

Direct Rapid Immunohistochemistry in diagnosis of rabies in animals

Shrikrishna Isloor,
Prof. & Lab. Director

KVAFSU-CVA Rabies Diagnostic Laboratory

WOAH (Founded as OIE) Reference Laboratory for Rabies

Dept. of Vet. Microbiology, Veterinary College, KVAFSU,

Hebbal, Bengaluru, INDIA

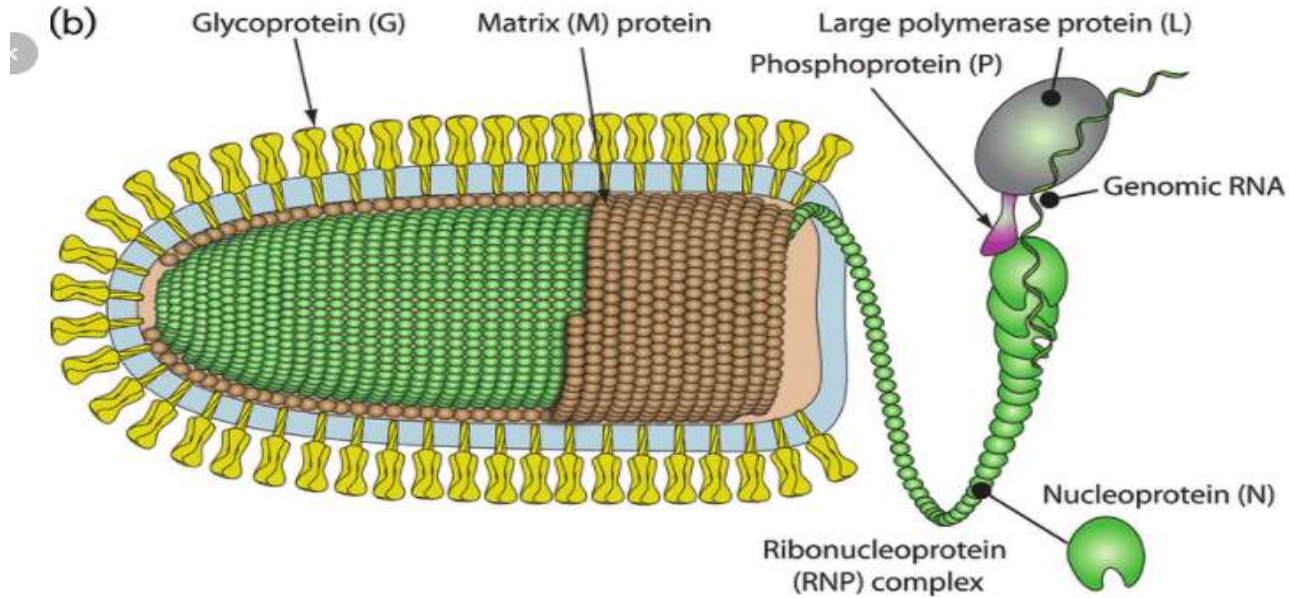


KVAFSU-CVA- Crucell Rabies Diagnostic Lab. Established in 2013

“Human Mab Combination for PEP of Rabies - Evaluation Versus Indian Natural Rabies Virus Strains”



N protein – Diagnosis



Rabies Genome



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



Laboratory diagnosis



Immunodiagnosics

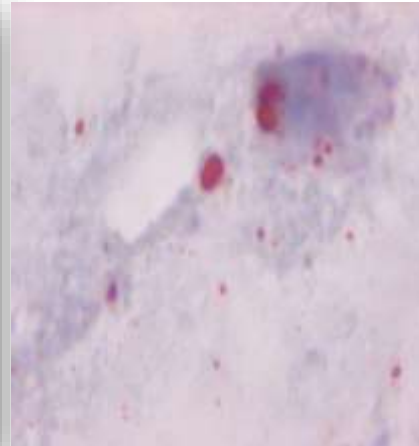
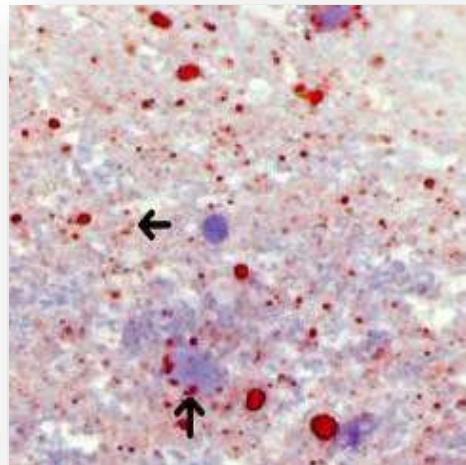
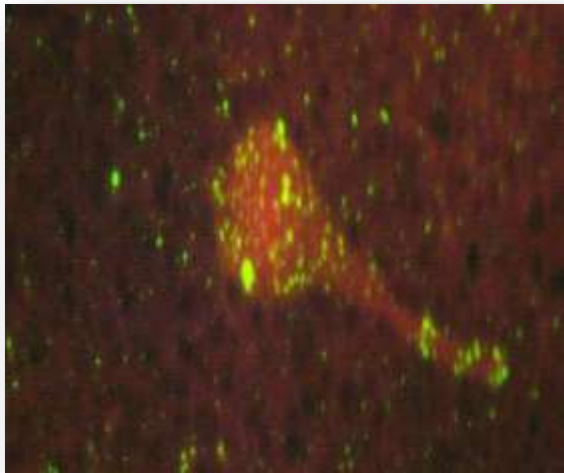
DFA (WHO-WOAH approved)



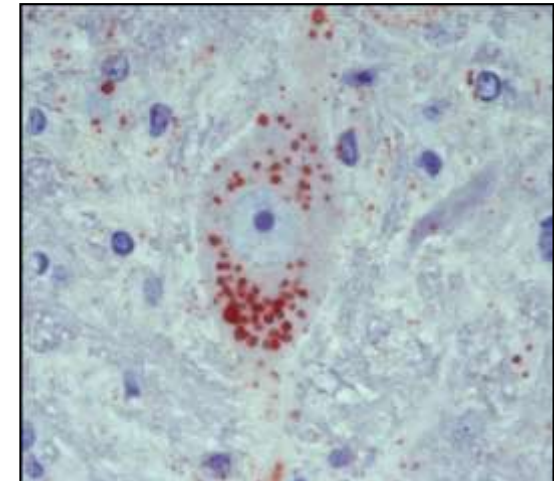
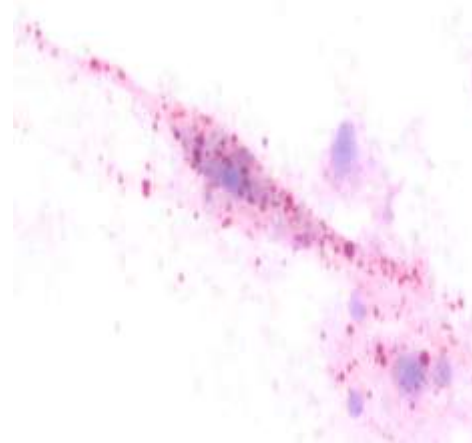
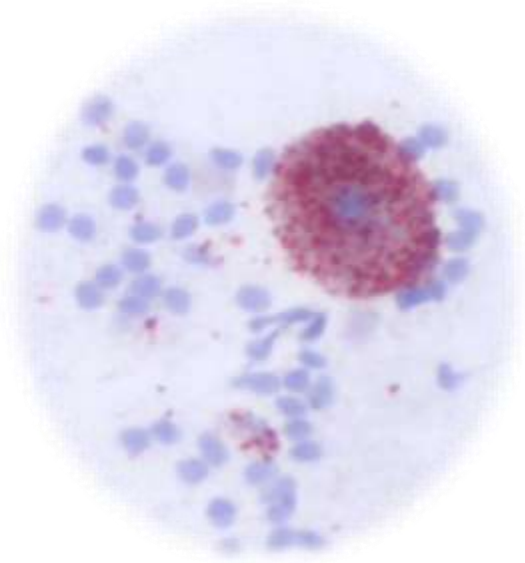
dRIT



LFA



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



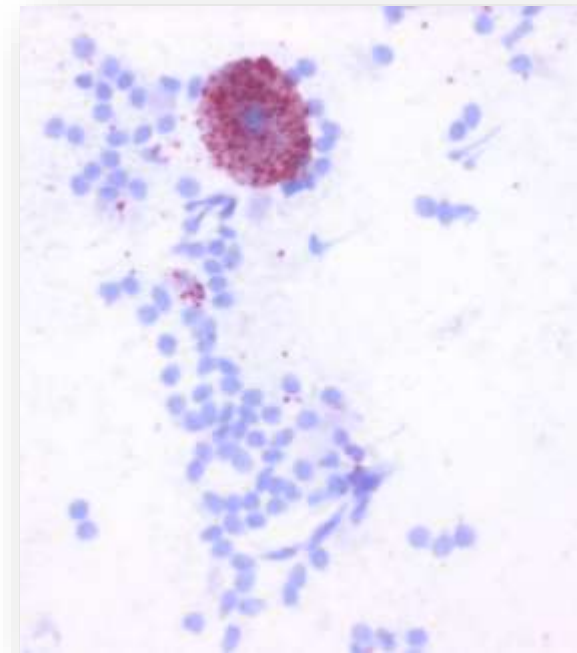
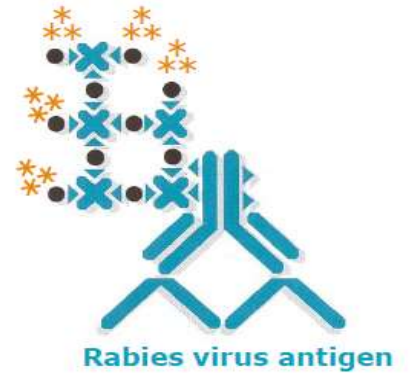
Direct Rapid Immunohistochemistry Test (dRIT)

- 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

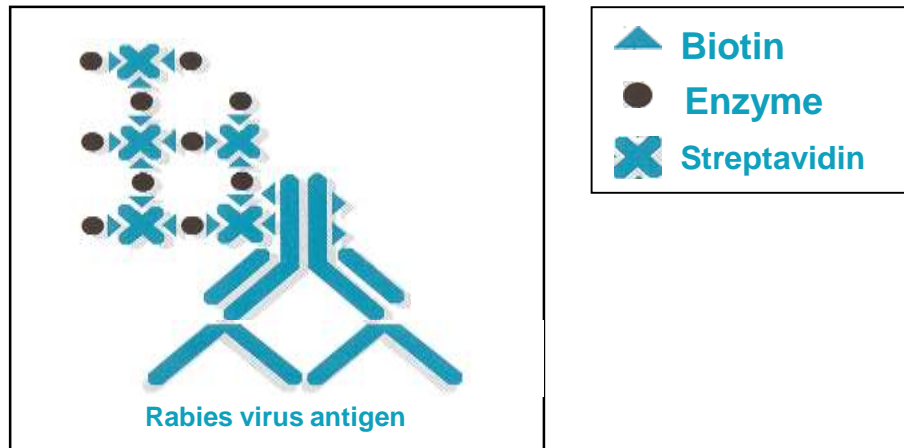


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Diagnosis for South Asia, 18th – 21st January, 2023

Direct RIT

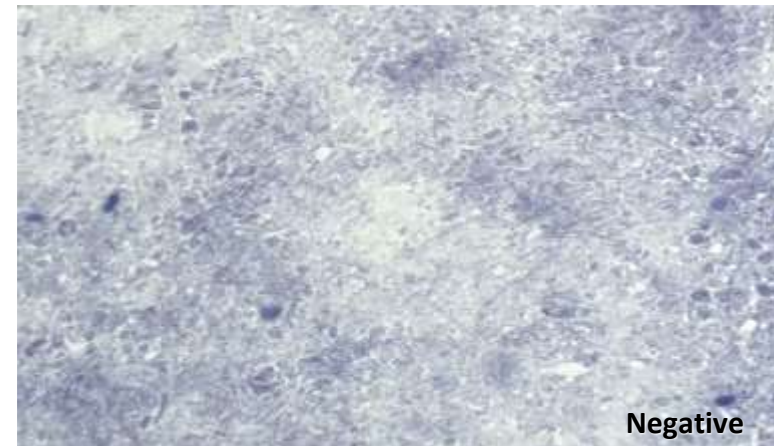


Direct rapid immunohistochemistry test (DRIT) for rabies diagnosis



General Procedure (52 minutes)

1. Brain touch impressions on slides.
2. Slides allowed to air-dry.
3. Fixation in 10% buffered formalin for 10 minutes.
4. Treat with hydrogen peroxide for 10 minutes.
5. Incubate slides with primary biotinylated antibody for 10 minutes.
6. Incubate with streptavidin-peroxidase 10 minutes.
7. Incubate with peroxidase substrate 10 minutes. (3-amino-9-ethylcarbazole, AEC)
8. Counterstain (Gill's hematoxylin) for 2 minutes.
9. Mount with water-soluble mounting media and read.



CDC SOP for dRIT

Make routine touch impressions



Air dry for 5 min. at RT



Inactivate and fix by immersing in 10 % Neutral buffered formalin at RT for 10 min.



Dip-rinse the slides in wash buffer TPBS (PBS with 1 % tween 80) (2 times)



Immerse in 3 % H₂O₂ for 10 min. to neutralize the endogenous peroxidases



Dip-rinse the slides in wash buffer TPBS (PBS with 1 % tween 80) (2 times)



Incubate in a humidity chamber at RT with biotinylated anti-rabies mAb for 10 min

Contd...

Dip-rinse the slides in wash buffer TPBS (PBS with 1 % tween 80) (2 times)

Incubate with streptavidin-peroxidase in a humidity chamber at RT for 10 min

Dip-rinse the slides in wash buffer TPBS (PBS with 1 % tween 80) (2 times)

Incubate with peroxidase substrate, amino-ethyl carbizole (AEC) in a humidity chamber at RT for 10 min.

Dip-rinse the slides in DW (2 times)

Counterstain with Gills Hematoxylin (diluted 1:1 with deionized/DW) for 2 min.

Dip-rinse the slides in DW (3 times)

Mount the slices with aqueous mounting medium with the help of cover-slip.

Microscopic observation by light microscopy

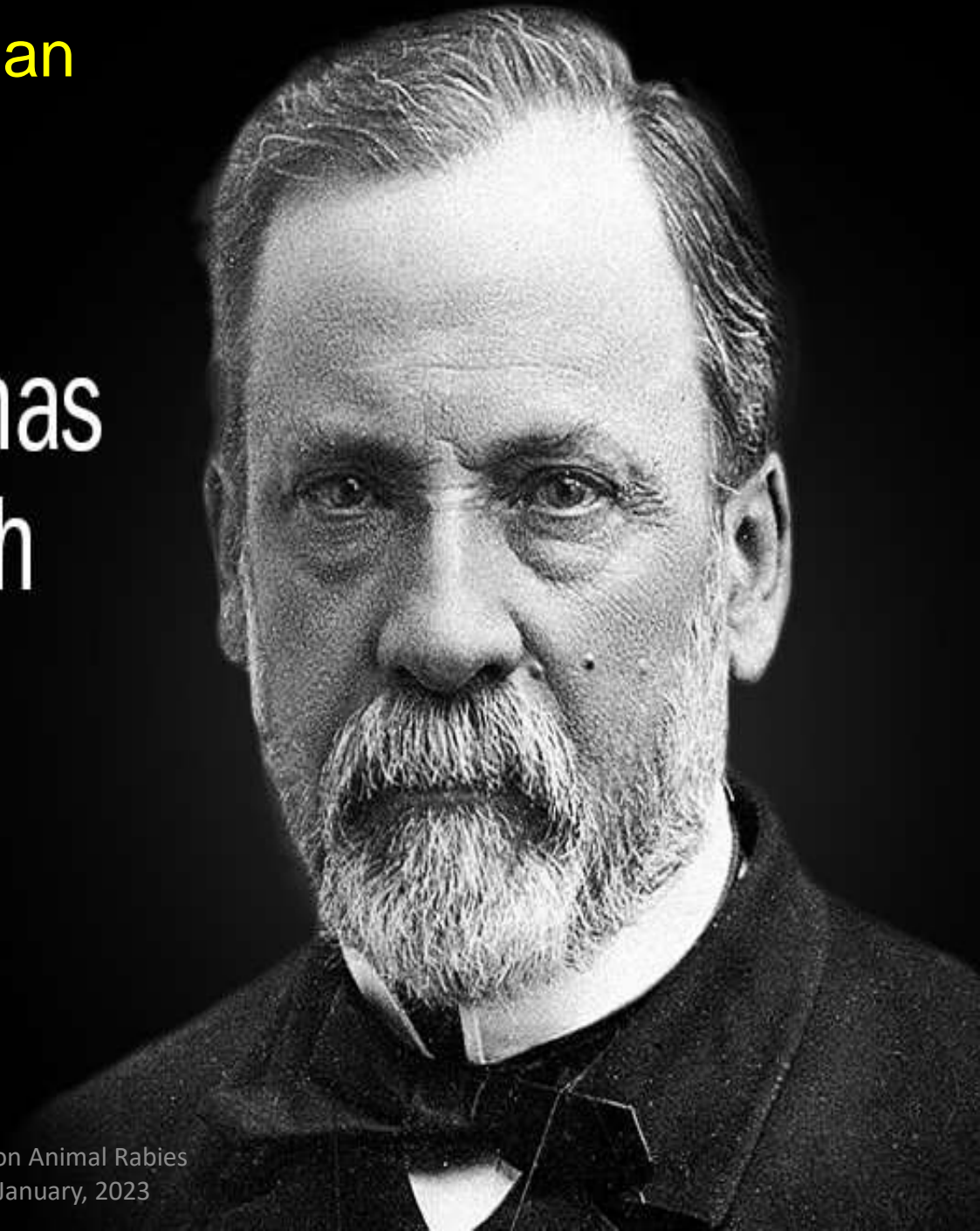
Conclusion

- ✓ 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

Lets work collectively against Rabies with an attitude of never give up....

Let me tell you the **secret** that has led me to my goal. My strength lies solely in my **tenacity**.

— *Louis Pasteur*



Salutations to Father of Microbiology / vaccinology...



WOAH Sub-Regional Training Workshop on Animal Rabies
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..at Pasteur Institute, Paris, France on 15th Dec. 2022



Global elimination of dog mediated rabies by 2030 – WOAHA , a leader

...at World Organization for Animal Health (WOAH); 12-14th Dec. 2022





Thank you



To make Rabies Elimination a reality!

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