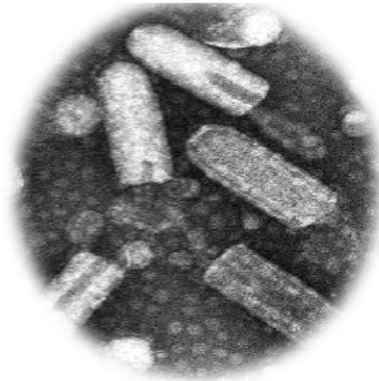




World Organisation  
for Animal Health  
Founded as OIE



## Occipital foramen approach for brain sampling, including sample preparation, sample transport and sample preservation



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## Preliminary Safety

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



- Do not aerosolize .

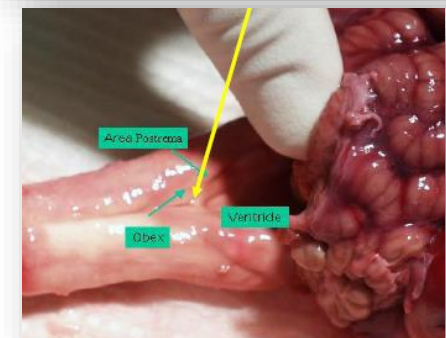
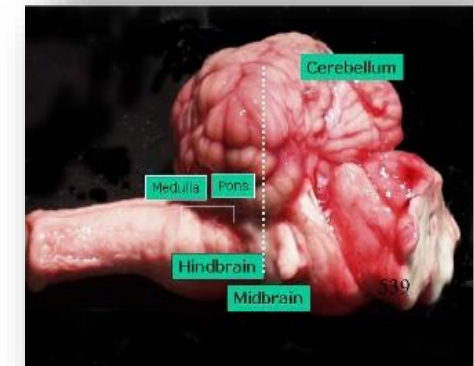
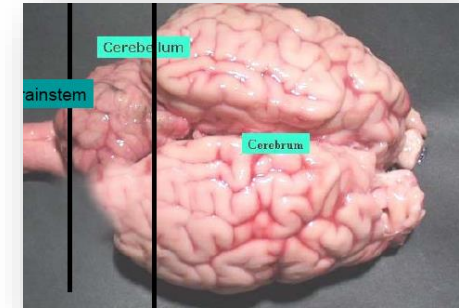
## Personal Protective equipment (PPE)

- 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

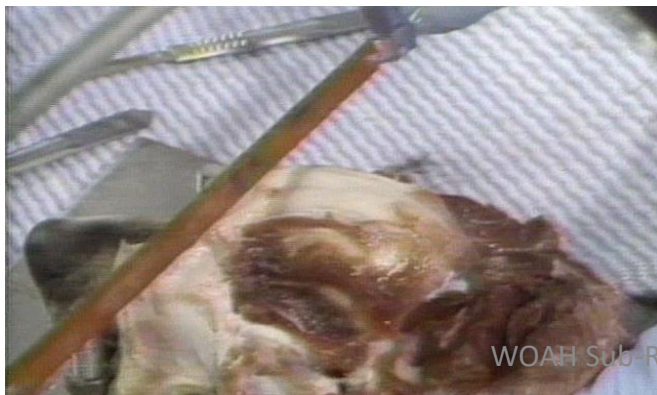
1. hammer & chisel
2. bone saw or hack saw
3. electric saw

# Removal of brain

## 1. Hammer & Chisel



## 2. Hack saw



## 3. Electric saw



# Retro-orbital route for brain sampling

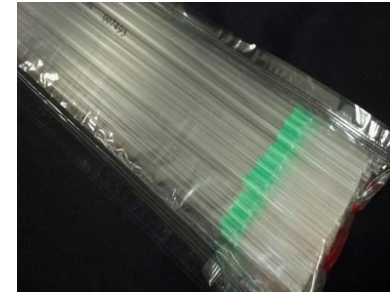
- A trocar used to make a hole in the posterior wall of the eye socket.
- A biopsy needle introduced through hole.





# 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 .**

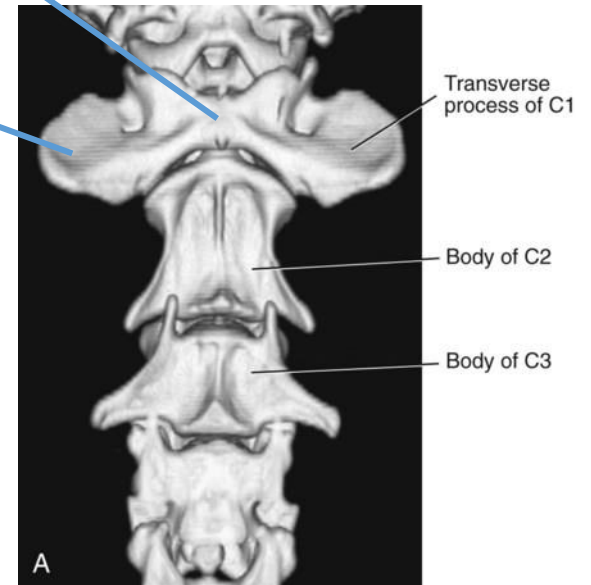
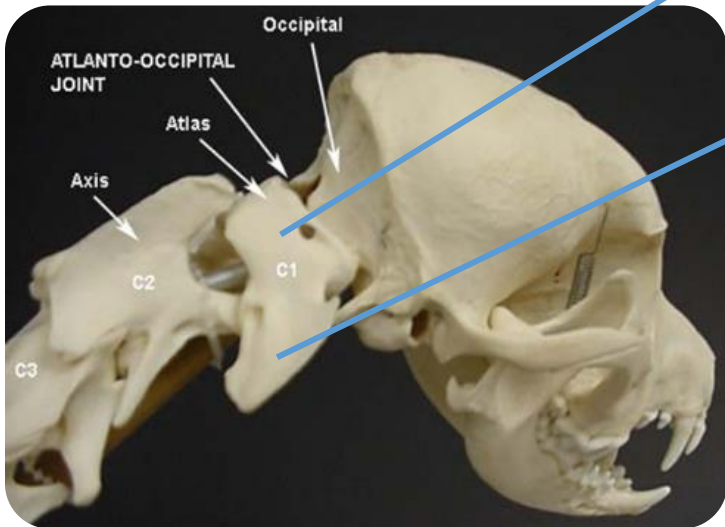
# Preservative Solutions

- **Brain sample:** 10% formal saline / Zenker's fluid / 50% glycerol saline
- **Salivary gland:** 50% glycerol saline
- CSF, saliva and urine: Tissue culture medium with 2% saline
- Specimens for cytology/**histopathology** : 10% neutral buffer formalin / Bouin's solution

# 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

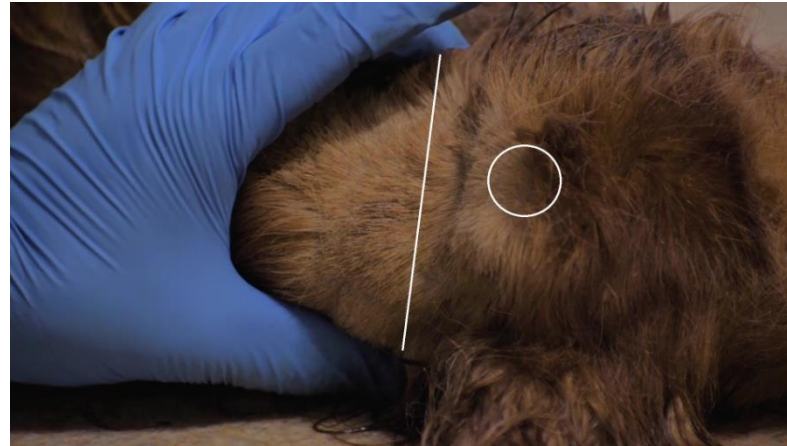


Old / preserved carcass:  
use syringe / AI sheath

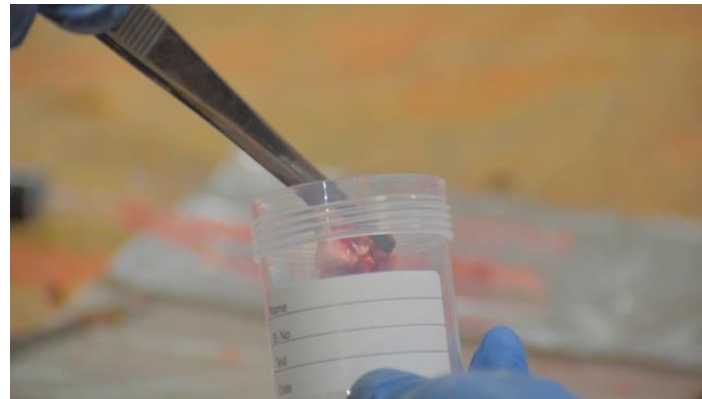




**Locate the transverse processes of the first C1 & external occipital protuberance. A deep cut be made just behind external occipital protuberance but anterior to imaginary line connecting the transverse processes of C1.**



**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**



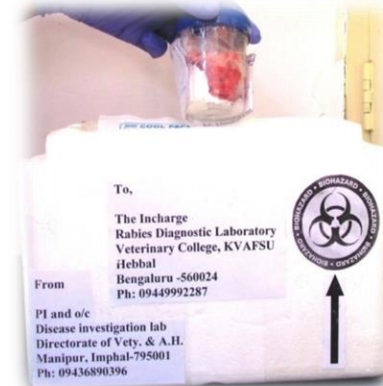
# Packing brain tissue in Primary, Secondary and tertiary containers



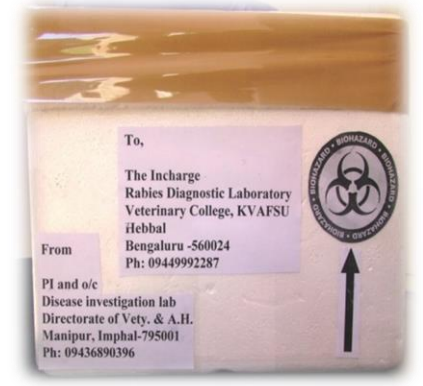
Primary container



Secondary container



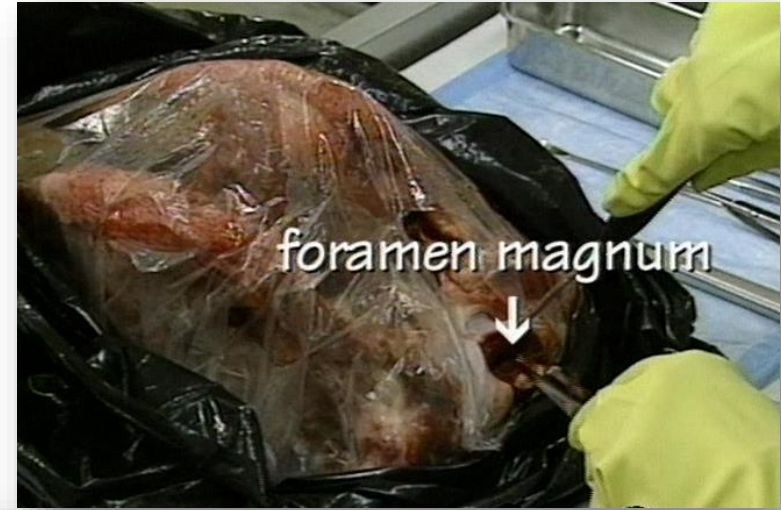
Tertiary container



# Occipital Foramen route for brain sampling routinely employed by trainees in the field



# Brain sampling if complete decapitation of head

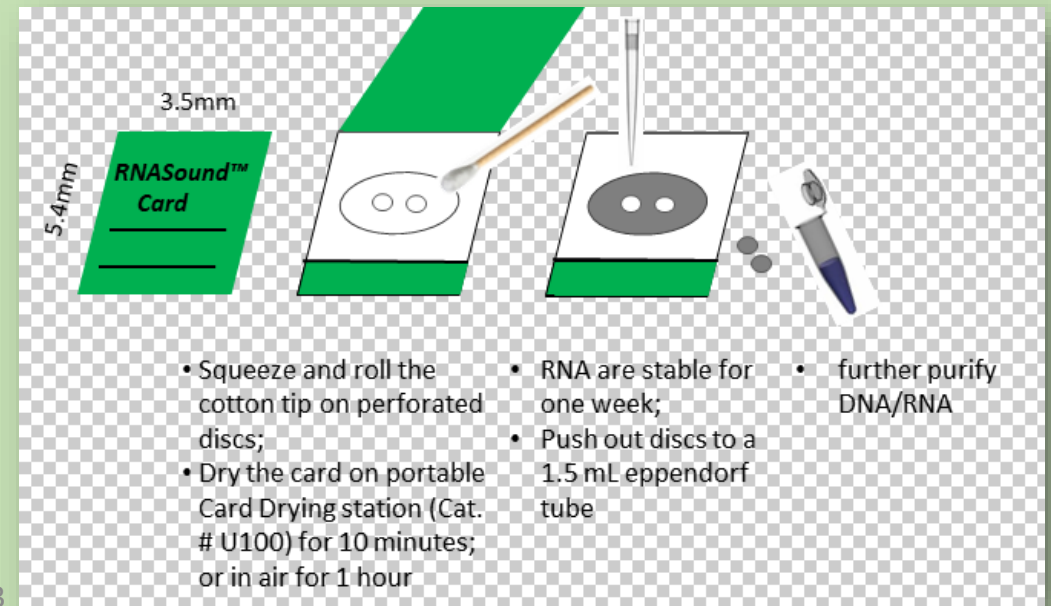


Wolf head



# Preservation for molecular techniques

- ✓ **Lysis buffers** for nucleic acid extraction and RNA preservation buffers **impregnated onto filter paper** (Picard-Meyer et al., 2007).
- ✓ Fit for **transport of specimens at ambient temperature without specific biohazard precautions** for detection of viral RNA and further genetic characterisation of rabies virus strains.



# 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.**