

Laboratory Diagnosis of Human Rabies



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Rabies: Global Disease Burden

- 'Rabies' is an acute, progressive, fatal encephalomyelitis
- Infection by viruses of genus Lyssavirus
- Annual number of human rabies deaths globally 61,000
- Vast majority of deaths (84%) in rural areas
- Estimated annual cost of rabies is US\$ 6 billion
- Maximum burden of human rabies in Asia and Africa

Year of estimate	Africa	China	India	All Asia	All Asia & Africa	World
2010	23 800	7450	16 450	34 500	58 300	61 000

WHO Expert Consultation on Rabies. Third report. Geneva, World Health Organization, 2018
WHO Technical Report Series, No. 1012

Reassessing the burden of human rabies-India

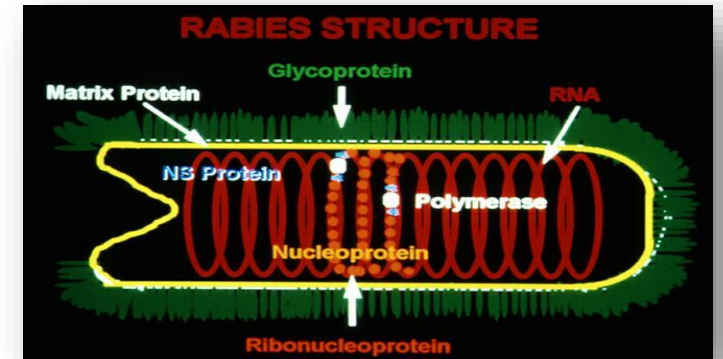
- A community-based nationwide cross-sectional survey with a multistage cluster-sampling design
- From March 2022 to August 2023, covering 60 districts in 15 Indian states
- Annual human rabies deaths were estimated using a decision-tree probability model
- The estimated human rabies deaths occurring annually in India: 5726



Thangaraj JWV et al. Estimates of the burden of human rabies deaths and animal bites in India, 2022-23: a community-based cross-sectional survey and probability decision-tree modelling study. Lancet Infect Dis. 2024 Sep 30:S1473-3099(24)00490-0.

The Virus

- Genus *Lyssavirus*
- Family *Rhabdoviridae*, order *Mononegavirales*
- Bullet shaped
- Measures 180 nm x 75 nm
- Enveloped (lipoprotein) surrounded by spikes
- Single stranded negative sense RNA of 12 kb
- Codes for 5 major proteins:
 - nucleoprotein N
 - phosphoprotein P
 - matrix protein M
 - glycoprotein G
 - polymerase L

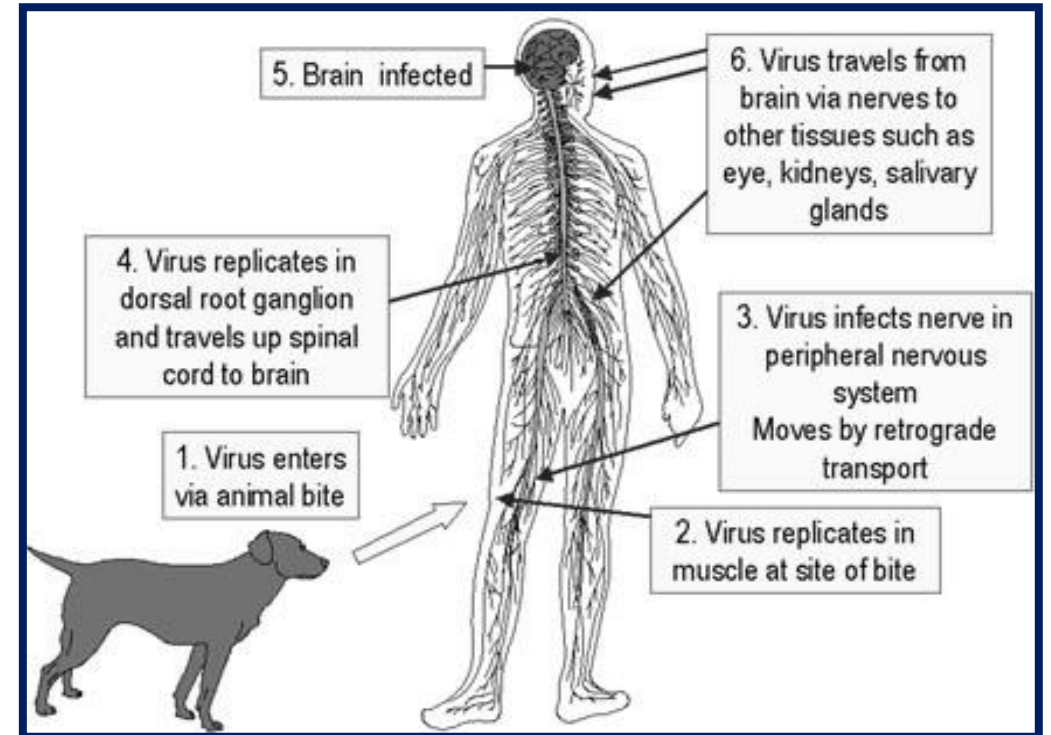


The Lyssaviruses

Species	Virus	Countries of virus isolation*	Most common reservoir based on virus detection
<i>Phylogroup I</i>			
Rabies lyssavirus	Rabies virus (RABV) [‡]	Global	All mammals
Aravan lyssavirus	Aravan virus (ARAV)	Kyrgyzstan	Bats
Australian bat lyssavirus	Australian bat lyssavirus (ABLV) [‡]	Australia	Bats
Bokeloh bat lyssavirus	Bokeloh bat lyssavirus (BBLV)	France and Germany	Bats
Duvenhage lyssavirus	Duvenhage virus (DUVV) [‡]	Kenya and South Africa	Bats
European bat 1 lyssavirus	European bat lyssavirus 1 (EBLV-1) [‡]	Belgium, Denmark, France, Germany, the Netherlands, Poland, Russia, Slovakia, Spain and Ukraine	Bats
European bat 2 lyssavirus	European bat lyssavirus 2 (EBLV-2) [‡]	Denmark, Finland, France, Germany, the Netherlands, Norway, Switzerland and the United Kingdom	Bats
Gannoruwa bat lyssavirus	Gannoruwa bat lyssavirus (GBLV)	Sri Lanka	Bats
Irkut lyssavirus	Irkut virus (IRKV) [‡]	China and Russia	Bats
Khujand lyssavirus	Khujand virus (KHUV)	Tajikistan	Bats
<i>Phylogroup II</i>			
Lagos bat lyssavirus	Lagos bat virus (LBV)	Central African Republic, Ethiopia, France [§] , Ghana, Nigeria, Senegal, South Africa and Zimbabwe	Bats
Mokola lyssavirus	Mokola virus (MOKV) [‡]	Cameroon, Central African Republic, Ethiopia, Nigeria, South Africa and Zimbabwe	Rodents and domestic animals
Shimoni bat lyssavirus	Shimoni bat virus (SHIBV)	Kenya	Bats
<i>Phylogroup III</i>			
Ikoma lyssavirus	Ikoma lyssavirus (IKOV)	Tanzania	African civet
Lleida bat lyssavirus	Lleida bat lyssavirus (LLEBV)	Spain	Bats
West Caucasian bat lyssavirus	West Caucasian bat virus (WCBV)	Russia	Bats

Transmission

- **Rabid animal bites/exposures**
- **Aerosols- caves inhabited by rabid bats**
- **Lab workers-Aerosols**
- **Handling/skinning infected carcasses of rabid animals (Skin, conjunctiva, mucous membranes)**
- **Corneal/Organ transplantation**



Clinical Course

- Incubation period: **20-90 days**
- Two clinical forms: **Encephalitic and Paralytic**
- Uniformly fatal with onset of symptoms
- No effective, validated treatment for rabies
- Treatment mainly symptomatic
- Focus on palliative measures to ensure prompt, effective and compassionate care to alleviate suffering
- Less than 30 rabies survivors reported world-wide



Need for Lab Confirmation

- **Differential Diagnosis**

- Autoimmune (NMDAR) encephalitis
- Guillain-Barre syndrome (GBS)
- Post-vaccinal encephalomyelitis
- Tetanus
- Other infections-JE, WNV, HSV, cerebral malaria
- Acute psychotic disorders
- Illicit drug use
- Scorpion/snake envenomations
- Organophosphate poisoning

Need for Lab Confirmation

- **Clinical Management & Infection Control**

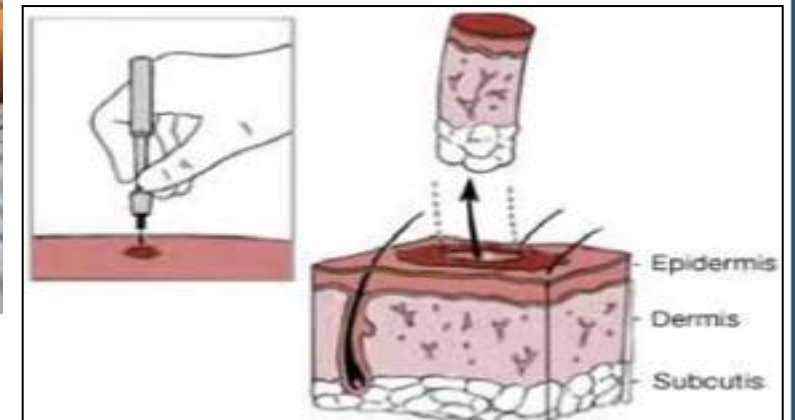
- Patient management/Barrier Nursing
- Prognostication; Case closure and grief counseling
- Prophylactic vaccination to close contacts
- Corneal/organ transplants-screening of donors
- Precautions-Autopsy/embalming/disposal

- **Disease Surveillance**

- Characterization of causative agent
- Surveillance, estimation of burden, disease dynamics
- Confirmation/Monitoring of 'rabies-free' areas

Sample Collection (Antemortem)

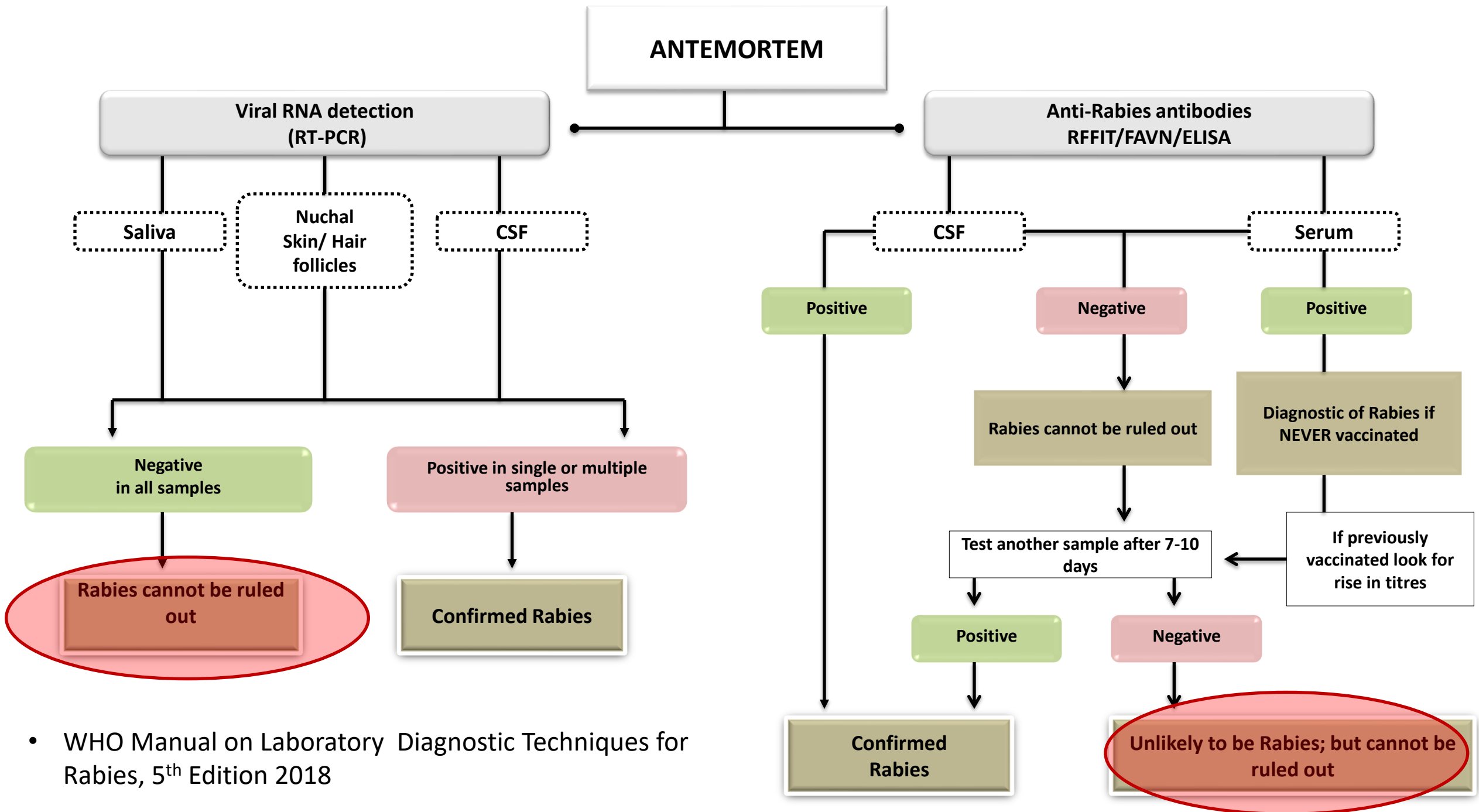
- **Saliva:** Pooled saliva; at least 3 serial samples at 3-6 hr interval
Pool saliva in a sterile leak-proof screw capped tube/container
 - **CSF:** 0.5-1 ml of CSF in a sterile vacutainer/container
 - **Nuchal skin biopsy:** 5-6 mm diameter with 10-12 hair follicles from the nape of the neck. Full thickness **Punch biopsy** preferred. Place the specimen in a sterile container.
 - **Serum:** Collect about 3 ml blood in a plain blood collection tube (without anticoagulants). Separate the serum by centrifugation. Transport serum (1ml) to the laboratory.
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- **Do NOT add formalin or any other liquid to the containers**
 - **Transport to the laboratory in cold chain at 2 - 8°C**
 - **Triple packaging; bio-safety measures**



Antemortem Diagnosis

	SALIVA	SKIN	CSF	BLOOD	CORNEAL SMEAR
• PCR for viral RNA	✓	✓	✓	✗	
• dFA for viral antigen		✓			✓
• Antibody detection			✓	✓	

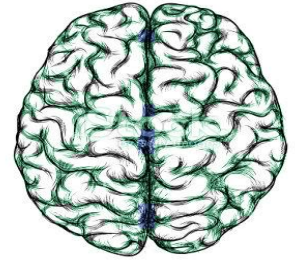
- Testing multiple/serial samples may be required to confirm diagnosis
- Negative results do not exclude diagnosis



- WHO Manual on Laboratory Diagnostic Techniques for Rabies, 5th Edition 2018

Sample Collection (Postmortem)

Postmortem brain tissue



- **Craniotomy: Consent for autopsy, challenging**
- **Orbital or transnasal route using biopsy needles**
- **Occipital route through the foramen magnum using lumbar puncture needle**
- **Place in a leak-proof rigid container with 50% glycerol in phosphate buffered saline (PBS) and transport to the laboratory in cold chain at 2 - 8°C.**
- **Can also be sent in formalin for histopathology (Negri bodies) or Immunohistochemistry**

Postmortem Diagnosis



- dFA for viral antigen



- PCR for viral RNA



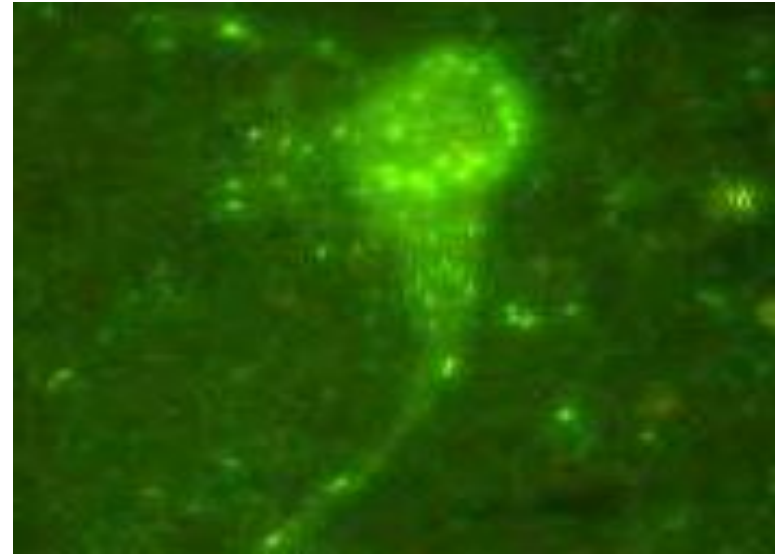
- Negri Bodies



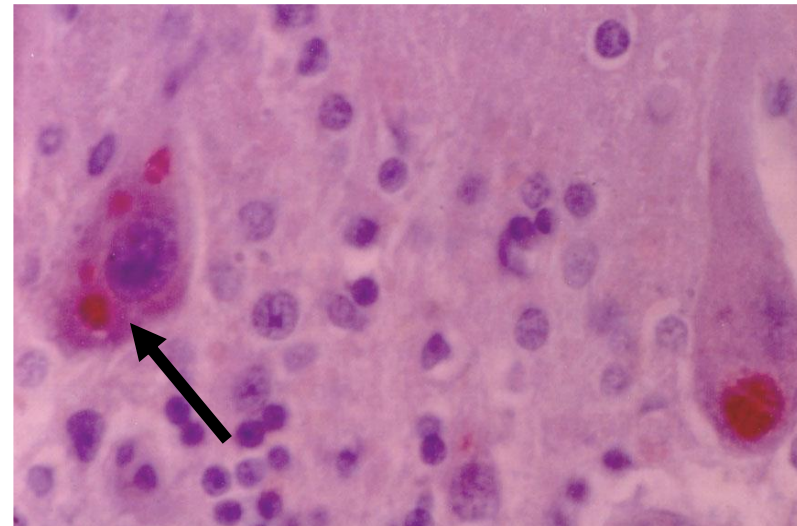
- Antibody detection

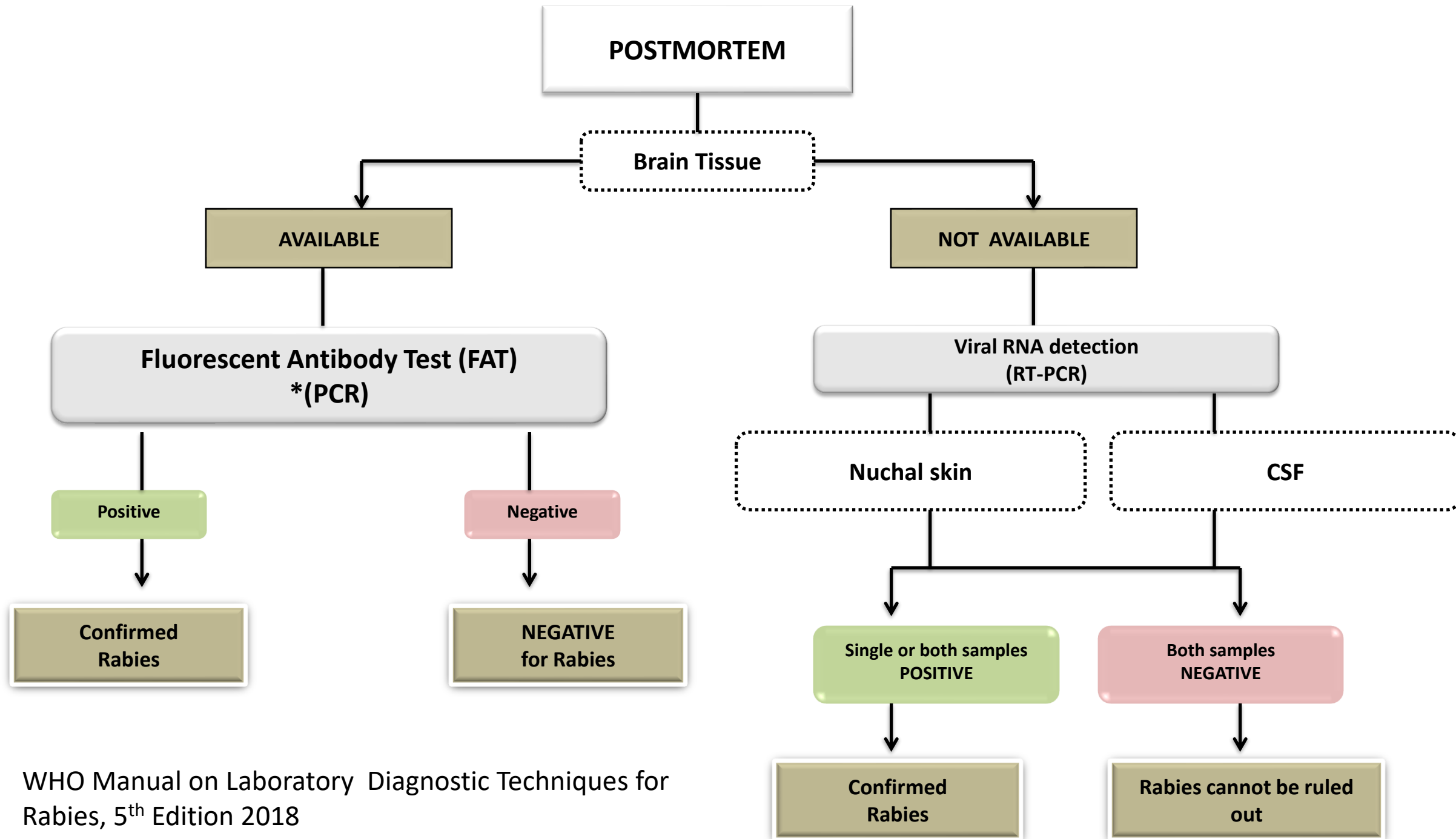


Direct Fluorescent antibody test (dFA)



Negri Bodies





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Challenges

Antemortem diagnosis

- Quality of samples vital; appropriate storage and transport
- Lab facilities for antemortem diagnosis few
- Sensitivity of antemortem diagnosis **low-Need for multiple tests & samples/serial sampling**
- Interpretation-Clinical correlation/duration of illness/vaccination history
- **Antemortem tests can 'rule in' Rabies but cannot 'rule out' Rabies**

Postmortem diagnosis

- 'Gold standard'-Antigen detection by DFA in brain tissue
- Obtaining postmortem brain tissue-challenge
- Lack of facilities; Biosafety concerns
- Transport to laboratories-Cost

Thank-you