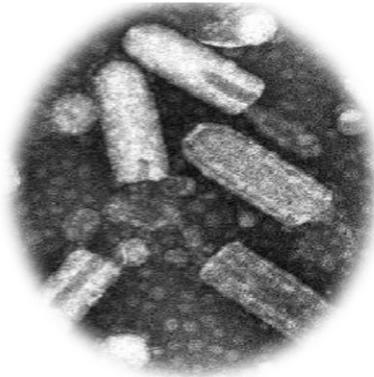




World Organisation
for Animal Health
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Occipital foramen approach for brain sampling and diagnosis of Rabies



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WOAH Reference Laboratory for Rabies

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Preliminary Safety

- Pre-exposure immunization and regular boosters.
- Personal protective equipment (PPE)



- Do not aerosolize .

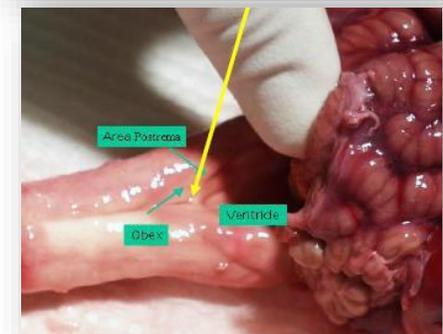
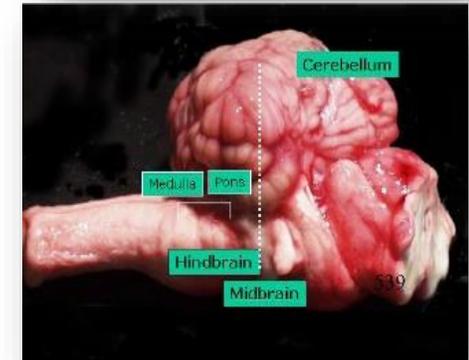
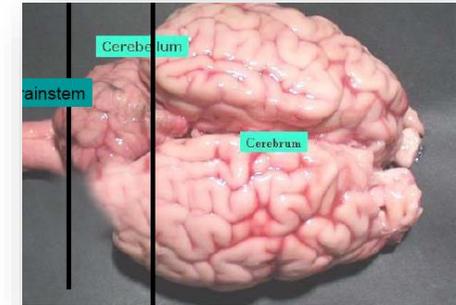
Personal Protective equipment (PPE)

- Disposable gloves
- Face mask and shield
- Eye goggles
- Hair cover /cap
- Disposable apron
- Shoe cover



Quality brain sample needed for accurate diagnosis of rabies

- Identifying the virus / its specific components using **reliable lab. tests**
- Laboratory **techniques vary in their efficiency, specificity and reliability**
- **Applied to brain tissue**, can also be applied to other organs (e.g. salivary glands)
- Proper packaging helps in proper maintenance of the samples to aid accurate diagnosis of rabies.



Collection of brain samples

- **Opening of the skull**
- **Retro-orbital route**
- **Occipital Foramen route**

Opening of the skull in necropsy room

- **Preferably brain stem, cerebellum ,Ammon's horn, thalamus, and medulla oblongata**
- **Prevent aerosols**
- **Cutting tools, scissors and scalpels, should be used with care to prevent injury and contamination**



Removal of brain from skull of dog



3 METHODS

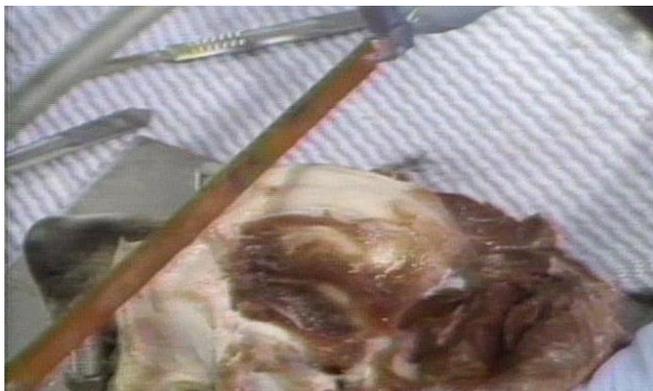
1. hammer & chisel
2. bone saw or hack saw
3. electric saw

Removal of brain

1. Hammer & Chisel



2. Hack saw



3. Electric saw



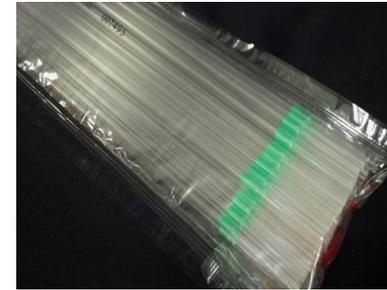
Retro-orbital route for brain sampling

- A trocar used to make a hole in the posterior wall of the eye socket.
- A biopsy needle introduced through hole.



Occipital Foramen route for brain sampling

- A 5 mm **Al sheath/ plastic drinking straw**,
- A **2 ml disposable plastic pipette**
- A 1–2 ml **truncated plastic syringe** introduced into the occipital foramen in the direction of an eye
- Samples from the **brain stem & cerebellum**
- When using a straw it should be pinched between the fingers to prevent material escaping when withdrawing.



Sample collecting equipment

- Scissors (sharp and blunt ended)
- Forceps
- BP blade plus holder
- AI sheath/ Straw
- Disposable syringes
- **Sample storage containers.**



Sample storage containers : Proper Handling of the Specimen

Primary Container

- ✓ Contains the clinical specimen
- ✓ A rigid one that can be tightly sealed.
- ✓ **Plastic containers of appropriate size**

Secondary Container

- ✓ Primary container is placed in a secondary container.
- ✓ A plastic or metal container with a lid or even a **suitable zip lock bag**.

Tertiary / Shipping Container

- ✓ Secondary container is placed in the **tertiary / shipping container: a thermocol box**.
- ✓ Frozen cool packs & cushioning materials put.
- ✓ Labelled as “BEWARE! BIOLOGICAL SPECIMEN FOR RABIES DIAGNOSIS. INFECTIOUS HAZARD!”

Transportation of Specimen without Preservatives

- Common method
- Strictly maintain cold chain
- The box should be labelled as **“BEWARE! BIOLOGICAL SPECIMEN FOR RABIES DIAGNOSIS. INFECTIOUS HAZARD!”**
- If the head of the animal to be submitted, immediately after decapitation, head has to be first cooled, wrapped in absorbing paper and placed in plastic bag.

Transport Using Preservative Solutions

- **Preservatives used if transit time long or refrigeration not possible.**
- Lab. technique determines the preservative to be used.
- Use of **formalin** safe since it inactivates rabies virus, but sample unsuitable for isolation / inoculation but suitable for histological studies.
- The use of **glycerine** solution does not inactivate the virus rapidly but is capable of inhibiting the growth of contaminants.
-
- **Note:** DFA test may be applied to glycerol-preserved specimens after washing. If preserved in formalin, DFA test may be used only after the specimen has been treated with a proteolytic enzyme. **However, the DFA test on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue .**

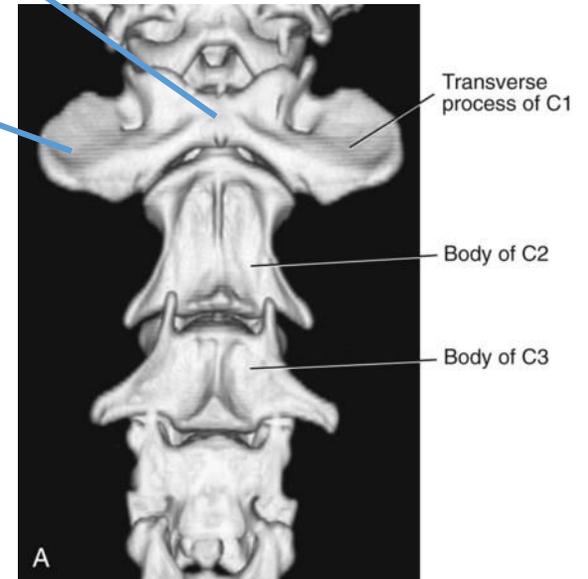
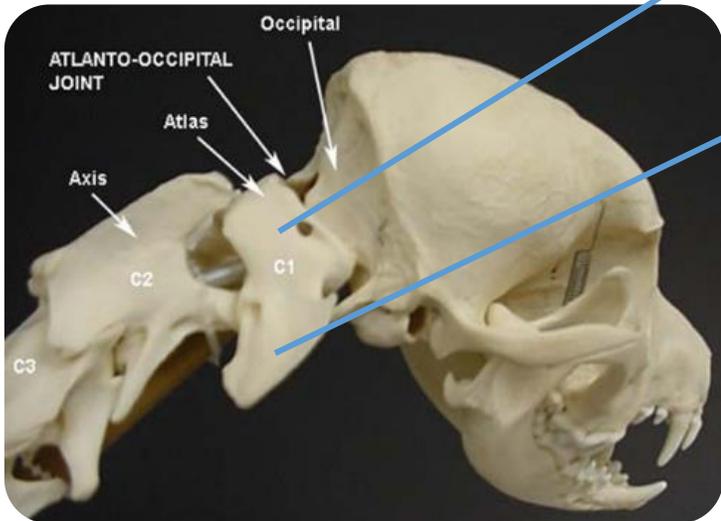
Preservative Solutions

- **Brain sample:** 50% glycerol saline
- **Salivary gland:** 50% glycerol saline

How to locate Foramen magnum / Occipital foramen

Atlas (first Cervical vertebra)

Wings of Atlas



Brain sample collection by field vets using Occipital foramen method – User friendly and simple approach

Fresh carcass:
use scissor / scalpel



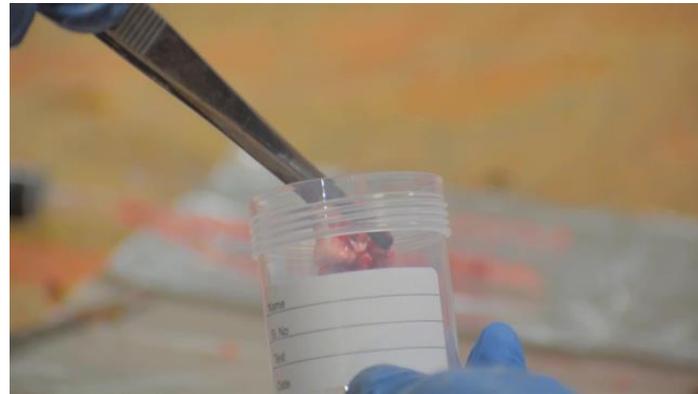
Old / preserved carcass:
use syringe / AI sheath



Locate the transverse processes of the first C1 & external occipital protuberance. A deep cut be made just behind external occipital protuberance but anterior to imaginary line connecting the transverse processes of C1.



Using the scalpel / BP blade, the brain stem portion visible is cut and the specimen collected into the primary container, closed & labelled.



Introduce an artificial insemination (AI) sheath connected to a disposable syringe deeply into the Occipital foramen and the tissue from brain stem aspirated



Packing brain tissue in Primary, Secondary and tertiary containers



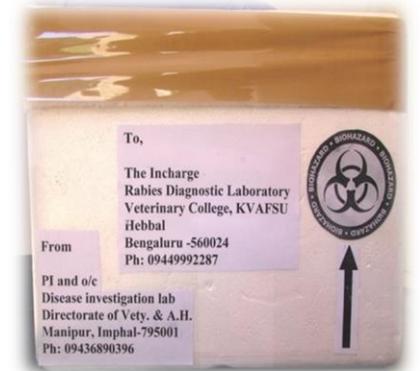
Primary container



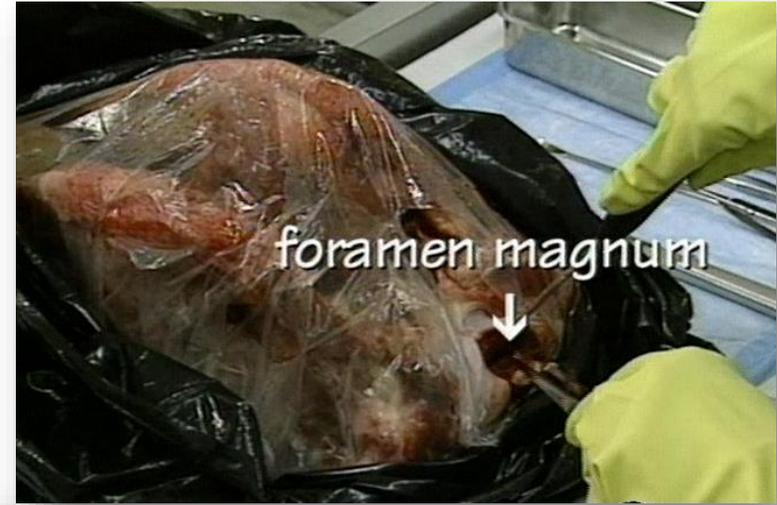
Secondary container



Tertiary container



Brain sampling if complete decapitation of head



Wolf head



Conclusion

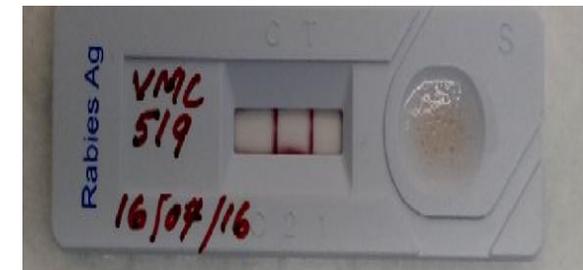
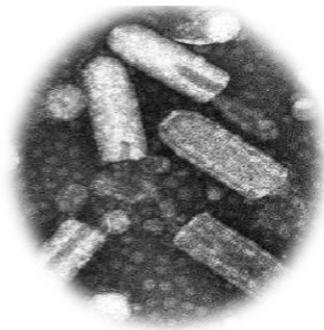
- Veterinarian collecting samples must have undergone **PreEP**
- Use **PPEs** during sample collection
- Take **precautions** while handling , transportation to ensure the safety of the personnel and the environment
- Samples should be transported **in cold chain** to prevent decomposition. Frozen **cold packs preferred over wet ice** since it may leak through the container.
- **Triple packaging** of the sample .
- **Quality sample aids accurate diagnosis of rabies.**



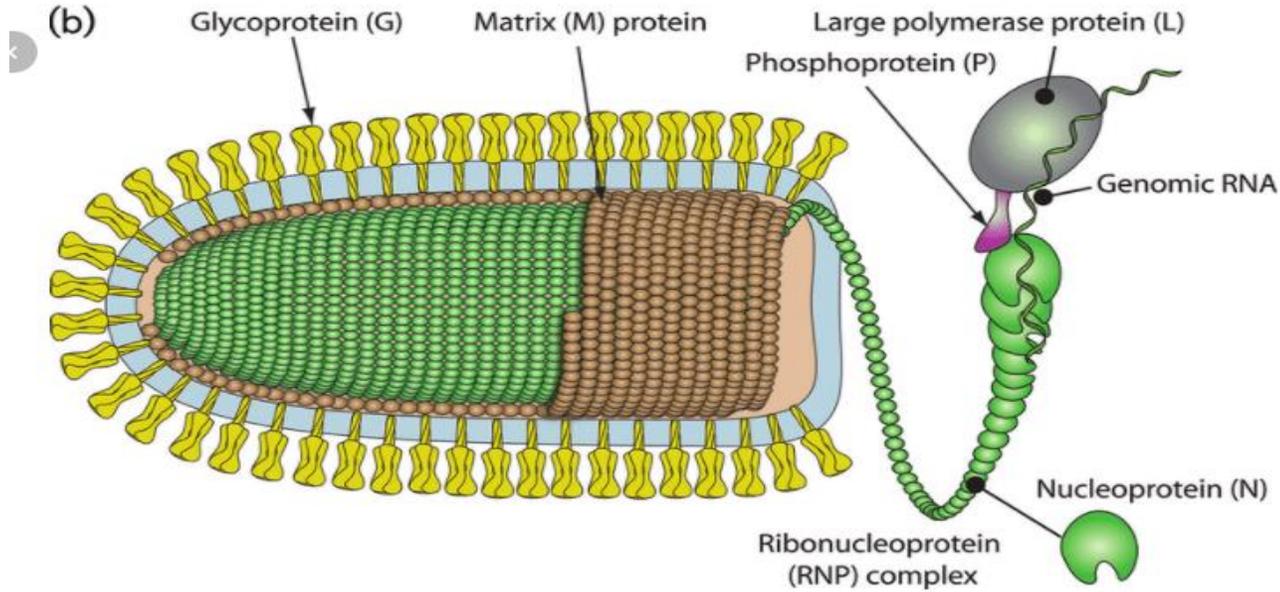
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LATERAL FLOW IMMUNOASSAY of Brain samples; principle, protocol, interpretation and reporting results



N protein – Diagnosis



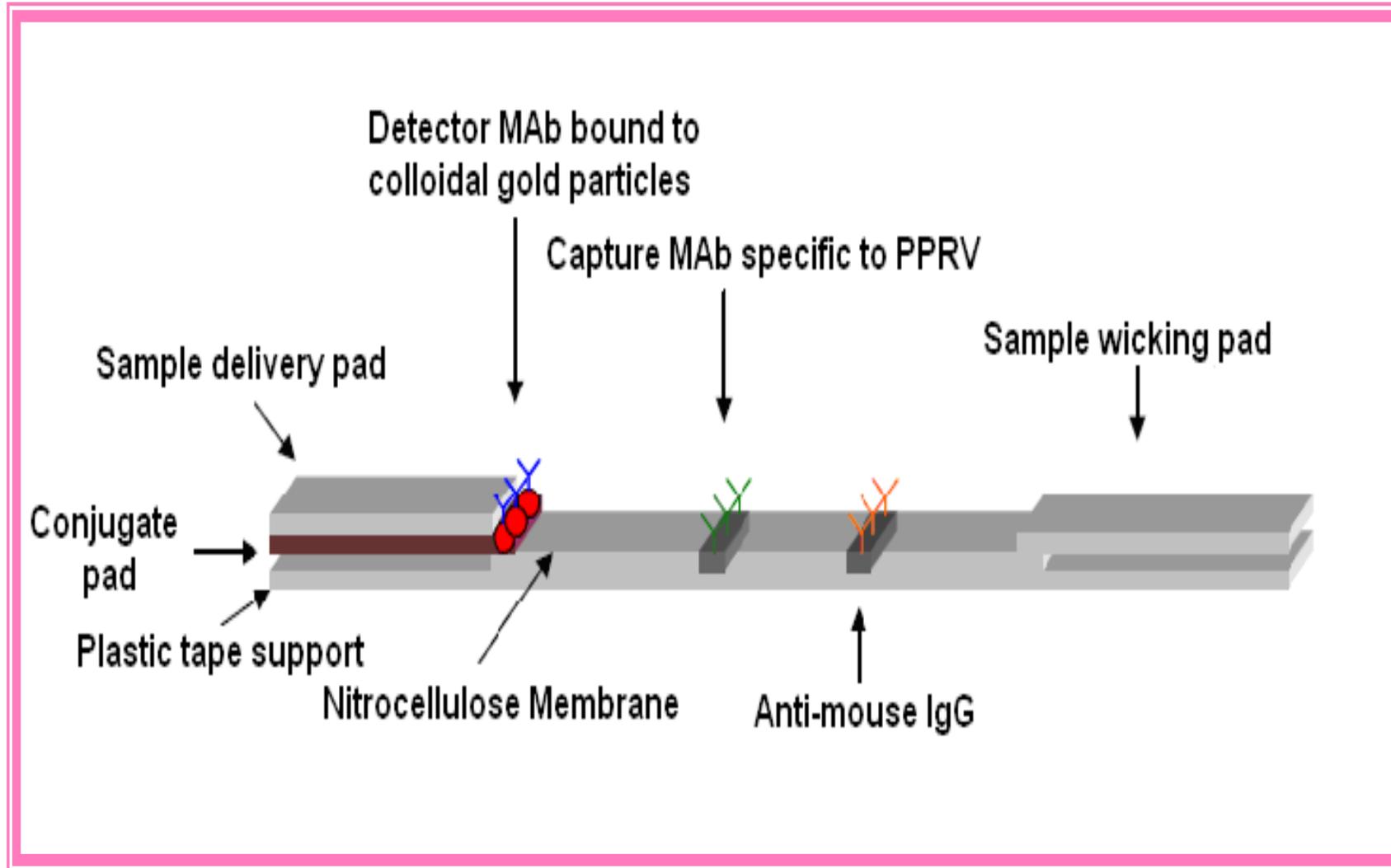
Rabies Genome



Timely and confirmatory diagnosis of rabies in dogs is a must for institution of strategies for its prevention, control.



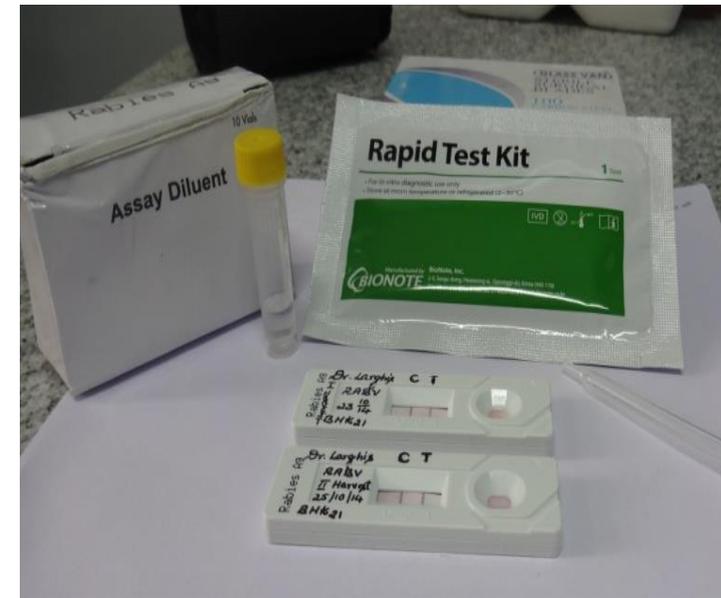
Principle and constituent components of LFA device



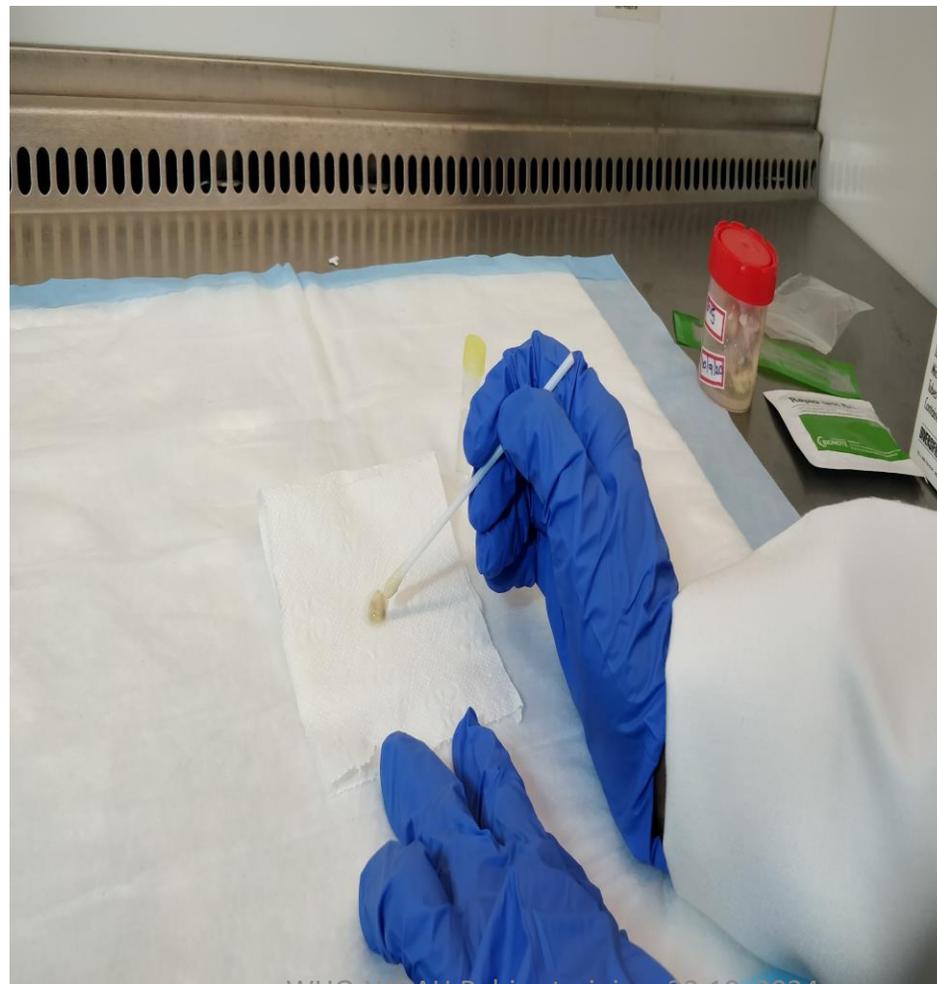
Lateral flow immunoassay 'Rapid Rabies Antigen Test kit' (Bionote, Hwaseong-si, Korea)

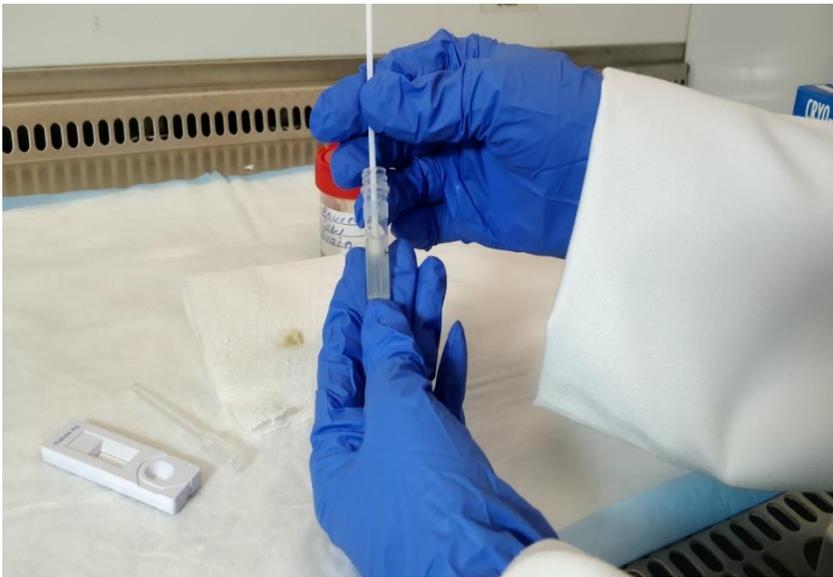
**Materials provided in the kit
(10 Tests/Kit):**

- Ten Rapid Rabies Ag Test devices
- Ten assay diluent tubes
- Ten disposable swabs
- Ten disposable droppers
- One instruction manual

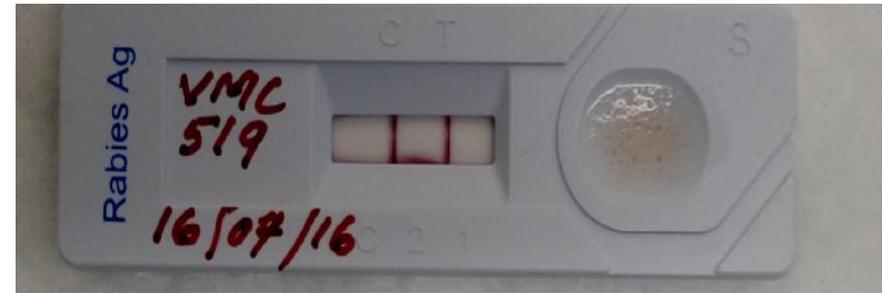


Assay Protocol

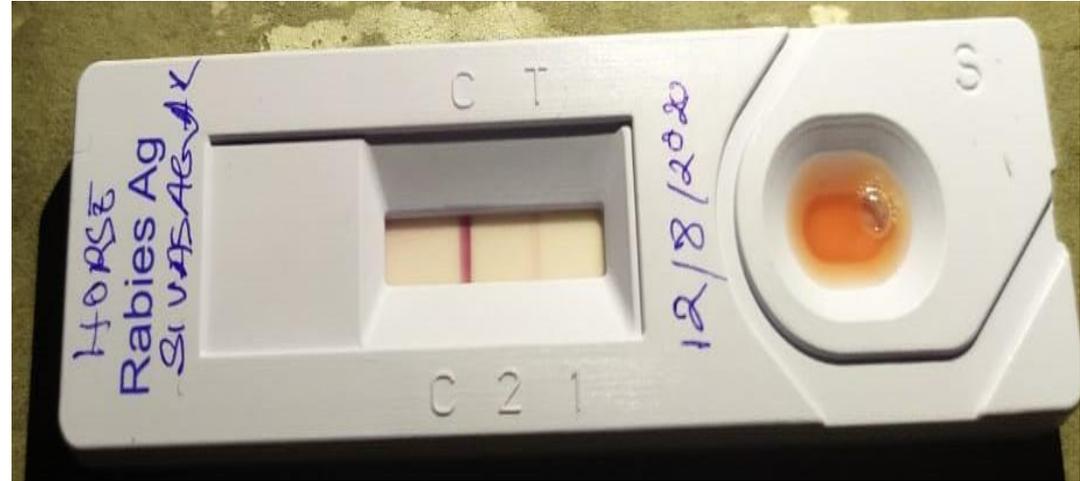




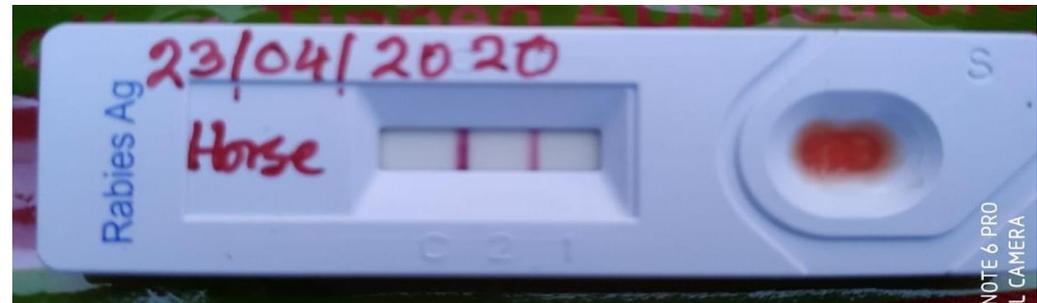
Interpretation of the LFA results:



Horse positive for rabies by LFA on 12th August, 2020 in Assam

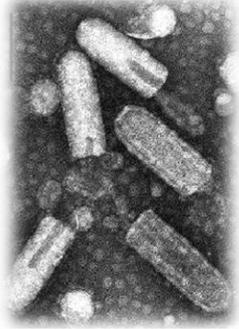


Horse from Assam Police Battalion
positive by LFA



LFA conducted at carcass side



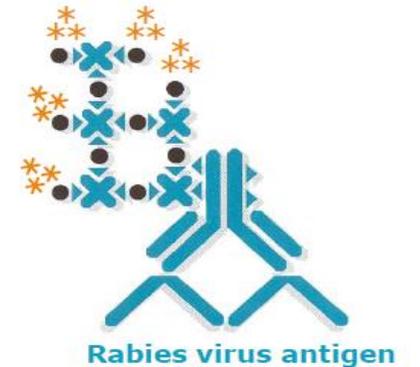


Direct Rapid Immunohistochemistry in diagnosis of rabies in animals

Direct Rapid Immunohistochemistry Test (dRIT)

- Staining time 1 hr.
- Done at bench-top at ambient temperature.
- Does not require a fluorescent microscope or incubator.
- Only a standard light microscope is required.
- Permits rabies diagnosis under field conditions.
- Cost effective - technology transfer to developing world

Direct RIT

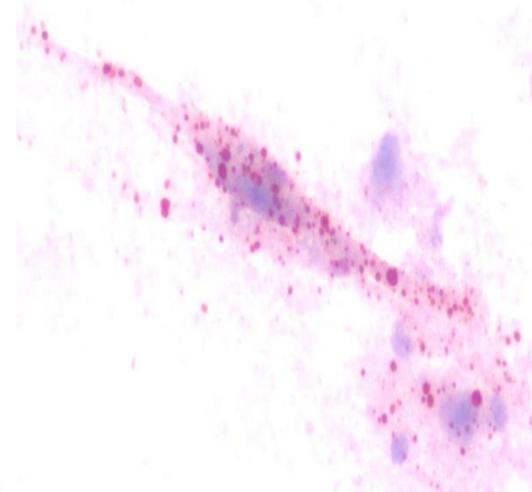
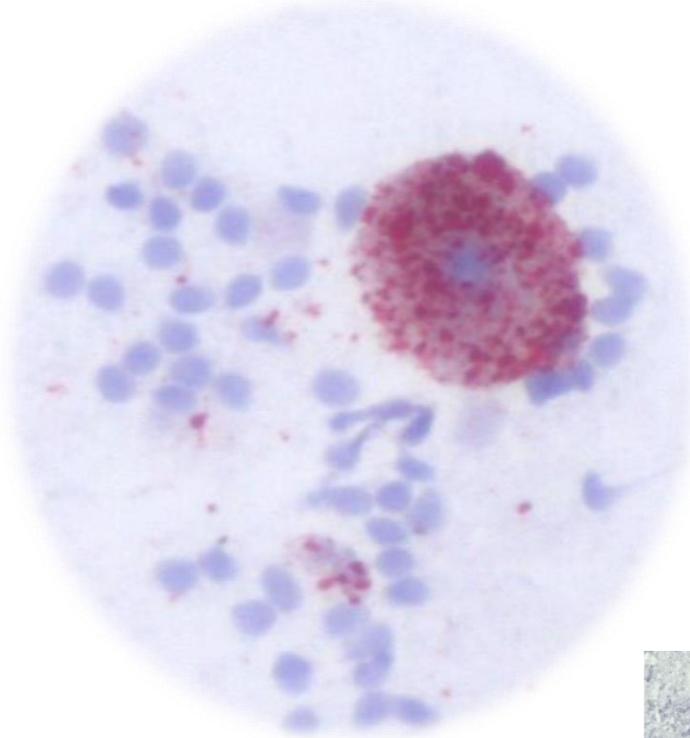


Contents of the kit:

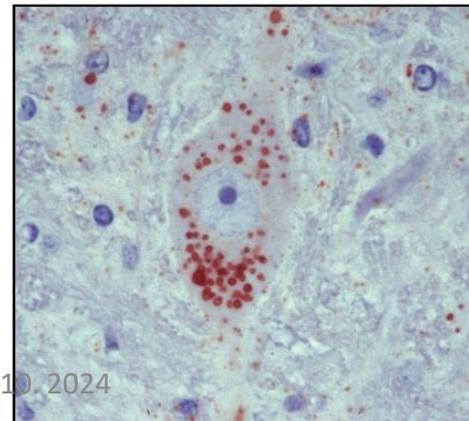
1. Tween 80
2. 30% hydrogen peroxide (H₂O₂) for step 2
3. Primary antibody: mouse anti-rabies biotinylated monoclonal antibody
 - i. CDC
 - ii. In-house
 1. Streptavidin peroxidase
 2. Acetate buffer
 3. Amino-ethylcarbazole (AEC) stock solution
 4. 3% H₂O₂ step 5
 5. Hematoxylin Gill no. 2
 6. Aqua mountant



Direct Rapid Immunohistochemistry Test (dRIT) For Detection of Rabies Virus Antigen



- Safe
- Independent of Fluorescent microscope, -20 deg.C, Incubator
- Feasible at District level



Conclusion

- ✓ Conventional Seller's staining replaced by DFA
- ✓ **dRIT** is userfriendly, sensitive & specific, be explored
- ✓ High time for **large scale training programmes for Vets on foramen magnum** method of brain collection, packing , shipping
- ✓ Robust surveillance



Thank you



To make Rabies Elimination a reality!