UNIVERSITEIT VAN PRETORIA
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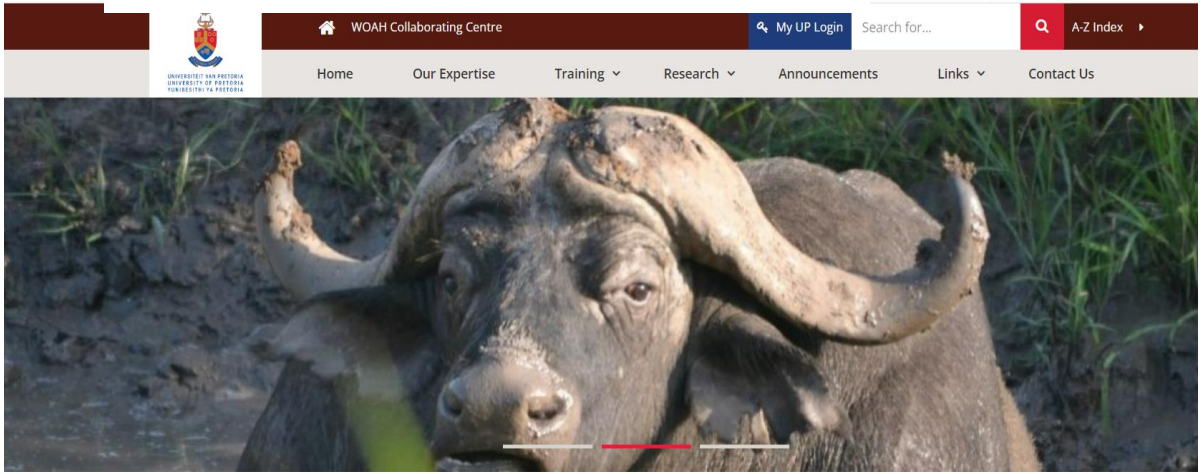
YUNIBESITHI YA PRETORIA

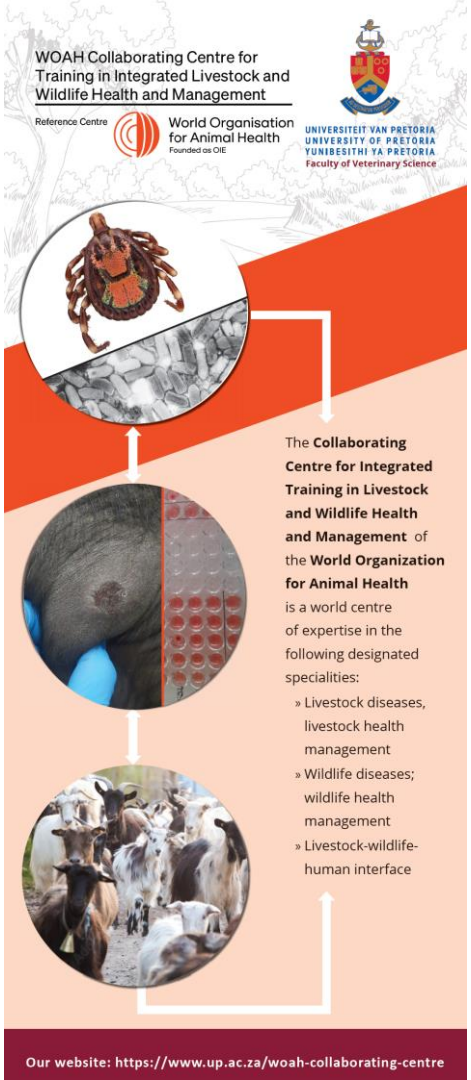
Faculty of Veterinary Science

WOAH Collaborating Centre for Training in Integrated Livestock and Wildlife Health and Management

Reference Centre
World Organisation for Animal Health
Founded as OIE

https://www.up.ac.za/woah-collaborating-centre





New laboratory approaches (antigen and antibody detection for rabies virus)

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Faculty of
Veterinary Science

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

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Make today matter

Presentation outline

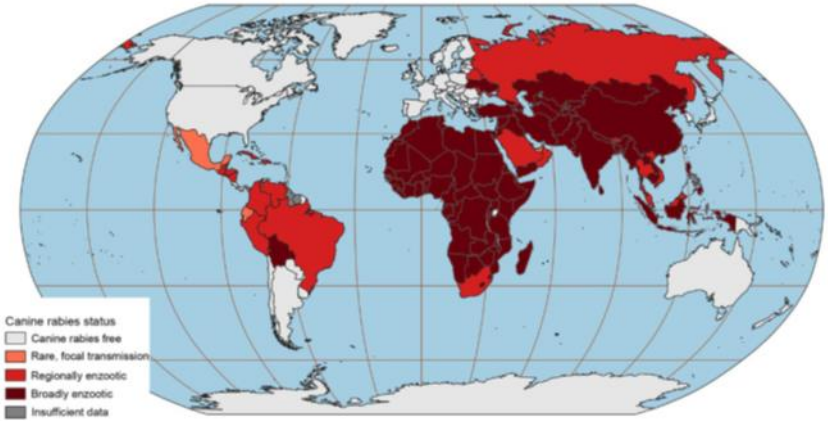
- Global rabies burden
- Lyssavirus diversity
- Current methods for rabies diagnosis
- Point of care in infectious disease diagnosis
- Findings of rabies diagnosis using point of care tests for rabies
- RabLab network
  - Registration of diagnostic tests by WOAH

Global rabies burden

- The majority of rabies cases in Africa and Asia are dog-mediated
  - bite contact

- At least 59 000 human deaths occur annually (India alone – 20 000 lives lost every year) (Hampson et al., 2015).
  - 50% of the human deaths are children under 15 years of age

- The burden of rabies is highest in Asia and Africa,
  - Human and economic costs of canine rabies are poorly known
  - the absence of reliable surveillance data
  - Official reporting of incidence data on rabies and rabies exposures remains desperatel



Underreporting of rabies …

- Death is inevitable following clinical onset
  - large number of rabies victims **never report** to health facilities
  - are **never diagnosed**

- **Misdiagnosis** to other neurological syndromes is frequent, especially in malaria endemic regions

- Shortages of life-saving PEP and centres that provide PEP for bite victims and
  - poorly monitored sales of PEP to private suppliers all

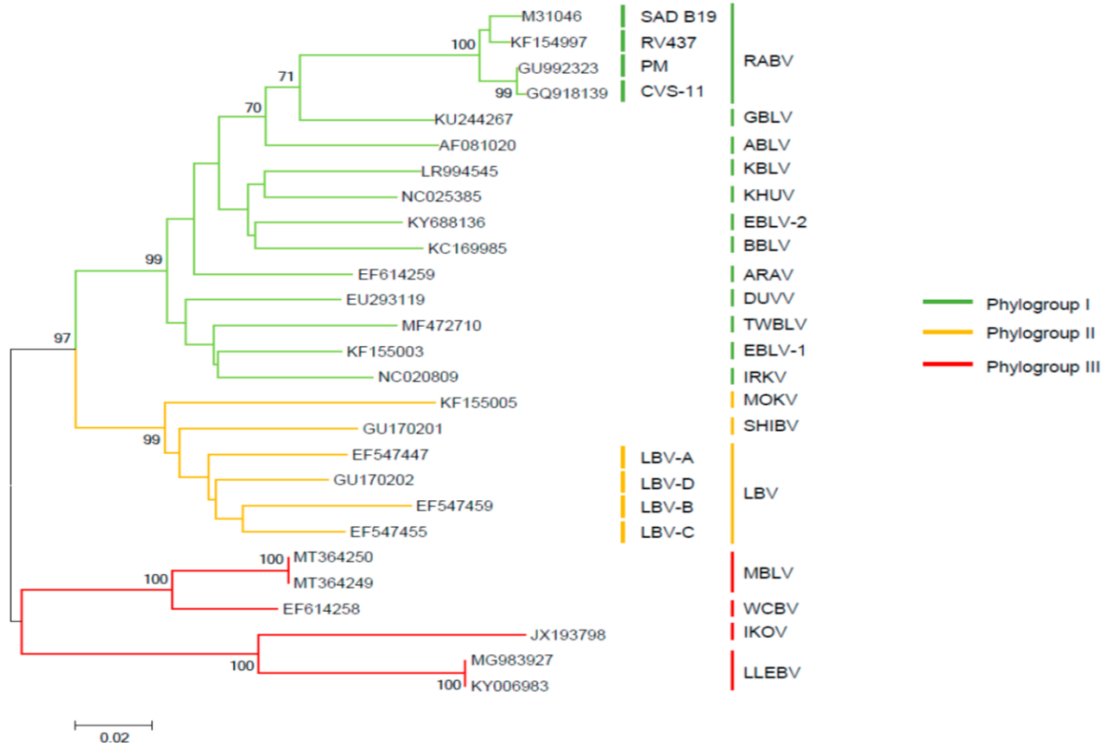
- poor infrastructure and a lack of personnel and facilities for rabies surveillance and diagnosis
  - only very limited data of questionable reliability are available.

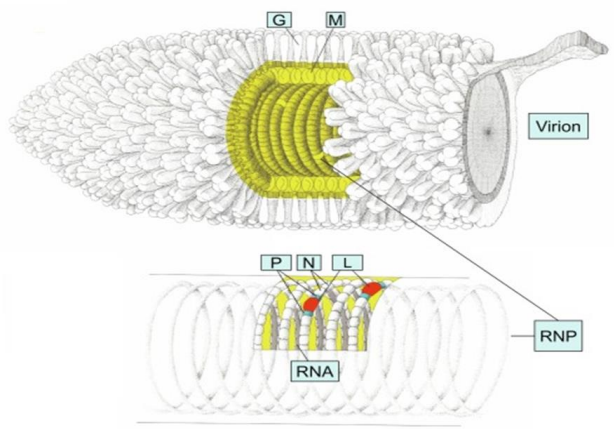
Lyssavirus diversity

- Until the 1950s, it was believed rabies was the sole cause of encephalitis.

- Discoveries of Lagos bat lyssavirus and Mokola lyssavirus – in the 1960s demonstrated existence of other lyssaviruses.

    - Serotypes
    - Genotypes
    - Viral species





- Primary rabies diagnosis can only provide information on whether **a specimen is positive(i.e. lyssavirus) or negative**.
- Even staining with the biological conjugates available may give different results, **e.g. i. ABLV stains dull compared to other lyssavirus species; may miss members of phylogroup III with FITC-labelled monoclonal antibodies**.
  - This therefore calls for the need to use panels of monoclonal antibodies or molecular typing.

Current methods for rabies diagnosis

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
DFA (antigen detection)	+++	n/a	+++	+++	+++	n/a
dRIT (antigen detection)	+++	n/a	+++	+++	+++	n/a
Sellers staining (antigen detection)	+	n/a	+	+	+	n/a
Cell culture (virus isolation)	n/a	n/a	+++	+++	+++	n/a
MIT (virus isolation)	n/a	n/a	+++	+++	+++	n/a
Conventional RT-PCR (RNA detection)	+++	n/a	+++	+++	+++	n/a
Real-time RT-PCR (RNA detection)	+++	n/a	+++	+++	+++	n/a
Detection of immune response						
VN	n/a	+++	+++	n/a	n/a	+++
ELISA	n/a	n/a	+++	n/a	n/a	+++

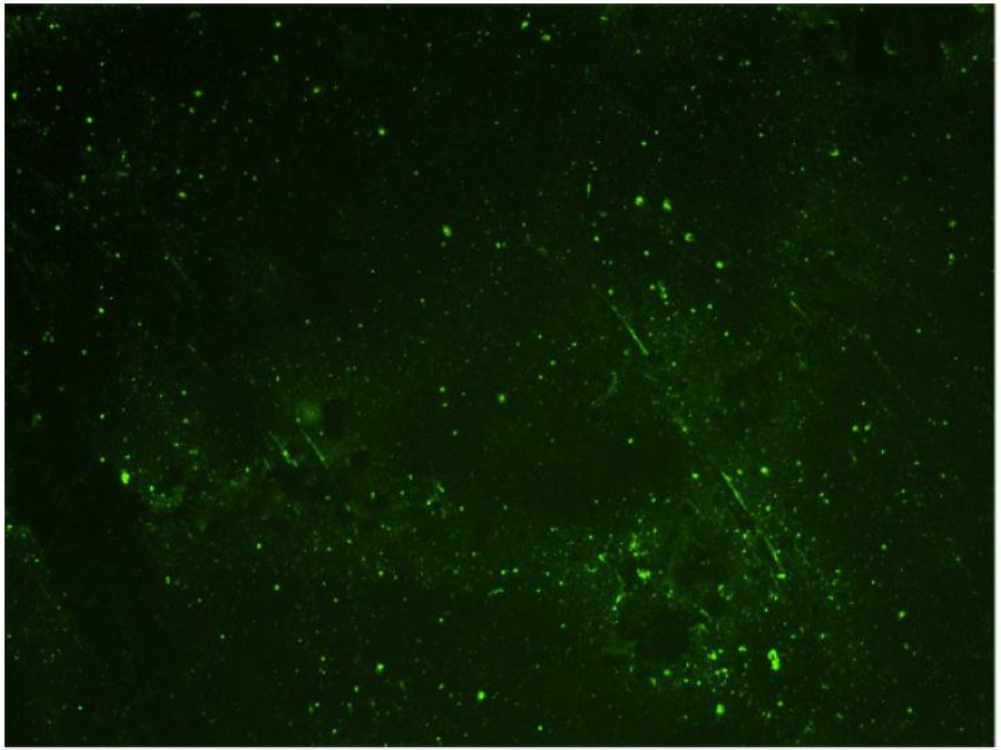
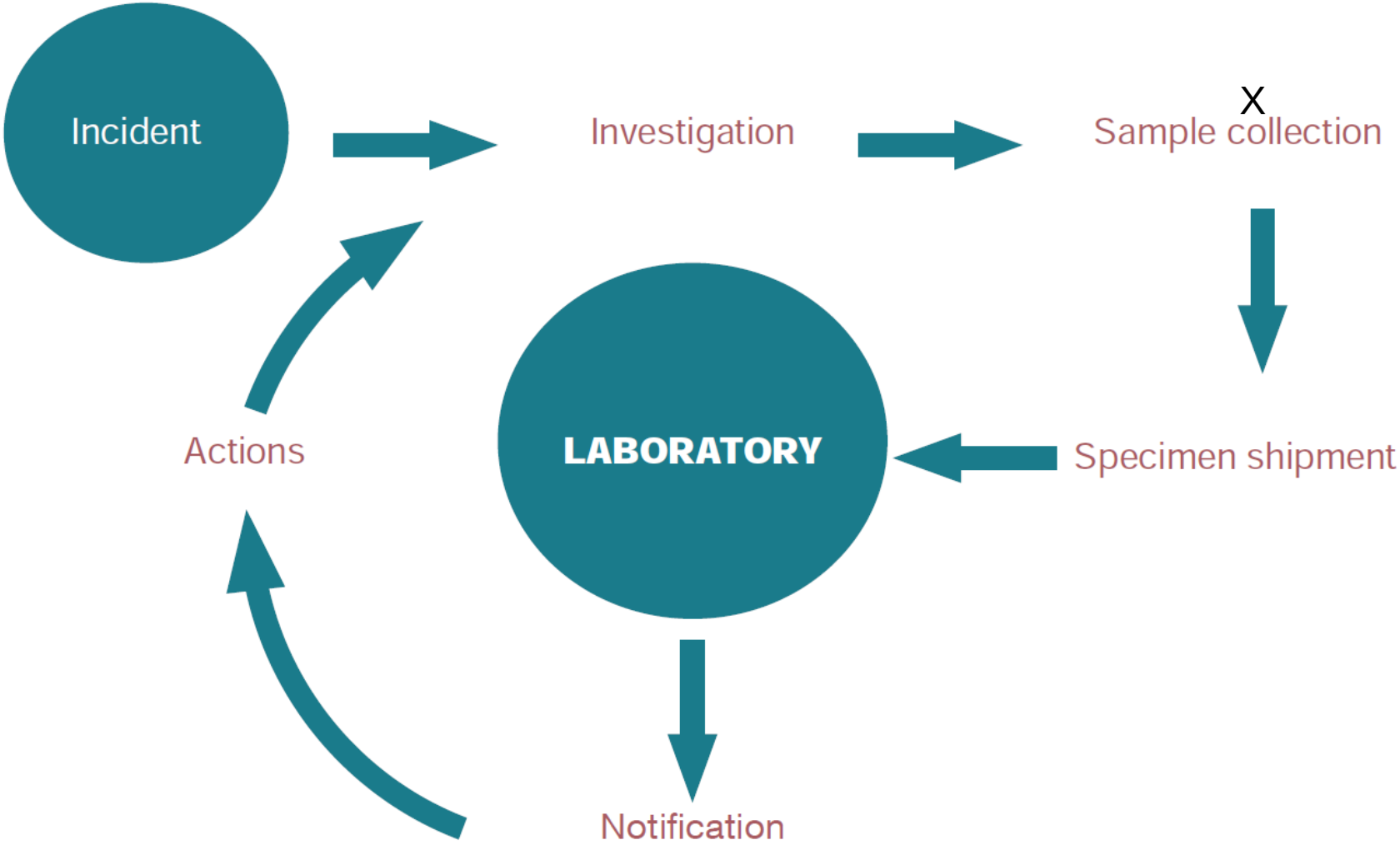


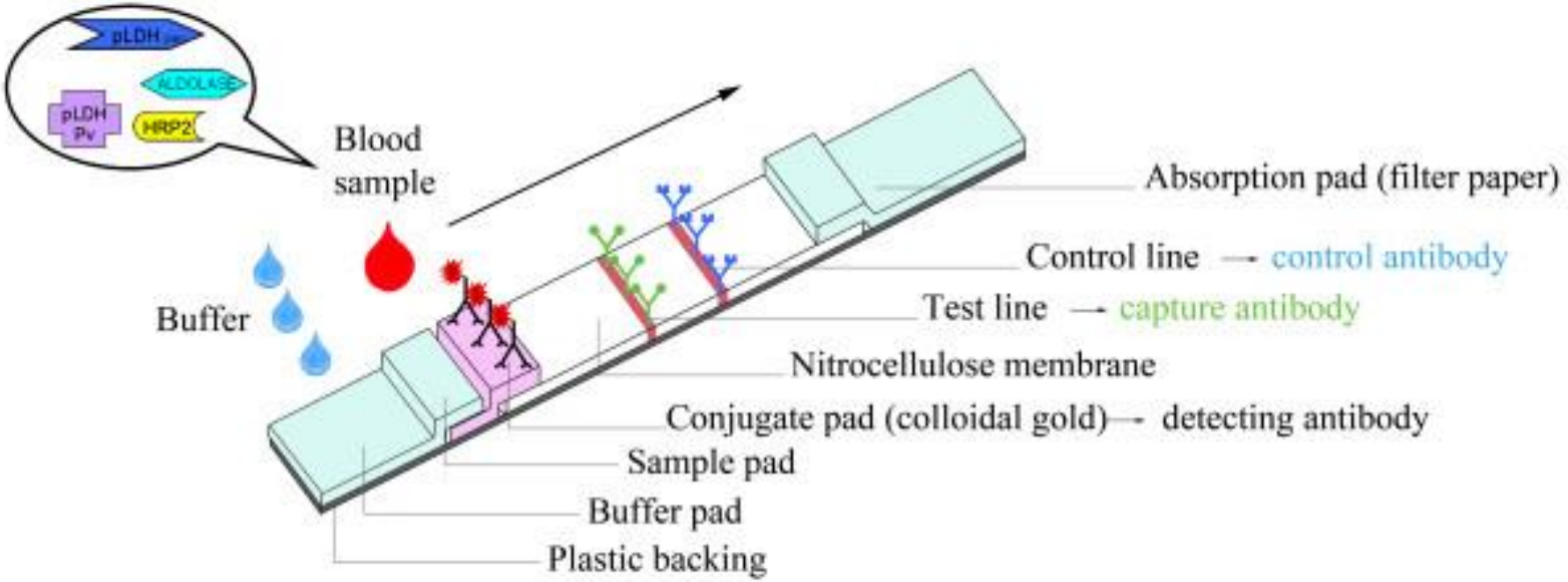
Figure: Apple green fluorescing Negri bodies (viral proteins)

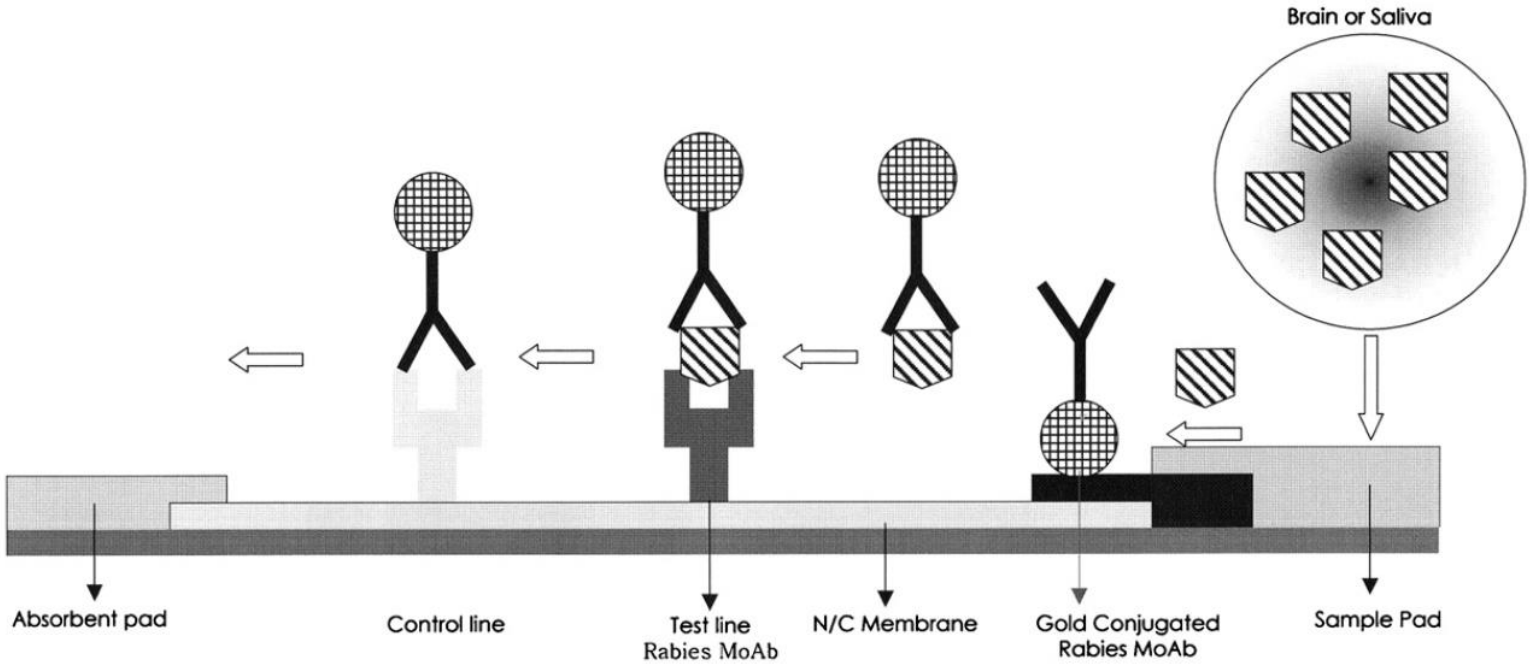

Incident
Investigation
X
Sample collection
Actions
LABORATORY
Specimen shipment
Notification

Point of care in rabies diagnosis

- Promising results obtained for the use of lateral flow assays
  - To detect rabies virus in brain, saliva and cell culture (sensitivity of 91.7% and specificity of 100%)
  - Cf with the direct fluorescent antibody test (**99.2%** and **99.9%** respectively)

- LFDs:
  - Several rapid immunodiagnostic test kits (RIDT) were developed
  - Rapid and simple
  - Do not require any specialized equipment or technical expertise
  - **Useful onsite test under field conditions (can be useful as screening tools)**
  - Could be used in developing countries with limited diagnostic resources


pLDH
ALDOLASE
pLDH Pv
HRP2
Blood sample
Buffer
Absorption pad (filter paper)
Control line → control antibody
Test line → capture antibody
Nitrocellulose membrane
Conjugate pad (colloidal gold) → detecting antibody
Sample pad
Buffer pad
Plastic backing


Brain or Saliva
Absorbent pad
Control line
Test line
Rabies MoAb
N/C Membrane
Gold Conjugated
Rabies MoAb
Sample Pad

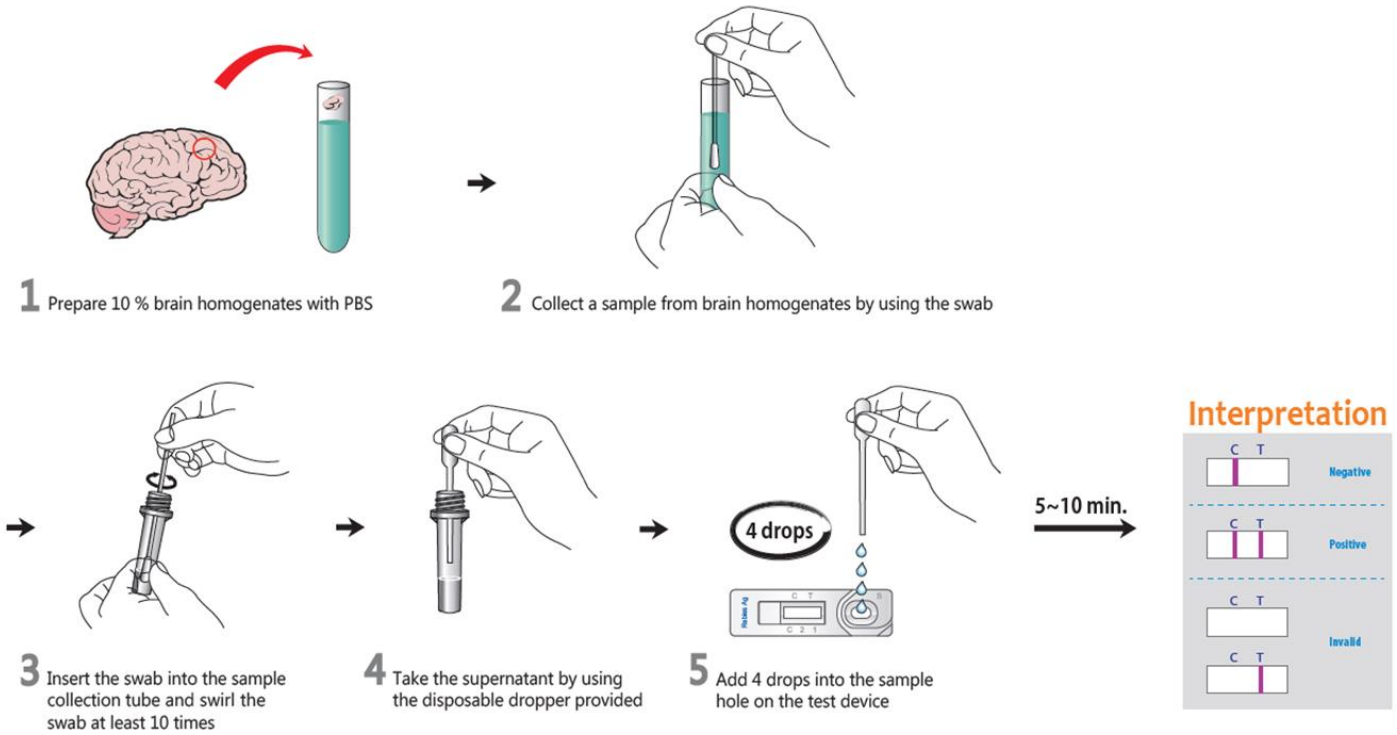


Figure . Schematic representation of rapid immunodiagnostic test procedure and results interpretation

Review of studies on LFDs

Markotter et al 2009. Onderstepoort Journal of Veterinary Research, 76:257–262 (South Africa)

- 25 field rabies viruses and 4 negative controls
    - Canid rabies biotype
    - Mongoose rabies biotype
    - LBV
    - Mokola lyssavirus
    - Duvenhage lyssavirus
- 20% brain suspension used
- Samples tested in duplicate
- Results compared with those obtained from DFA – 100% concordance

Kang et al., 2007. Evaluation of a rapid immunodiagnostic test kit for rabies virus (South Korea)

- 51 clinical samples and 4 virus isolates
- **Cross-reactivity tested** against:

  Escherichia coli, Clostridium perfringens, Salmonella suis; and viruses: canine, distemper virus, pseudorabies virus, infectious bovine rhinotracheitis virus, porcine encephalomyocarditis virus, and Japanese encephalitis virus
- Monoclonal against the nucleoprotein gene (anti-N Mab)
- Results:
  - 91.7% **sensitive** and 100% **specific**

Table 1. Lyssavirus positive brain samples included in the evaluation of the rapid immunodiagnostic test (RIDT)

Sample number	Laboratory number	Host species	Geographic location
1	001/19	Bovine	Clocolan, Free State
2	022/19	Mongoose	
3	044/19	Jackal	
4	045/19	Dog	
5	049/19	Jackal	
6	065/19	Dog	
7	076/19	Bovine	
8	081/19	Meerkat	
9	384/18	Dog	Nkomazi, Mpumalanga
10	391/18	Dog	Klerksdorp, North West
11	396/18	Rhino	Windhoek, Namibia
12	505/18	Dog	Schwezernaicker, NW
13	541/18	Bat Eared fox	Kuruman, Northern Cape
14	551/18	Dog	Madidi, North West
15	555/18	Dog	Tzaneen, Limpopo
16	557/18	Dog	Bushbuckridge, MP
17	560/18		Makhado, Limpopo

Table . Results of the Rapid immunodiagnostic test (RIDT) after positive brain samples

LABORATORY NUMBER	RIDT Kit 1	RIDT Kit 2	RIDT Kit 3	RIDT Kit 4	RIDT Kit 5
001/19	+ve	-ve	-ve	-ve	-ve
022/19	+ve	-ve*	+ve	-ve	-ve
044/19	+ve	+ve	+ve	-ve	-ve
045/19	+ve	-ve	-ve	-ve	-ve
049/19	+ve	+ve	-ve	-ve	-ve
065/19	+ve	-ve	-ve	-ve*	-ve
076/19	+ve	-ve	-ve*	-ve	-ve
081/19	+ve	-ve*	-ve	-ve	-ve
384/18	+ve	-ve	-ve	-ve	-ve
391/18	+ve	-ve	-ve	-ve	-ve
396/18	+ve	-ve*	-ve	-ve	-ve
505/18	+ve	-ve*	-ve	-ve	-ve
541/18	+ve	-ve	-ve	-ve	-ve
551/18	+ve	-ve	-ve	-ve	-ve
555/18	+ve	-ve*	-ve	+ve	-ve
557/18	+ve	-ve	-ve	-ve	-ve
560/18	+ve	-ve	-ve	-ve	-ve

Table . Results of the Rapid immunodiagnostic test (RIDT) after positive brain samples – interlaboratory participation

Test	Sensitivity exp. inf. animals	Sensitivity field samples	overall Sensitivity	Specificity
1	2,3%	0,0%	1,2%	100,0%
2	15,9%	41,4%	28,7%	100,0%
3	0,0%	3,4%	1,7%	100,0%
4	38,8%	69,0%	53,9%	100,0%
5	22,7%	48,3%	35,5%	100,0%
6	0,0%	0,0%	0,0%	100,0%

49
Rabies Ag
C T
S 4
Rabies Ag
C 2 1
C T
384
S
Rabies Ag
C 2 1
C T
391
S
Rabies Ag
C 2 1
C T
505
S
Rabies Ag
C 2 1
C T
22
S

SUMMARY

- The intensity of the test lines varied between different virus samples
- All the tests were clearly readable
- There were no cases of doubtful interpretation observed
- Most samples reacted between 3-5 min
- None of the samples indicated any non-specific reactions
- RIDT Kit 2 and 3 were able to detect 2 positives
- RIDT Kit 4 was able to detect 1 positive
- RIDT Kit 5 did not detect any positive
- This study indicated that most of these kits still require more validation before they could be released in the market.

Summary of findings from literature

LFD			
Manufacturer	Sensitivity	Specificity	Source
1	0%	100%	Klein
2	1%	100%	Klein
3	3%	100%	Klein
4	20%	100%	Klein
5	62%	100%	Klein
6	88%	100%	Kimitsuki
7	95%	100%	Kimitsuki
4	0%	100%	Kimitsuki
6	94%	100%	Kimitsuki
5/7	92%	79%	Freuling

WOAH RABLAB NETWORK – strategic objectives

1. The correct implementation of WOAH international standards and guidelines which are the foundation for OIE Member Countries' efforts to eliminate rabies;

2. Improve awareness through effective communication of rabies elimination strategies;

3. Assess and, if appropriate, strengthen the impact of the WOAH regional rabies vaccine banks;

4. Adequate governance of the WOAH activities targeting rabies is established and ensures coordination with inter-sectorial partners;

5. Leverage resources to mobilise funding and foster political and community engagement in rabies elimination.

WOAH RABLAB NETWORK – expected outcomes

a. WOAH international standards are reviewed and correctly implemented

b. Responsible dog ownership is promoted as part of dog population management practices

c. The WOAH rabies expert network is enhanced

d. Dog vaccination remains the foundation for the global human rabies elimination

e. Regional and inter-sectorial collaboration is enhanced

f. The regional rabies vaccine banks are sized in accordance with the OIE policy document on vaccine banks following the vaccine bank think-tank

g. Member countries are accountable for the use of vaccines received through the regional vaccine banks

h. Global awareness and understanding of rabies elimination efforts are improved by an effective communication strategy

i. Long-term political and social commitment is achieved resulting in sustainable resource mobilisation

RabLab statement on LFDs

- There are a number of rapid immunochromatographic tests (lateral flow devices) for RABV available on the market.
- Amendment in Chapter 3.1.18 – Rabies (infection with rabies virus and other lyssaviruses) of the WOAH Terrestrail Manual.
    - The routine use of these tests in the context of **statutory rabies surveillance is currently not recommended:**
        - General lack of quality control,
        - Clarity on assay methods
        - Compliance with international standards for validation as a test kit.
    - RabLab can only recommend the routine use of diagnostic methods that have been **by WOAH through the Manual or the WOAH Register of Diagnostic kits**.
    - WOAH recommends transparency in assay methods
    - Performance of LFDs is largely dependent on the monoclonal (polyclonal) antibodies and their affinity to bind to rabies virus variants.
    - Veterinary authorities should review product details
    - Negative and positive rabies controls cannot be performed with these tests in parallel as required for any other diagnostic test.

RabLab statement on LFDs

- Rapid immunochromatographic method does not comply with standards for a laboratory test method, recognition must be made through the WOAH register of Diagnostic kits.

- Manufacturer to validate the kit based on the package leaflets according to international standards.

- **LFDs for research purposes**:
    - Considering the ease of performance, and the need for improved surveillance, LFDs can be useful for research purposes.
    - **Only tests with a high performance should be used**.
    - All test results should be confirmed by standard tests.

Thank You