

Guidelines for Collection and Shipment of Samples to NIHSAD for NIPAH VIRUS testing:

Safety Guidelines:

All the personnel involved in the sample collection should read the safety guidelines given in the chapter 2 of FAO manual available at <http://www.fao.org/3/ah690e/ah690e00.pdf>.

In brief, the personnel involved should wear complete Personnel Protective Equipment's including FFP2/N95 mask, safety goggles, boots/disposable shoe covers, strong impenetrable gloves, disposable overalls etc. at the site of sample collection during sampling and also during any sample handling in local laboratories.

The closed premises used for temporary sample handling and packaging should be fumigated using 10 gram of potassium permanganate plus 25ml Formalin per cubic meter of space. A 1:100 dilution of bleach or 5% Lysol solution or any other equivalent virucidal solutions available should be used for surface decontamination.

Target species for collection of samples:

1. Bats primarily Indian Flying Fox also known as Greater Indian Fruit bats (Genus *Pteropus*) is a reservoir of Nipah virus. Colonies of these bats present close to the epicenter (approx. 5-10 km radius) of human outbreak needs to be identified for sample collection. It is preferable to sample the colonies nearest to the epicenter on priority. Oropharyngeal Swabs, Rectal Swabs and Blood/Serum samples should be collected from different colonies present in the vicinity.
2. Pigs are susceptible to Nipah virus infection. Clotted and unclotted blood as well as Oropharyngeal/Nasal Swabs of Pigs around the epicenter of Human Outbreak should be sent for testing.

Sampling Procedure and Transient storage:

Blood collection:

The bat captured should be properly restrained wearing hard non-penetrable gloves. One-two ml blood is collected from brachial or cephalic artery or from the vein by using 2 or 5 mL syringe and 24-gauge needle. After collection in syringe blood is transferred to vacutainers (Heparinized or with EDTA) for further storage and transport. The vacutainers should be packed in plastic bags, and then placed in bigger plastic container (for two layer packing) and stored in refrigerator at +4°C till further transport to NIHSAD.

Blood from adult pigs should be collected from ear veins and from piglets from anterior vena cava using 1.0 to 1.5 inches/20 to 22 gauge needle. After collection one to two ml blood each should be transferred in vacutainers with EDTA and without EDTA. The vacutainers should be packed in plastic bags, and then placed in bigger plastic container (for two layer packing) and stored in refrigerator at +4°C till further transport to NIHSAD.

Swab collection:

For bats, two separate sterile swabs should be inserted in the oropharynx and rectum and taken out after screwing/rotating movement. The swab tip is broken/cut off and placed in a 2 ml sterile screw cap or cryovial and tightly closed. The screw cap vials should contain 0.5 ml Viral Transport Medium or PBS with antibiotics. The individual screw cap vial is sealed with parafilm and placed in larger plastic container.

For pigs, ensure the animal is adequately restrained with the head positioned upward. Young pigs may be restrained by holding animals against the handler's body or larger animals may be restrained with a snare. The snare should be positioned back in the mouth so that nasal openings aren't closed off. Wipe off snout and

insert sterile swab deep (at least 3 cm) into nasal cavity, avoiding contact with the outside of the nostril. Rotate swab hard enough on the inside of the nose to collect the sample. Repeat the process with the other nostril, using the same swab. Place the swab in the sample tube containing the medium and stir the swab in the medium to facilitate the release of sample and break off the shaft within the tube and securely close the tube.

Labelling of each sample containers:

Number each sample tube individually with a permanent marker. For each sample appropriate information must include the animal or group identification, Premises Identification Number, date, & species.

If the delivery of the collected samples is possible within 72 h, then store them in refrigerator at +4°C, else for longer transient storage, store in freezer at - 80°C (-20 °C, if - 80°C not available).

Composition of virus transport media (VTM):

Viral transport medium for collection of oropharyngeal or rectal swabs can be made as follows: Add 10g veal infusion broth and 2g bovine albumin fraction V to sterile distilled water (to 400 ml). Add 0.8 ml gentamicin sulfate solution (50 mg/ml) and 2 ml amphotericin B (250 µg/ml). Sterilize by filtration. Dispense in 0.5 to 1 ml in sterile container and store at 4 °C until used.

Alternatively, PBS (pH 7.2 to 7.4) with antibiotics can be used for storage and transport of swabs. The final concentration of antibiotics to be used in PBS is Penicillin 1000 IU/ml, Streptomycin 500 µg/ml, Gentamycin 500 µg/ml and Amphotericin 25 µg/ml.

Summary of Sample collection, transient storage and transport:

Sample	Transient Storage up to 72 hours	Transient Storage more than 72 hours
Blood (In EDTA or Heparin)	Refrigerator +4°C	Refrigerator +4°C
Serum (without anticoagulant)	Refrigerator +4°C	Freezer at -20 °C (- 80°C if available)
Swabs (VTM or PBS)	Refrigerator +4°C	Freezer at -20 °C (- 80°C if available)
Carcass	Freezer at -20 °C	Freezer at -20 °C (- 80°C if available)
TRANSPORT TO NIHSAD	On Coolpacks	On Coolpacks

Packaging and Transport of Samples:

All samples collected for Nipah investigation should be transported to NIHSAD with personnel messenger only.

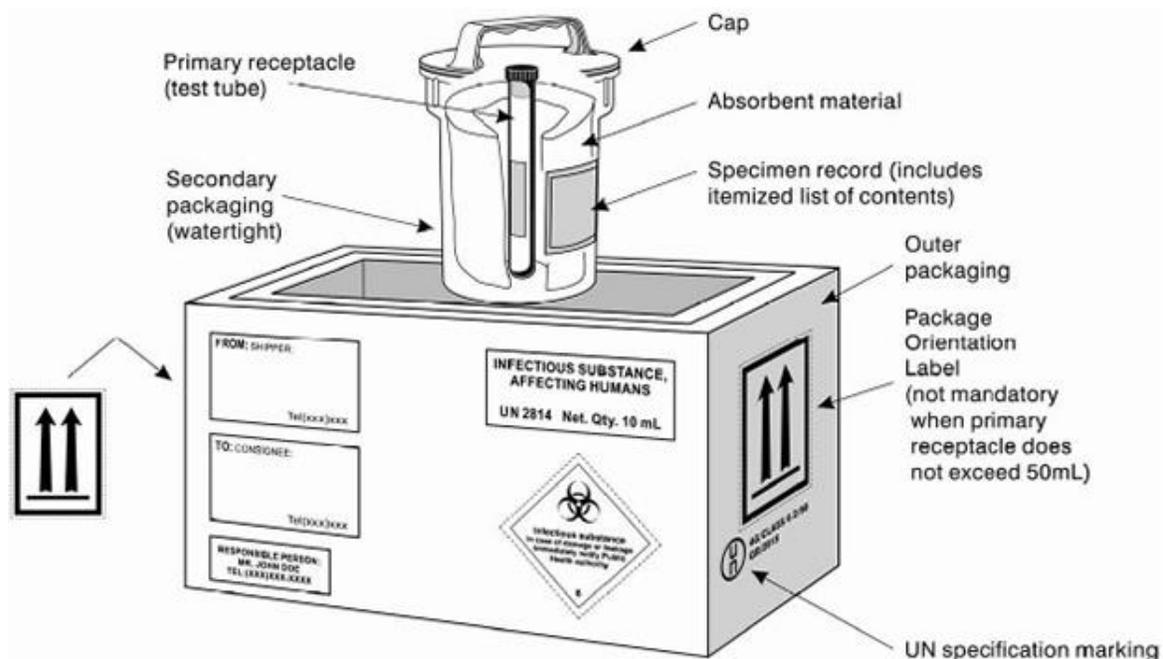
The safest method of transporting samples is using triple layer container & cold chain should be maintained throughout the period. Containers illustrated below can be commercially obtained (eg. Fischer Scientific).

However, in the areas where obtaining such container is difficult the same principal of triple layer packing should be followed and customized package should be made with suitable materials available at their ends. In brief the packaging and transportation should be done as follows:

1. All the samples should be packaged in triple layer packing. The packaging should consist of at least three components: water-proof primary container(s) (this directly contain samples); (an absorbent layer) a water-proof secondary packaging; an outer packaging of adequate strength and insulation (to contain coolpacks or dry ice).
2. Different set of samples should be individually packed and properly labelled in primary containers. Each sample containing vials should be preferably individually sealed with parafilm.

3. The case sheets with complete information should be placed in a plastic bag or envelop and be pasted outside of the outermost thermocol or vaccine container. The case sheets with complete information about the samples should be completely filled in Case report Form (separate sheet) and provided along with the samples. The information should necessarily include the details of source, location and date of collection. See the sample information sheet below.
4. Cold Chain: The outermost insulated layer of container should be filled with dry ice if samples are to be transported frozen, or with coolpacks if the samples are to be transported at +4°C. Use dry ice or coolpacks in the ratio of at least for 6 volumes to 1 volume of sample. (Refer the table above for which samples to be shipped on dry ice and which ones on coolpacks).
5. Person handling the sample should wear gloves, mask and a gown, to avoid direct contact with the infectious material. After completing the packing of samples, person should thoroughly wash hand with disinfecting solution. Before dispatching the container, Bleach can be used for disinfection. A 1:100 dilution of bleach or 5% Lysol solution should be used to clean the outer surfaces of the container.
6. Please notify the National Institute of High Security Animal Diseases (NIHSAD), India of the shipment details before dispatch. Transport must be as fast as possible and should not exceed 72 hours otherwise, there is a risk of interruption in the cold chain and loss of sample integrity.
7. SHIPPING ADDRESS: National Institute of High Security Animal Diseases (NIHSAD), Hathaikheda Dam Road, Anand Nagar, Bhopal (MP), PIN: 462022, India.
8. CONTACT DETAILS: Director's Office: +91 755 2759204, EPABX: +91 755 2754674-75, Fax +91 755 2758842. director.nihsad@icar.gov.in

In view of the fact that NIPAH VIRUS is a high-risks zoonotic pathogen the guidelines prescribed by OIE for collection and shipment of diagnostic samples should be strictly followed. The link for guidelines is as below: (http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/1.1.01_COLLECTION.pdf)



Sample Submission Form (Data sheet) for NIPAH VIRUS investigation at NIHSAD, Bhopal

Sender's Name, Designation and Address:								
Date of dispatch of samples and letter no:								
S No	Location(s) of sampling (Coordinates) (State/District/Village/Block)	Date(s) of sampling	Species (Bat/Pig/Others)	Type of Sample (Blood/Swabs/Carcass/Tissue)	Number of Samples	Storage Media used (VTM/PBS)	Transient Storage temp (+4°C / -20 °C) and duration	Shipping condition (Coolpacks /Dry Ice)
1								
2								
3								
4								
5								