

Animal Rabies Testing in South Dakota

South Dakota One Health Seminar
October 30, 2017

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Objectives

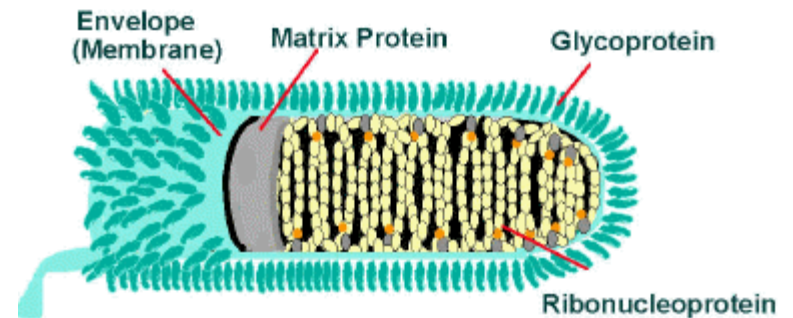
Preamalytical: overview of specimen collection, and packaging and shipping

Analytical: review contemporary methods for rabies detection

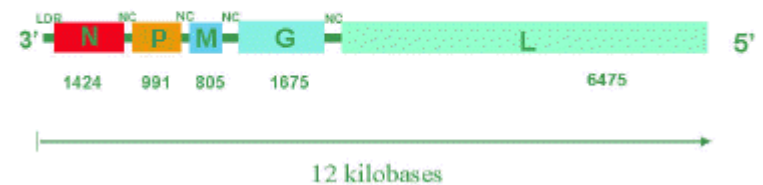
Postanalytical: reporting results and additional testing as needed

What is the Rabies Virus???

- Lyssavirus (Family Rhabdoviridae)
- Structure:
 - 180x75nm
 - Bullet-shaped
- Genome:
 - ssRNA
 - Nucleoprotein (N)
 - Phosphoprotein (P)
 - Matrix Protein (M)
 - Glycoprotein (G)
 - Polymerase (L)



Rabies Genome



Why is Rabies Important?

- For all the reasons discussed by Lon, Dustin, and others
 - Profoundly affects the central nervous system
 - Nearly always fatal
 - Significant impact on wildlife and domestic animals including pet animal and livestock

Rabies in South Dakota: an unclear but present danger

South Dakota ONE HEALTH
Sioux Falls
30 October 2017

- Rabies is a nearly always fatal, viral disease affecting the central nervous system.
- Transmitted when the virus is introduced into bite wounds or open cuts in skin, onto mucus membranes or by transplant.
- Infectious agent: RNA Lyssavirus

Lon Kightlinger, MSPH, Ph.D., State Epidemiologist
South Dakota Department of Health
Pierre, South Dakota



SOUTH DAKOTA
DEPARTMENT OF HEALTH

RABIES:
**DEALING WITH HUMAN
EXPOSURES**

SD One Health Seminar • October 30, 2017

Dustin Ortbahn, MPH
Disease Surveillance Coordinator

The poster features a central silhouette of a person falling or being pulled back, with several bats flying above. Silhouettes of a cat, a dog, and a raccoon are positioned at the bottom. The background is white with orange curved borders on the left and right sides. The South Dakota Department of Health logo is in the top left corner.

How is Rabies Virus Detected?

1. Identification of animals that meet defined criteria
2. Humane euthanasia when possible
3. Removal of head and/or brain tissue
4. Transport to an appropriate laboratory
5. Diagnostic testing of brain tissue
 - Direct fluorescence assay (gold standard)
 - Histopathology
 - Immunohistochemistry
 - Electron microscopy
 - Nucleic acid amplification

Specimen Criteria

- **The SDPHL accepts the following specimens:**
 - Small animal heads, bats, brains, brain material



Ring-tailed Lemur



Specimen Collection

- **The SDPHL does not accept:**
 - Whole animals
 - Livestock heads
 - Large breed dog heads
 - Severely decomposed specimens



Packaging and Shipping

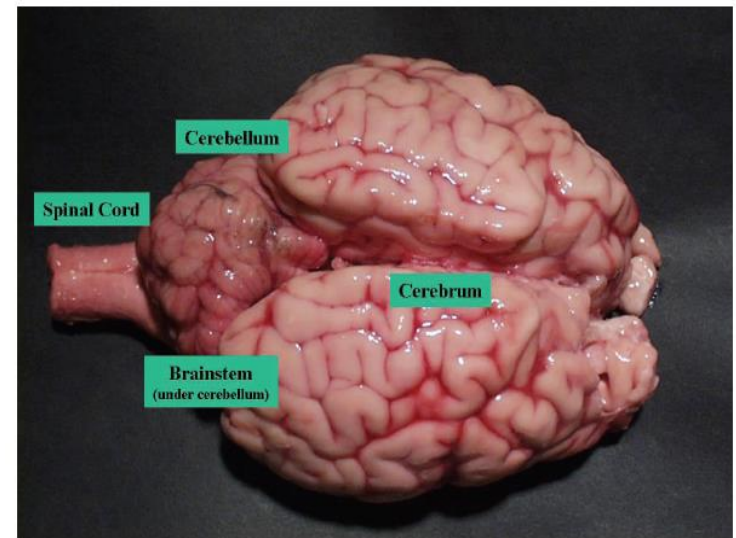
- Appropriate packaging materials include foam containers with cardboard overpack with cold packs



- At a minimum:
 - Make sure contents are secure
 - Packing materials are not leaking or soaked in fluids
 - Specimen is kept cold but not frozen

Why so much emphasis on specimen collection, packaging, and shipping?

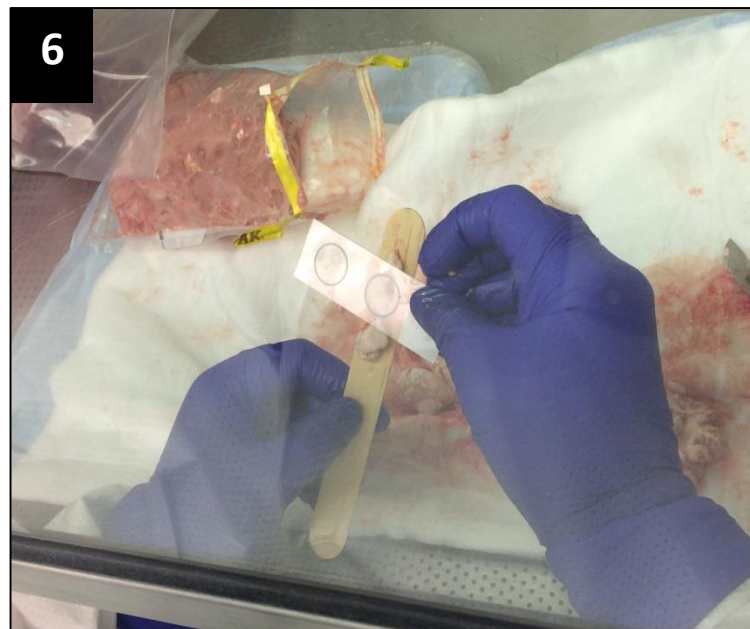
- Specimen quality is critical!
 - Avoid freeze:thaw cycles
 - Avoid deterioration
 - Avoid dispatching animal by head trauma
- Brainstem and cerebellum are most important for testing but the hippocampus can also be tested



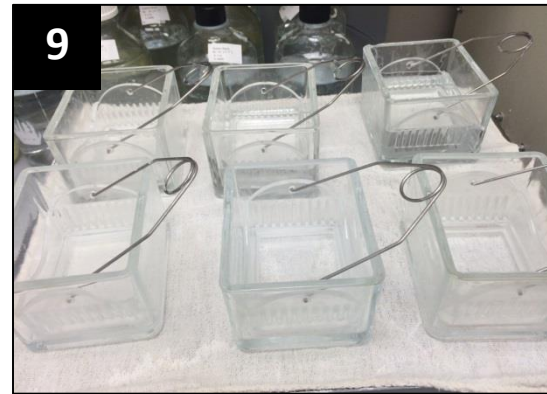
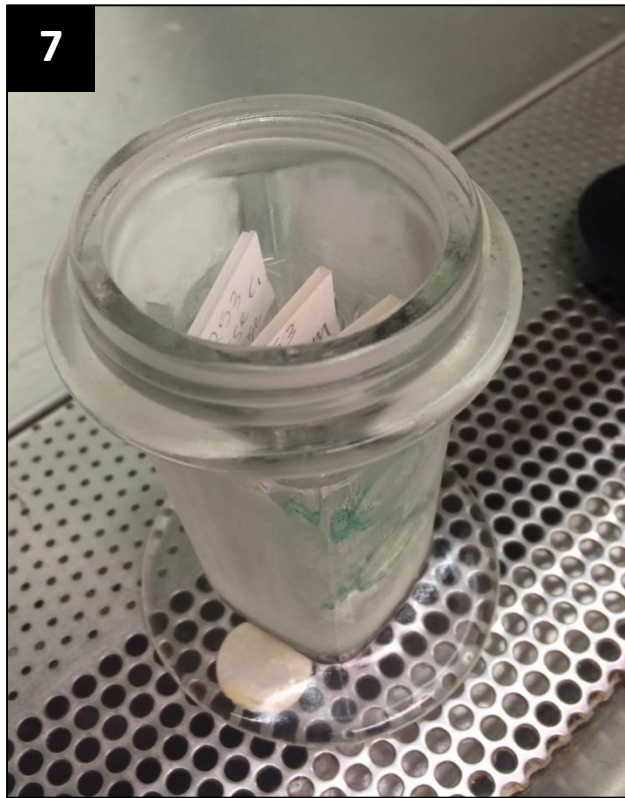
Specimen Preparation



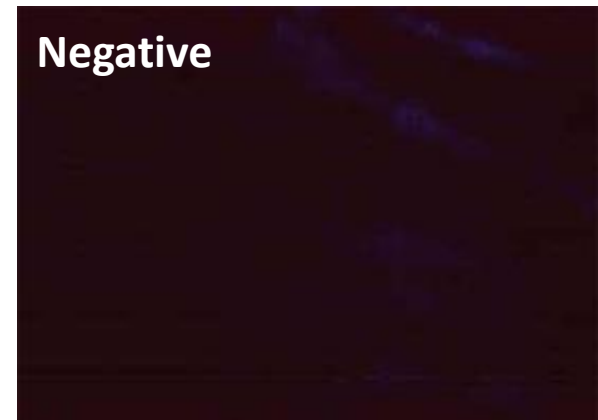
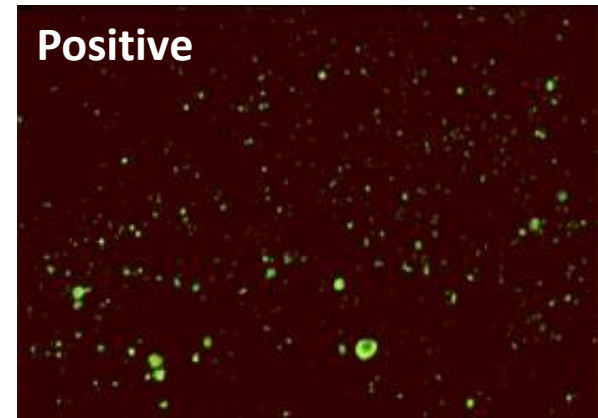
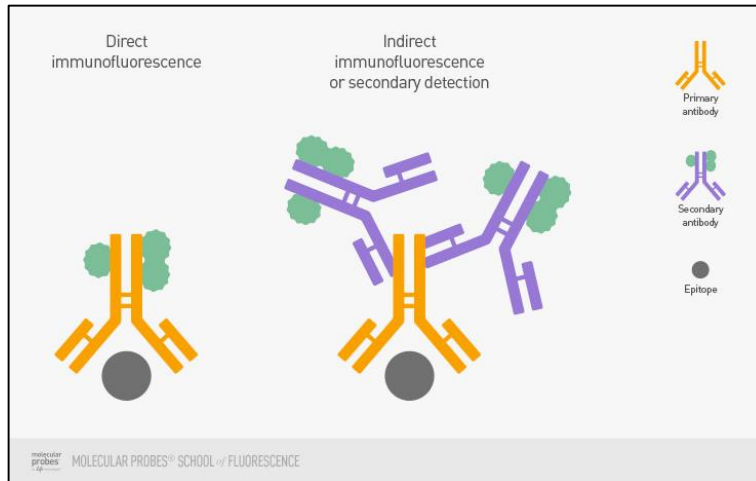
Impressions



Fixation and Staining

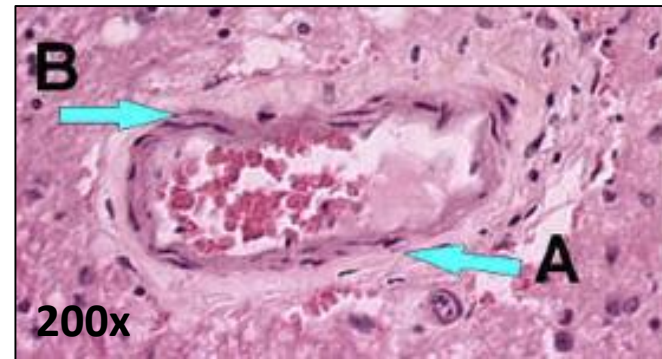
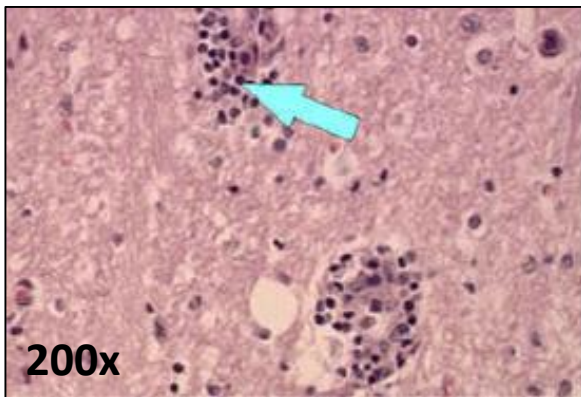
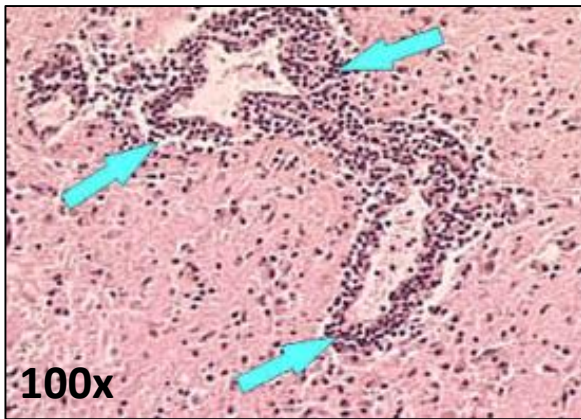


Direct Fluorescence Assay & Microscopy



- Microscopy is performed in duplicate by two trained scientists
- Positive and negative controls are also performed

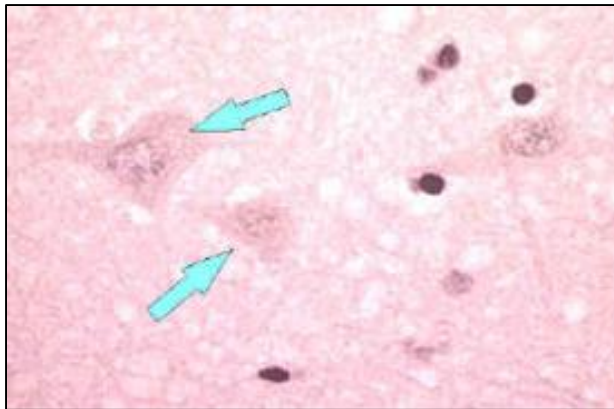
Other Methods for Rabies Detection: Histopathology



- H&E stained brain tissue
- Evidence of inflammation
- Perivascular cuffing of lymphocytes and PMNs

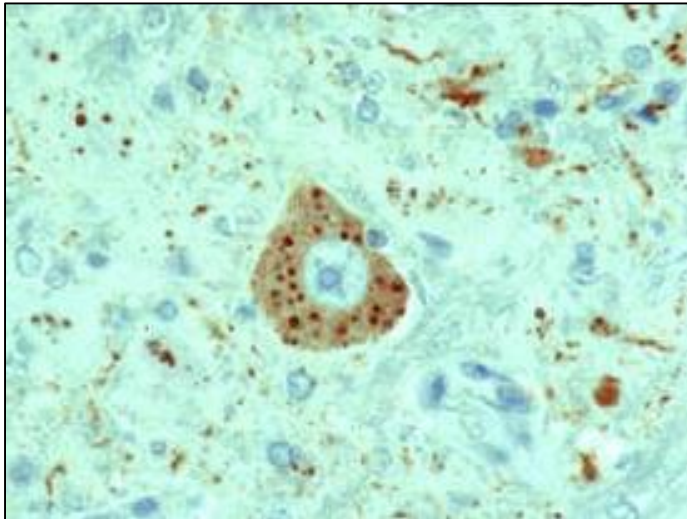
Other Methods for Rabies Detection:

Histopathology



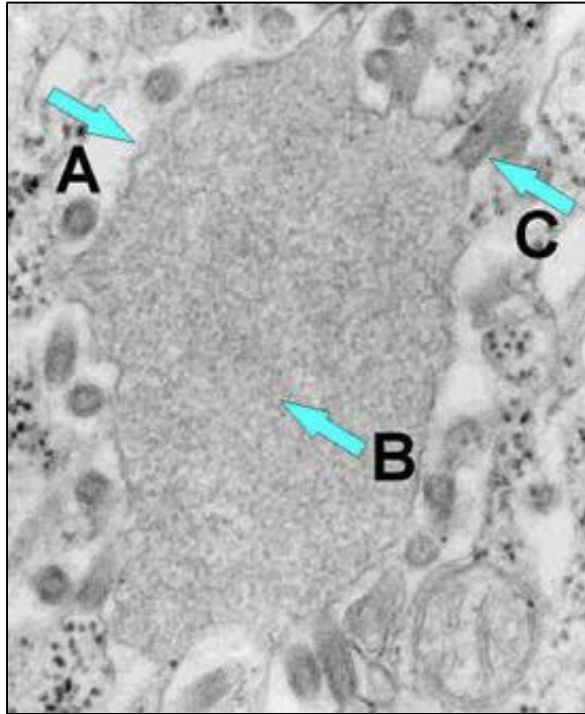
- Negri bodies
- Round/oval inclusions
 - Pyramidal cells of Ammon's horn
 - Purkinje cells of the cerebellum
 - Various other ganglia
- Mann's, Sellers or giemsa stains
- Detected in 50% of rabies cases
- Not diagnostic for rabies!!
- False positives do occur!!

Other Methods for Rabies Detection: Immunohistochemistry

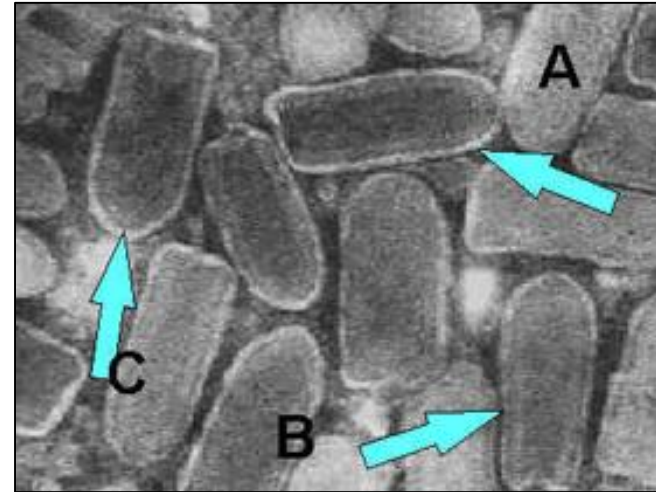


- Detection of rabies viral antigens using virus-specific antibodies
- Improved sensitivity compared to histological staining

Other Methods for Rabies Detection: Electron Microscopy

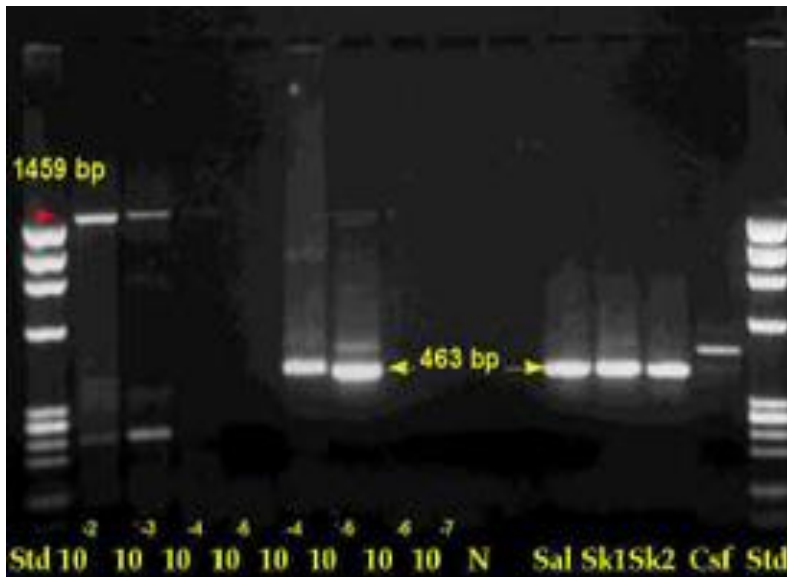


- Negri body (A)
- Viral ribonucleoprotein (B)
- Virus budding (C)



- Bullet-shaped virions with glycoprotein spikes (A-C)

Other Methods for Rabies Detection: Nucleic Acid Amplification



- Numerous assays in development
- Mostly PCR and rtPCR assays
- Detect specific gene sequences in the rabies virus genome

How are test results reported?

1. Rabies results are considered “critical values” so the SDPHL calls both ODPS and the veterinarian with positive results.
2. For clients that use our secure fax option, a report will be released the same-day between 3pm and 5pm.
3. For clients that do not use our secure fax option, a report is mailed the next business day.

Is additional testing ever performed?

1. Indeterminate results may warrant additional testing.
2. The SDPHL will send specimens to the CDC upon request.

Review

1. Identification of animals that meet defined criteria
 - ✓ Contact the Department of Health following human exposure
2. Humane euthanasia when possible
 - ✓ Avoid trauma to the brain
3. Removal of head and/or brain tissue
 - ✓ Avoid trauma to the brain; seek veterinarian assistance
4. Transport to an appropriate laboratory
 - ✓ Prompt; no leaking fluids; cold but not frozen
5. Diagnostic testing of brain tissue by DFA
6. Results to veterinarian and ODPS

