urine = 2ml at 2-8ºc

At the time of Birth

1. CSF specimen = 3ml CSF in a tube at 2-8ºc storage
2. Infant serum = 2-3ml at 2-6ºc
3. Infant urine = 2ml at 2-8ºc
4. Amniotic fluid/cord blood = 3-5ml at 2-6ºc.
5. Fixed tissue samples (This is to be considered for any gestation for which placenta is available):
   - Placenta & Fetal membranes - Collect at least 3 full thickness pieces (0.5-1cmx3-4cm in depth) from middle third of placental disk and at least 1 from the placental disk margin. Additionally one 5x12cm strip of fetal membrane can be collected.
   - Umbilical cord: At least four 2.5cm segments of the cord must be collected obtained proximal, middle, and distal to umbilical cord insertion site on the placenta. All enter specimens are labeled approximately to identify location of the sample.

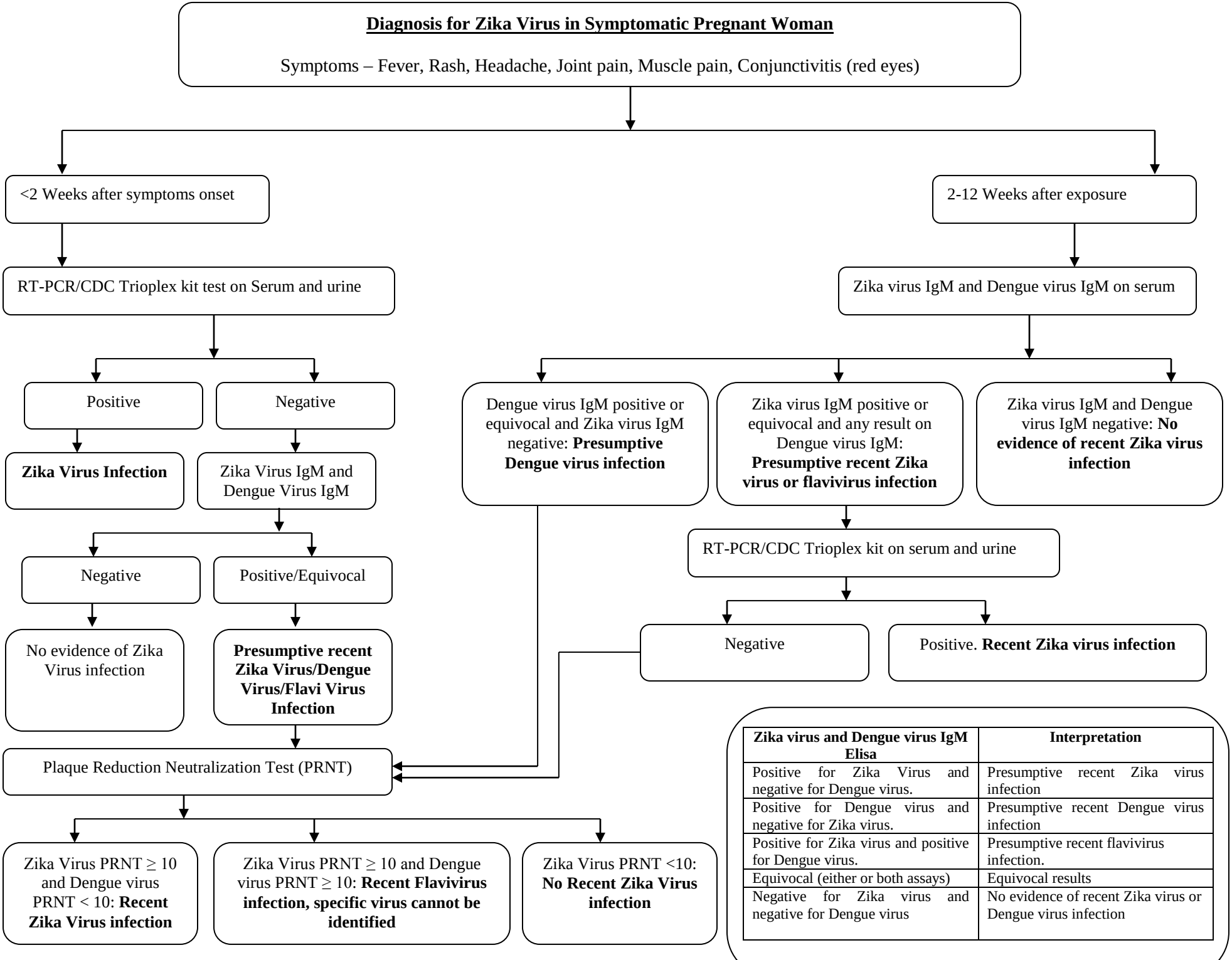
Pregnant Women

Serum: 4-8 ml
Urine : 5-15ml

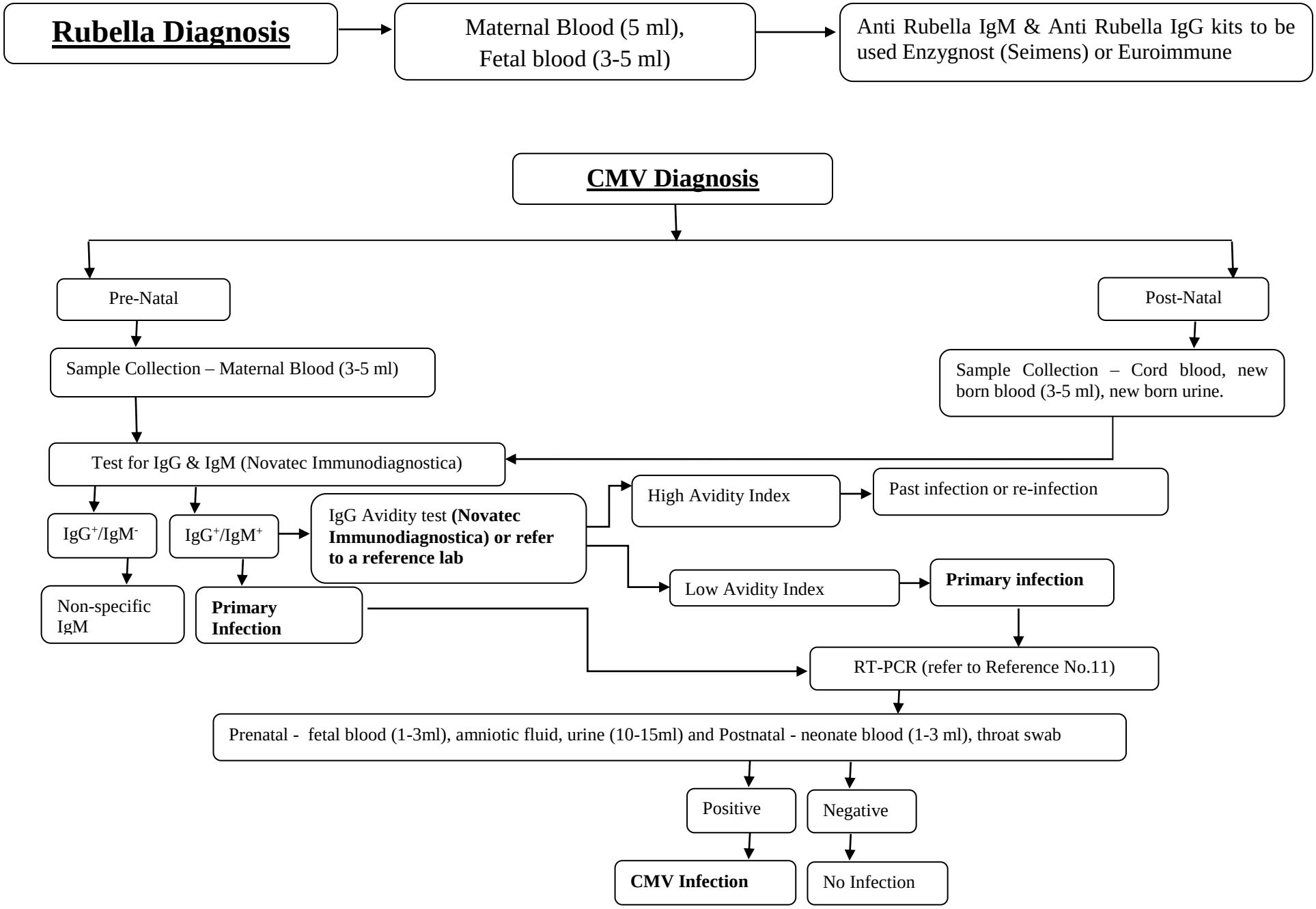
Transportation of the sample to the reference laboratory

- Triple layer packaging (as described at Annexure 1).
- Ship within 48 hours of collection under cold chain (dry ice or at least with cooling pack gels to maintain temperature of 2-8ºc)
- Samples should be packed labeled and marked.
- Include the completely filled out clinical and epidemiological record.
- Fixed tissues may include formalin – fixed wet tissues and/or formalin fixed paraffin embedded tissues.

Note: A volume of formalin used to fix tissues should be 10X the volume of tissue.



Zika virus and Dengue virus IgM Elisa	Interpretation
Positive for Zika Virus and negative for Dengue virus.	Presumptive recent Zika virus infection
Positive for Dengue virus and negative for Zika virus.	Presumptive recent Dengue virus infection
Positive for Zika virus and positive for Dengue virus.	Presumptive recent flavivirus infection.
Equivocal (either or both assays)	Equivocal results
Negative for Zika virus and negative for Dengue virus	No evidence of recent Zika virus or Dengue virus infection


Rubella Diagnosis
Maternal Blood (5 ml),
Fetal blood (3-5 ml)
Anti Rubella IgM & Anti Rubella IgG kits to be used Enzygnost (Seimens) or Euroimmune
CMV Diagnosis
Pre-Natal
Post-Natal
Sample Collection – Maternal Blood (3-5 ml)
Sample Collection – Cord blood, new born blood (3-5 ml), new born urine.
Test for IgG & IgM (Novatec Immunodiagnostica)
IgG+/IgM-
IgG+/IgM+
IgG Avidity test (Novatec Immunodiagnostica) or refer to a reference lab
High Avidity Index
Past infection or re-infection
Low Avidity Index
Primary infection
Non-specific IgM
Primary Infection
RT-PCR (refer to Reference No.11)
Prenatal - fetal blood (1-3ml), amniotic fluid, urine (10-15ml) and Postnatal - neonate blood (1-3 ml), throat swab
Positive
Negative
CMV Infection
No Infection

Guidelines for Sample packing and Transport

The regulations for the transport of infectious materials (by any mode of transport) are based upon the United Nations Model Regulations on the Transport of Dangerous Goods.Sample has to be packed by standard triple packaging system (**Annexure-1 and 2**).

Information to be sent by filling case report form (**Annexure-3**)

- Every sample should be accompanied by appropriate Case Report Form (CRF)
- **Filling of following details are mandatory in** Case Report Form**:**

1. **Date of onset** of symptoms
2. **Date of specimen** collection
3. Any **pertinent travel history** (3 months prior to the date of symptoms onset)
4. Any details available of DEN, CHIK/Rubella/Cytomegalovirus testing
5. If female patient, details of LMP, pregnancy, if any

ANNEXURE-1

Packaging System

a. Serum, CSF and urine to be sent on dry ice (Frozen) for Real Time RT PCR and serology

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 for any logistic problem or suggestion, kindly contact RCVRDL, NIV, Pune.*

1. Primary container: Individual screw capped vials, tightened and properly seald with tape/parafilm to be used for sending serum/Urine/CSF and whole blood/placental tissue. Vials should be labeled with the sample number and test required. Keep the vials in upright position.

2. Secondary Container: should be durable, watertight and leak proof to enclose and protect the primary containers. (plastic storage boxes of good quality/zip lock bags etc) There should be enough absorbent material (paper napkins/old newspaper) packed around the vials to absorb all fluid in case of breakage or leakage. More than one vial can be placed in secondary container.

3. Outer Container: Place the secondary container inside the outer container (thermocol box/durable cardboard box) maintain upright position of vials. There should be enough absorbent material (paper napkins/old newspaper) packed around it to absorb any leakage/spillage. The smallest overall external dimension shall be 10 x 10 cm.

4. Ice, ice pads shall be placed outside the secondary container, within outer container or if wet ice is used, it should be in a leak-proof container.

5. All forms, documents to be placed inside a sealed plastic cover within the outer container.

6. Label the outer container as follows:

- The sender's, name, address and telephone number
- The receiver's name, address and telephone number
- **"BIOLOGICAL SUBSTANCE, CATEGORY B"**
- Whom to contact in case of emergency with telephone number

7. Documents 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.
- 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.

The triple packaging system, the choice for the transport of infectious and potentially infectious substances, is exemplified in Figure 1 and 2. Infectious substances are classified in Division 6.2 and assigned to UN 2814, UN 2900, UN 3291 or UN 3373, as appropriate.

Category B

An infectious substance which does not meet the criteria for inclusion in Category A. Infectious substances in **Category B shall be assigned to UN 3373** (Fig 7).

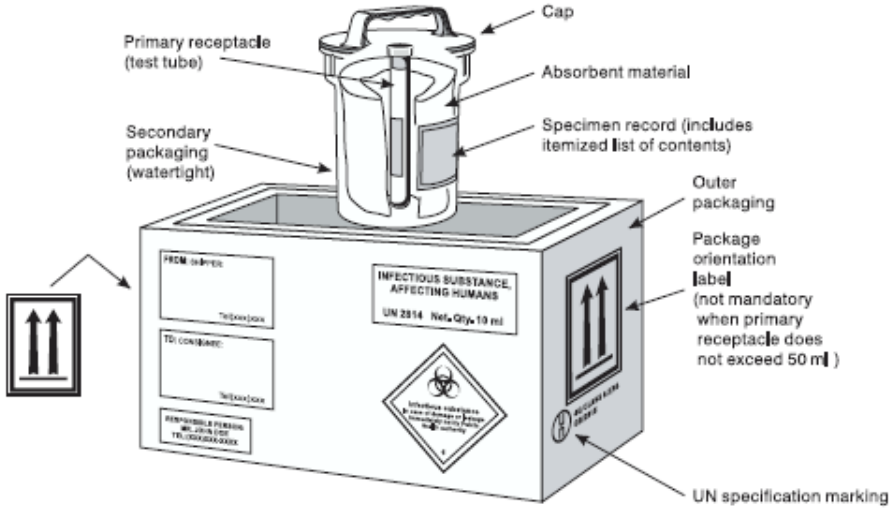


Fig-1: Packing and labeling of Category A Infectious substances

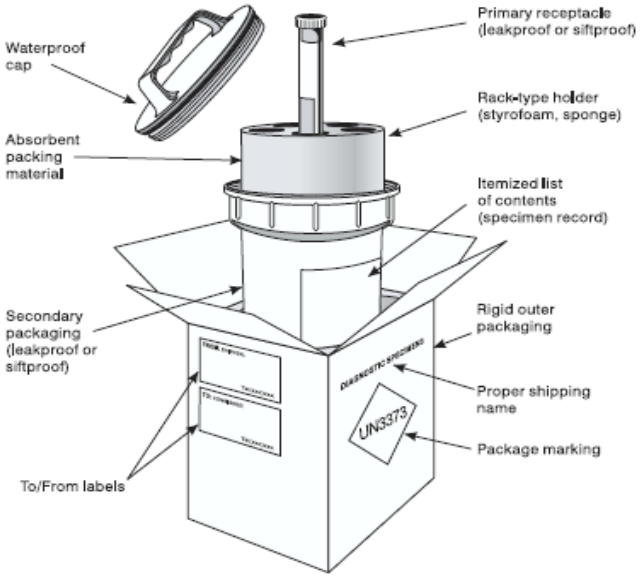


Fig-2: Packing and labeling of Category B Infectious substances

(Graphics by IATA, Montreal, Canada)

ANNEXURE-2

General Guidelines for Sample Handling

1. Specimen Acceptance Criteria

Ensure that the specimens are properly labeled with unique identification number and date. Cross check the specimen label (unique identification number and date) with the request form.

1. Ensure that the specimen received is in good condition and cold chain is maintained.
2. Record all primary specimens in the laboratory register /computer after receiving them in the laboratory.

2. **Specimen Rejection Criteria:**

- Unlabelled Specimens
- Incorrectly Labeled (Mislabeled) Specimens.
- Incorrect Container or Preservative

Insufficient Specimen for test(s)

- Recollect the sample if insufficient specimen is received for all procedures requested and the specimen is easily recollectable.
- If the specimen is not easily recollectable (CSF, Serum, Urine, etc.), the ordering physician will be contacted to establish a priority order of tests to be performed.

Unsuitable Specimen for Procedure(s)

- Reject the specimen which are received and are unsuitable (e.g. leaking/broken specimen container, haemolysed, lipimic specimen etc.) for the procedure requested or if the specimen has been in transit too long for a valid result.

Clinical Specimens

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

3. Guidelines for specimen Collection

1. A BSL2 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.

4. 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.

Annexure-3
Case Report Form for Newborn Screening for Possible Congenital Zika Virus Infection

B/o	Father's name
Date of Birth	Gender
House no	Street/Taluka
Village/Area	District /Pincode
Phone no	email
Occupation of mother	Occupation of father
Education of mother	Education of father
Income group	Maternal travel history in past 2 months:
Baby Hospital Reg No	Mother's hospital Reg No.
Date of collection	Age of baby ---yrs---months---days
Birth weight(gms)	Crown to heel length cm
Gestational age(wks)	Head circumference cm
lethargy	Respiratory distress
jaundice	Skin rash(petechaiae)
hepatomegaly	Splenomegaly
seizures	Hypotonia
Any other: Any history of congenital abnormality in previous pregnancy , Age of mother, Whether normal delivery or cesarean section or instrumental, Any addiction history such as tobacco or alcohol consumption in mother, Any history of drug intake by mother during pregnancy or h/o hospitalization. Whether the baby is preterm or Small for date or full term	

BIRTH ABNOMALITIES: Please complete this section in full even if no abnormalities were present

Cranial Abnormalities : Yes/No	If yes, details:
Eye Abnormalities: Yes/No	If yes, details:
Ear Abnormalities: Yes/No	If yes, details:

Neural tube defects, e.g. spina bifida, meningocele : Yes/No	Cleft lip/Plalate:Yes/No
Upper limb Abnormalities: Yes/No	If yes, details:
Lower limb Abnormalities: Yes/No	If yes, details:
Any other : specify	

Supportive Investigations with Date

Hb	Platelet count
TLC	DC
Bilirubin	Blood group
ALT	AST
USG Brain	USG abdomen:
Cranial CT	Any other

Any Investigations for Other Congenital Infections with Date

CMV
Rubells
Toxoplasmosis
Herpes Simplex
Syphilis
Any other

Management

NICU care required
Details of Treatment
CRF completed by:……..(signature)
Name: Date:

References:

1. Rabe B.I et al., 2016, Interim Guidance for Interpretation of Zika Virus Antibody Test Results, Morbidity and Mortality Weekly Report, Vol.65 (21), Pages- 543-546.

2.Muriel Bowser, Mayor, Zika Sample Collection Guidance, Department of Forensic Sciences, Government of the District of Columbia, Pages - 1-4.

3. ZIKA VIRUS: Collection and submission of Placental and Fetal Tissues For Zika Virus Testing, CDC, UD Department of Health and Human Services, July, 2017.

4. MDHHS Zika Virus Specimen Collection and Transport Guidelines, May, 2017.

5. Oduyebo T et al., 2016, Update: Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus Exposure, Morbidity and Mortality Weekly Report, Vol 65 (29), Pages – 739-744.

6. Laboratory testing for Zika Virus infection, Iterim Guidance, World Health Organization, 2016.

7. Marrs Caroline et al., 2016, Zika Virus and Pregnancy: A Review of the Literature and Clinical Considerations, American Journal of Perinatology, Vol 33(7), Pages – 625-639.

8. Sztajerowska J, et al., 2016, Microcephaly Associated with Zika Virus Infection – Prevention, Diagnosis and Treatment, Vol IV(3), Pages -8-11.

9. Mendelson E et al., 2006, Laboratory Assessment and Diagnosis of Congenital Viral Infections: Rubella, Cytomegalovirus (CMV), Varicella Zoster Virus (VZV), Herpes Simplex Virus (HSV) , Parvovirus B 19 and human immunodeficiency virus (HIV), Reproductive Toxicology, Vol 21, Pages – 352-382.

10. Walker S.P eta l., 2013, Cytomegalovirus in pregnancy: to screen or not to screen, BMC Pregnancy and Childbirth, Vol 13 (96), Pages 1-8.

11. Habbal W et al., 2009, Comparative evaluation of published cytomegalovirus primers for rapid real time PCR: which are the most sensitive, Journal of Medical Microbiology, 58, pages - 878-883.