ICMR-NATIONAL INSTITUTE OF VIROLOGY
20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411001, Maharashtra, INDIA
Tel: ICMR-NIV, Camp: +91-020-26127301/26006290; Fax: 26122669/26126643
ICMR-NIV, Pashan: +91-020-26006890; Fax: 25871895/25870640
Email: director.niv@icmr.gov.in; Website: www.niv.icmr.org.in

INSTRUCTIONS FOR COLLECTION, STORAGE AND TRANSPORT OF SAMPLES FOR RABIES TESTING

Purpose and Scope	This document describes the processes for collection, storage, packaging and transport of clinical specimens from suspected cases of rabies, for laboratory testing.
Responsibilities	The processes described in this document should be performed by trained personnel, under the supervision of a trained clinician/microbiologist. The personnel handling the specimens should have completed a course of rabies vaccination (using a vaccine approved by Drugs Controller General of India) and must strictly observe biosafety protocols.
Biosafety Precautions to be followed	Personnel handling the clinical specimens must wear a full-sleeved laboratory coat/gown, 2 pairs of non-sterile latex/nitrile gloves, cryogloves (if handling dry ice for packing), surgical/N95 mask, goggles/face shield, shoe cover/boots and hair cap.
Types of specimens to be collected	**Ante-mortem testing for rabies:** Suitable samples include saliva, neck skin biopsy, serum and cerebrospinal fluid. **Post-mortem testing for rabies:** Brain tissue is the preferred specimen for confirmatory testing of rabies and may be collected at autopsy. Cerebrospinal fluid, neck skin biopsy and serum samples may also be collected and sent.
Collection and storage of samples	1. **Saliva:** Three to four samples of saliva (clear liquid portion, 1-2mL each) may be collected (using a sterile syringe or Pasteur pipette) at intervals of 3-6 hours, into a sterile, screw-capped, leak-proof container. The samples may be pooled, if desired. *Sputum samples are not suitable for testing, and should be strictly avoided.* 2. **Cerebrospinal fluid:** At least 1-2mL of cerebrospinal fluid may be sent. 3. **Serum/whole blood:** At least 2mL of serum (preferably collected into a yellow-capped Gel Separator Tube) or 5mL of whole blood (collected in a plain red-capped collection tube) should be sent in a screw-capped, leak-proof, polypropylene tube with an O-ring. 4. **Neck skin biopsy:** A full thickness punch/excision biopsy (5-6mm in diameter and 5-7mm in depth) may be obtained from the nape of the neck, at the hairline, ensuring the inclusion of at least 10-15 hair follicles. The material should be placed on a piece of sterile gauze moistened with sterile normal saline, overlaid with another piece of sterile gauze, and placed inside a sterile screw-capped container. No preservative/viral transport medium should be added to the vial. 5. **Brain tissue:** Sections of cerebellum, cerebral cortex, brainstem, medulla and hippocampus may be submitted in **glycerol-phosphate buffered saline** (50:50 mixture) in a screw-capped, leak-proof container. **Do not add formalin to the brain tissue specimens.**
Storage of specimens	The samples, after collection, should be sent immediately to the laboratory for testing. In case of unavoidable transport delays, the samples may be stored at 2-8˚C for upto 24 hours. Storage at -20˚C is recommended for delays up to 72 hours, and for delays beyond this, the samples should be frozen at -80˚C.

Packaging of samples	Specimens should be packaged in Standard Triple Packaging, in accordance with the national/international guidelines. **1. Primary container:** The clinical samples should be collected in screw-capped, leak-proof, polypropylene vials (preferably with O-ring). The vials should be kept in an upright position, and their neck sealed using parafilm or adhesive tape to prevent leakage. Sufficient amounts of absorbent material (e.g., cotton, paper towels or newspaper) should be placed around each sample vial to contain accidental leakage. **2. Secondary container:** The primary vials with the samples should be enclosed in a sturdy, leak-proof secondary container (e.g., Zip-lock bag, 50mL centrifuge tube, or other plastic containers with a tight lid). More than one primary vial can be arranged inside a secondary container. **3. Outer container:** Zip-lock pouches, if used as a secondary container, should be further placed inside a sturdy plastic container, and its lid should be sealed. Hard board or thermocol boxes or vaccine carriers can be used as an outer container. The dimensions of the outer container should not be less than 10cm x 10cm x 10cm (length, height and width). **4. Cold chain:** Sufficient amounts of coolant material (gel packs/dry ice) should be placed between the secondary and outer containers. The primary vial should not come in contact with the coolant material. The gel packs should have been hard-frozen for several hours at the appropriate temperature, and thawed at room temperature for 30 minutes prior to use. Precautions must be taken while handling dry ice to minimize the risks of frost bite, explosion and asphyxiation. **5. Documents and labeling:** The completely filled-in Test Request Form must accompany each set of samples, and should be placed inside a sealed plastic envelope on the outer container. The outer container should be labeled with details including name, full address and telephone numbers of the sender and the recipient, and the name and contact number of an emergency contact person. A label indicating 'Biological Substance, Category B' should be attached to the outer container. If dry ice is used, a label to indicate it should be affixed. For shipment via Air, Airway Bill and other documents may be prepared in consultation with the agency. **6.** Before dispatching the shipment, the outer container may be wiped using 5% Lyzol or 0.1% sodium hypochlorite solution. **7. Shipping address:** The specimens packaged as above and accompanied by a completely filled-in **Request Form for Rabies Testing (Human/Animal Samples, as appropriate)** (available at **www.niv.icmr.org.in**, under the tab 'Current Activities' → 'Services/Outbreak Investigations') should be sent to: **The Virus Registry, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411001, Maharashtra, India (Tel: 020-26006231/290).** The samples should be sent only after prior intimation to the **Rabies Laboratory** (Tel: **020-26006890/807/893).** For further information, please contact the **Encephalitis Group (020-26006893/890)** of ICMR-NIV, Pune, or email us in ***encephalitisgroup2016@gmail.com***.