



*Michigan Department of Community Health
Bureau of Laboratories*

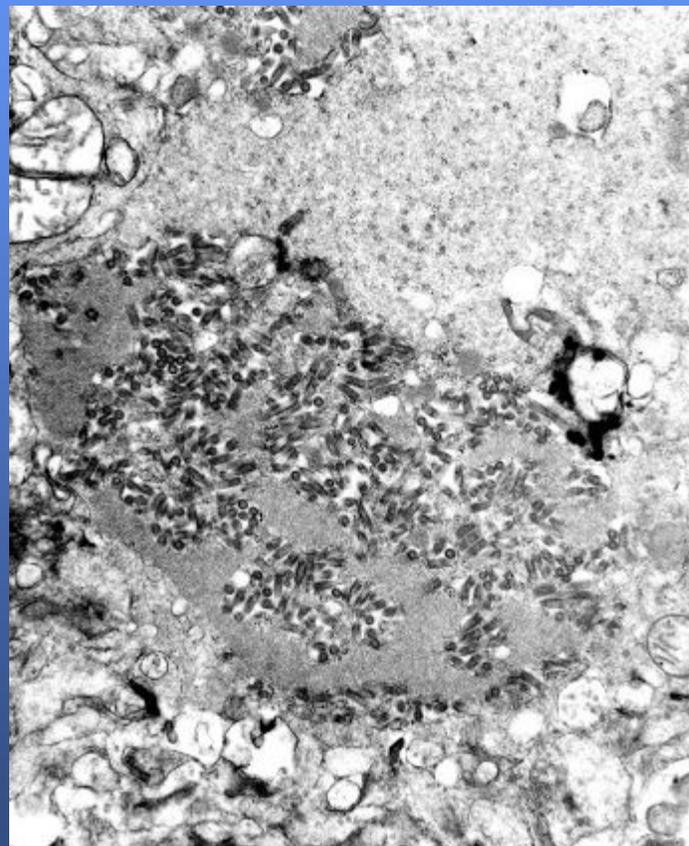
*Laboratory Issues
Good Specimens Lead to Good
Results*

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Overview

- Recent CDC Protocol Changes
- Proper specimen collection
- Proper specimen submission and transport
- Emergency weekend & holiday testing



CDC, PHIL

CDC Procedural Changes

- Rabies Direct Fluorescent Antibody (DFA) Test protocol revisions instituted in 2002.
- Resulted from the findings of the National Working Group on Rabies Prevention and Control (Hanlon et al., JAVMA, 215 (10):1444-1446, November 15, 1999).



Reasons for Recommended Changes

- Survey of labs revealed modifications to almost all steps of the FA test.
- Quality of commercial diagnostic reagents and a decreasing number of producers.
- Lack of vital communication between labs.
- Need for transfer of advanced diagnosis of rabies to state labs.



Goal of New Recommendations

- The improvement of the overall quality of rabies testing through the formulation of guidelines and standards for equipment, reagents, training, laboratory protocols, quality assurance, and laboratory policy for rabies diagnosis.
 - Establish a single protocol by which all other methods could be validated by comparison.
 - Avoid diagnostic dilemmas in routine rabies testing.



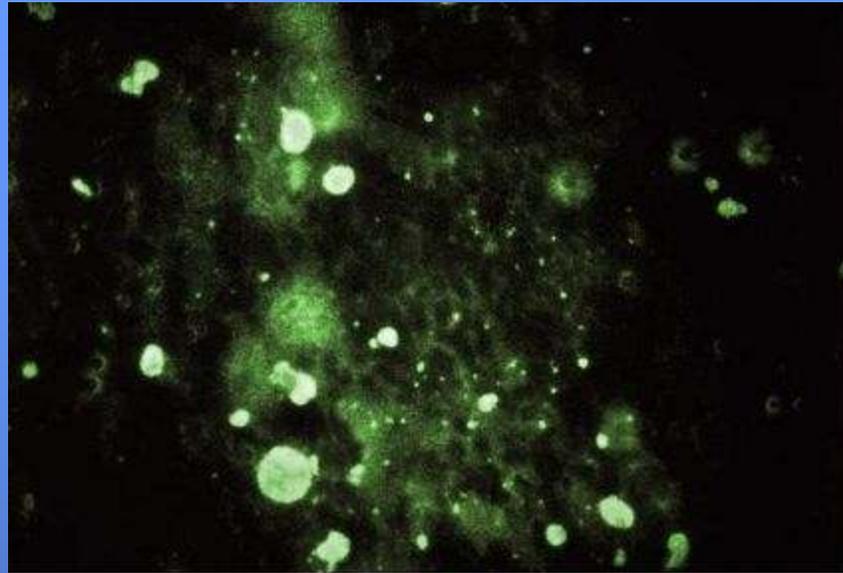
New Standard Protocol Requires:

- Adequate sampling of brain tissues from the brain stem (cross section through pons or medulla) and from cerebellum (vermis, right and left lobes).
- Acetone fixation in a separate container for each sample's slides.
- Use of 2 anti-rabies conjugates in every test.



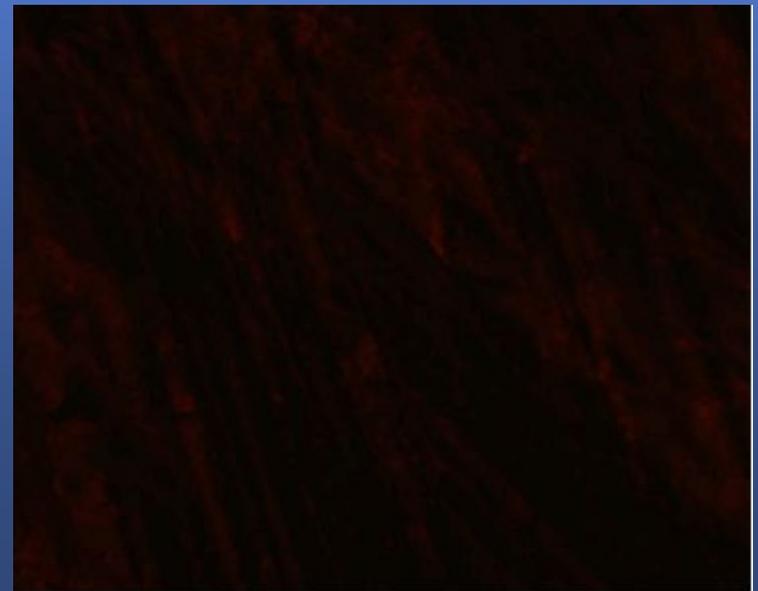
New Standard Protocol Requires:

- Rinse slides in separate containers.
- Use of 20% glycerol Tris buffered mountant.
- Two readers to evaluate test slides.
- Repeat testing of weakly positive and inconclusive tests using a rabies conjugate and specificity (negative) control conjugate.



Rabies
Positive
DFA. From
CDC, PHIL.

Rabies Negative
DFA. From CDC.



Rabies Pathogenesis – Rational for Specimen Collection

- Rabies viral antigen is widespread in most infected animals.
- BUT, may be unilateral especially in large animals (livestock).
- Thorough examination of the brain stem and cerebellum is necessary for accurate diagnosis.
- A cross-section through the brain stem will maximize ascending and descending nerve tracts.

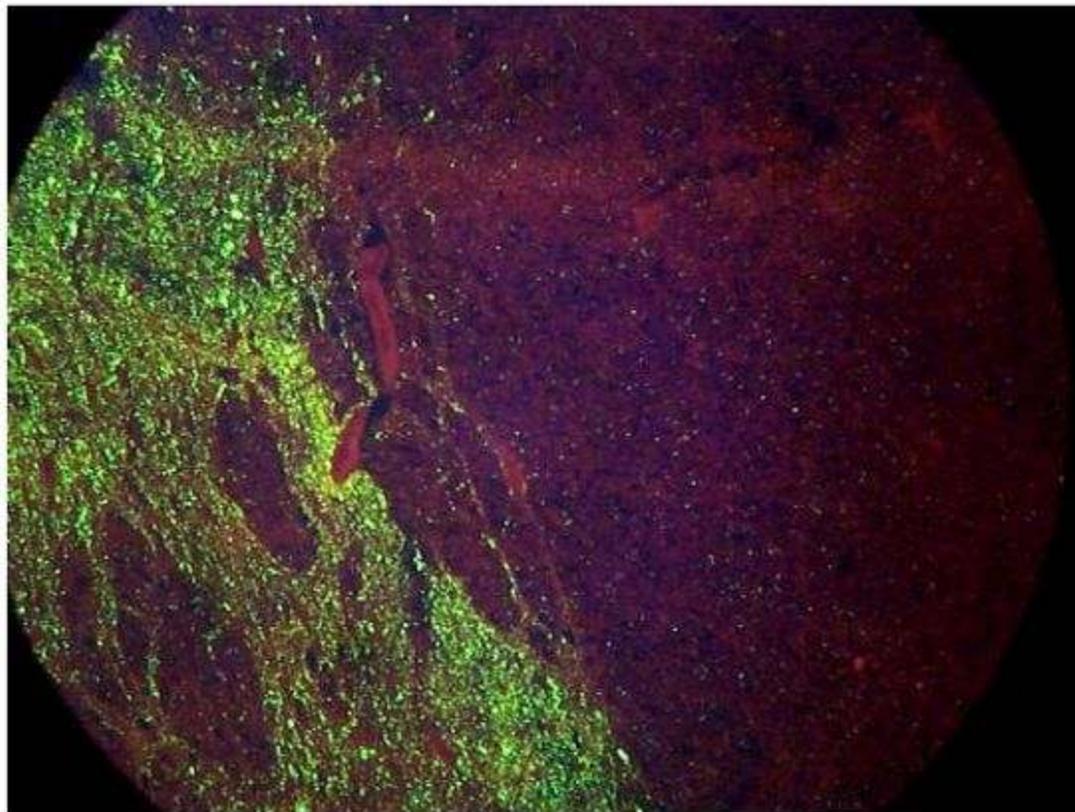


Figure 1. DFA test of a transverse section (cross-section) of Brain stem from a rabies infected donkey showing unilateral virus spread (200X magnification, frozen section).

From CDC

Specimen Collection

- Avoid cross-contamination.
- Mistakes made at necropsy cannot be easily resolved by repeat testing and/or performance of confirmatory tests.
- Rabies diagnosis requires fresh (NOT FIXED) brain tissue from the brainstem and cerebellum.
- A full cross-section of the mid-cerebellum and subjacent rostral brain stem are required for a valid test.

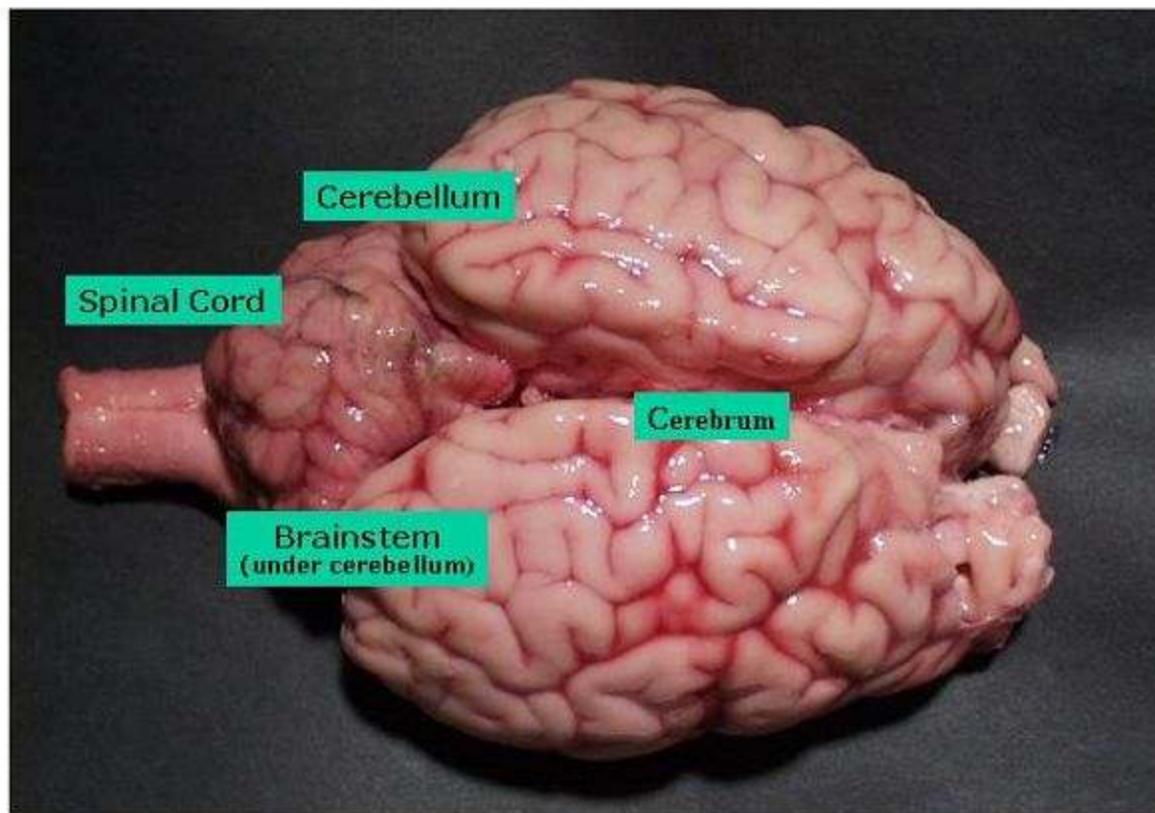


Figure 2. Dorsal view of brain.

From CDC

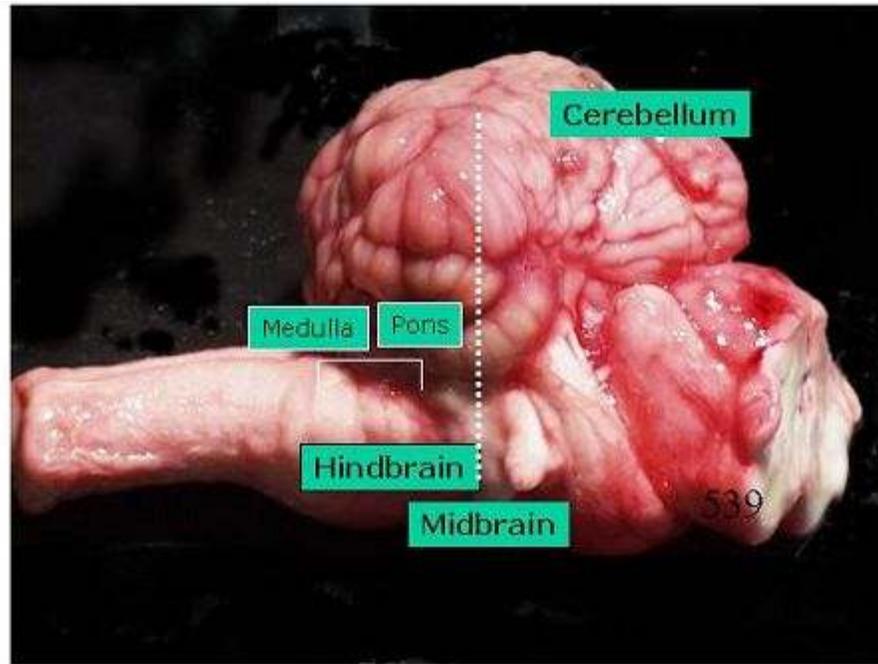


Figure 4. Lateral view of brain with cerebrum removed to show the extension of brain stem beneath the cerebellum. A rabies diagnosis should include an observation of the cut surface of a cross section of the brain stem (through the medulla, pons, or midbrain area) and the cerebellum (through each hemisphere and the vermis). For example, a cross section of the midbrain area (dashed line) would include all tissues necessary for rabies diagnosis.

From CDC



Specimen Collection Pitfalls

- Damage to brain from improper euthanization techniques.
 - Shooting in head.
 - Stepping on bat.
- Damage to brain from improper storage after death.
 - Burial for days or weeks.
- Freezing the brain.
- Incomplete spinal cord or cerebellum.

Specimen Submission

- Appropriate packaging.
 - Must not be leaking.
 - Place in securely sealed plastic bag.
 - Place test request form in small plastic bag.
 - Ship in plastic bucket in cardboard box.
- Label as diagnostic specimen.
- Ship by fastest means to lab: Express Mail, private courier or FedEx.

Specimen Submission

- Be aware of ambient temperatures.
 - Shipping in extreme temperatures can result in either an unsatisfactory sample or a delay in testing.
- Emergency State-Wide Transport System
 - Available for emergencies only.
 - Must be pre-approved by calling MDCH at 517-335-8063 or 517-335-9030.

Unacceptable Specimens

- Deteriorated or decomposed samples.
- Formalin-fixed tissues.
- Specimens with incomplete cross sections of spinal cord or cerebellum.
- Repeated freeze-thaw cycles may reduce test sensitivity.



Weekend & Holiday Testing

- Must be pre-approved.
 - 517-335-8063 during normal work hours
 - 517-335-9030 after hours
- Animal not current on its vaccination.
- Human exposure.

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Questions?

www.michigan.gov/mdchlab