

## Collection of Samples for Diagnosis of Rabies Antigen in Humans

1. **Patient History:** Physicians should provide a complete history of the patient and appropriately fill the clinical proforma of the Haffkine Institute stating the details of the patient.
2. **Sample Packaging and Transport**
  - All samples should be considered potentially infectious.
  - All specimens should be collected in a primary container that is watertight and leak-proof and must be securely sealed (tape around the cap will ensure that the containers do not open during transit). **If immediate transport is not possible, samples should be stored frozen at -20°C or below.**
  - The primary container should be put in a secondary container such as zip-lock plastic bag with an insulating material between primary and secondary containers.
  - The secondary container should be put into a rigid outer packaging box during transport.
  - **Please ensure maintenance of cold chain (2-8°C) while the sample is being transported.**
3. **Sample Collection** (Ante-mortem diagnosis)

### **3.1 SALIVA**

Saliva may be collected using a sterile eyedropper pipette and placed in a small sterile container which can be sealed securely.

No preservatives or additional material should be added.

Tracheal aspirates and sputum are not suitable for rabies tests.

**Laboratory tests to be performed:** Detection of rabies RNA by Nested Reverse transcriptase Polymerase Chain Reaction- RT PCR.

### **3.2 CORNEAL IMPRESSION SMEARS**

Impressions of the cornea of the left and the right eye may be taken on a clean grease free slide. Allow the smear to dry. Transport the slides in a closed container.

**Laboratory tests to be performed:** Detection of rabies antigen by direct Fluorescent Antibody Test for staining for viral antigen in frozen sections of the biopsy.

### **3.3 NUCHAL BIOPSY**

A section of skin 5 to 6 mm in diameter should be taken from the posterior region of the neck at the hairline.

The biopsy specimen should contain a minimum of 10 hair follicles and be of sufficient depth to include the cutaneous nerves at the base of the follicle.

Place the specimen on a piece of sterile gauze moistened with sterile water and place in a sealed container.

**Laboratory tests to be performed:** Detection of rabies RNA by Nested Reverse transcriptase Polymerase Chain Reaction- RT PCR and direct Fluorescent Antibody Test for staining for viral antigen in frozen sections of the biopsy.

### **3.4 CEREBRAL SPINAL FLUID (CSF)**

At least 0.5 ml of serum or CSF should be collected; no preservatives should be added.

**Laboratory test to be performed:** Detection of rabies RNA by Nested Reverse transcriptase Polymerase Chain Reaction- RT PCR.

N.B.: For anté-mortem diagnosis, a negative test does not rule out Rabies Virus infection.

## 4. **Sample Collection** (Post-mortem diagnosis)

### **BRAIN BIOPSY**

Portions of the medulla (brain stem), the cerebellum, and the hippocampus are recommended for diagnosis. The biopsy may be placed in a sterile sealed container containing 50% glycerol saline.

Preservation of tissues by **fixation in formalin is NOT recommended** if rabies diagnosis is desired.

**Laboratory tests to be performed:** Detection of rabies RNA by Nested Reverse transcriptase Polymerase Chain Reaction- RT PCR and direct Fluorescent Antibody Test for staining for viral antigen in frozen sections of the biopsy.

### **REFERENCES:**

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