ARC • LNR
Excellence in Research and Development

Rabies diagnostic techniques/methods and surveillance in animals.

Claude Sabeta, PhD

23 September 2020

OIE Rabies Reference Laboratory

Onderstepoort Veterinary Research, Pretoria, Republic of South Africa

Presentation outline ……

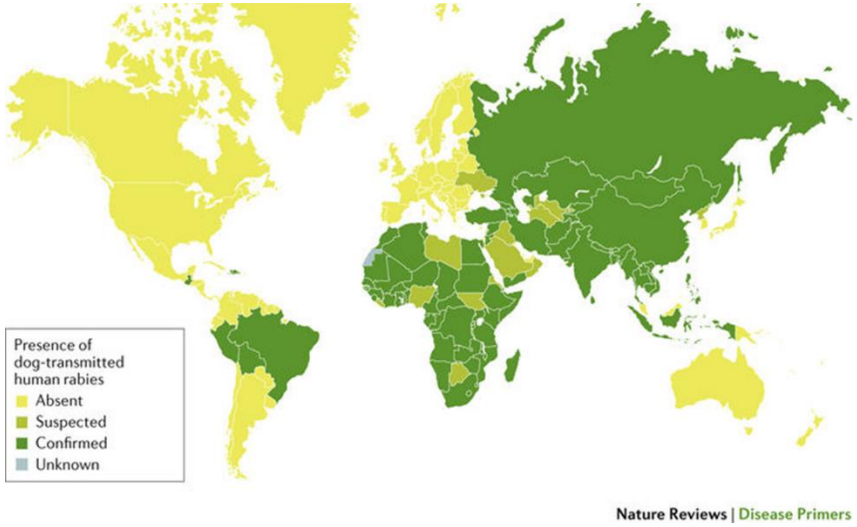
- Introduction – rabies burden
- Lyssavirus genus
- Rabies testing in RSA
  - Other regional countries
- Sample submissions
- Test methods commonly used in rabies testing on the continent
  - Back up and confirmatory tests available
- Rabies testing in RSA - 2018
- Summary of test methods (advantages/disadvantages)

Rabies is a neglected tropical disease and poses a veterinary & public health threat

- Commonly encountered in dog populations in endemic regions of Asia and Africa
  - the burden of the disease is highest here.

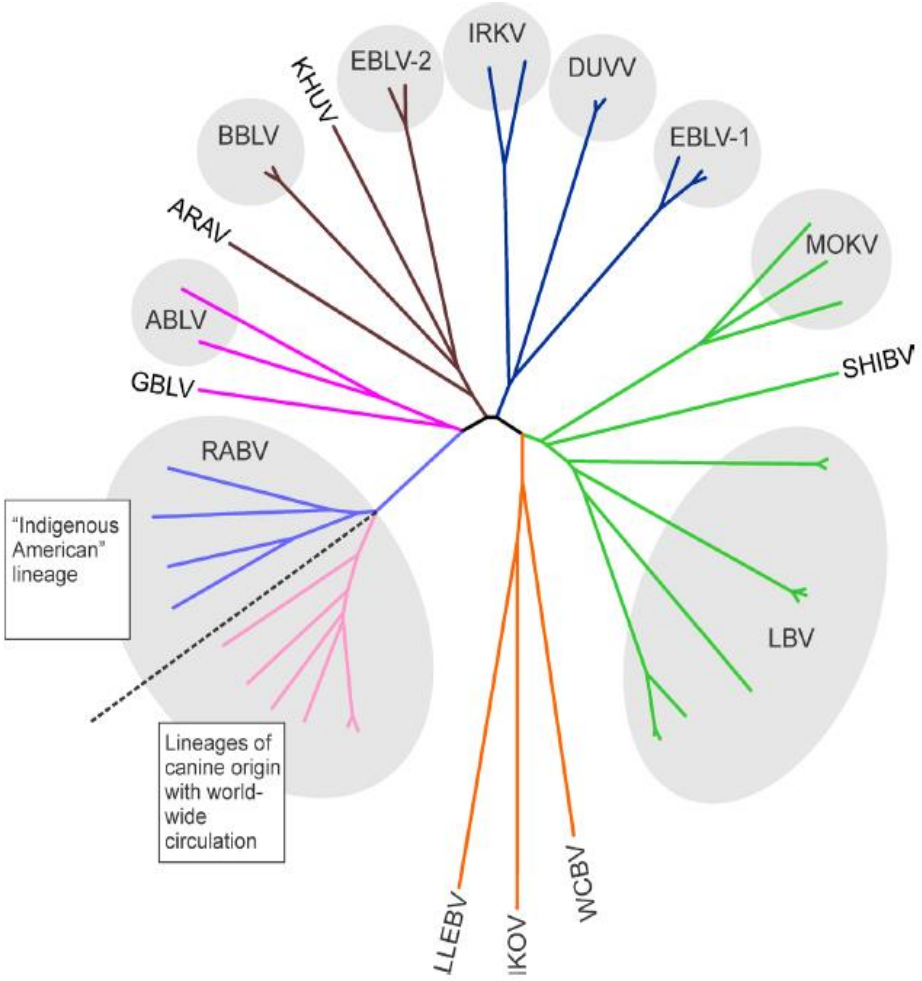
- At least 60 000 human deaths occur annually (India alone – 20 000 deaths annually).
  - 50% of the human deaths are children under 15 years of age

- Under-reporting of human cases due to limited:
  - Laboratory capability
  - Qualified personnel
  - Medical and veterinary infrastructure



Fooks et al., 2017

Lyssaviruses are divided into 3 phylogroups ……

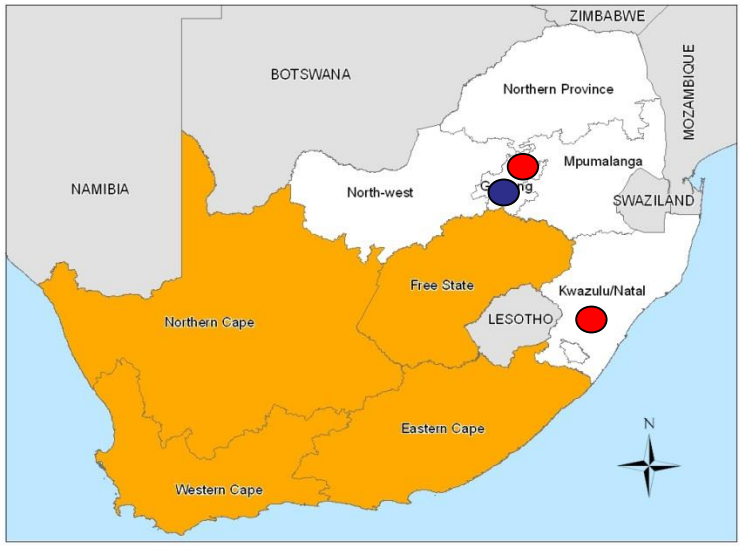


Rupprecht et al., 2017

- Pathogenicity – intracerebral (i,.c) and intramuscular (i.m.)
- Cross – protection: current vaccines based on phylogroup I viruses

ARC • LNR
Excellence in Research and Development

Rabies testing in South Africa …



3 designated labs (Gauteng (2) and KZN

Animal diagnostic laboratories at Onderstepoort,

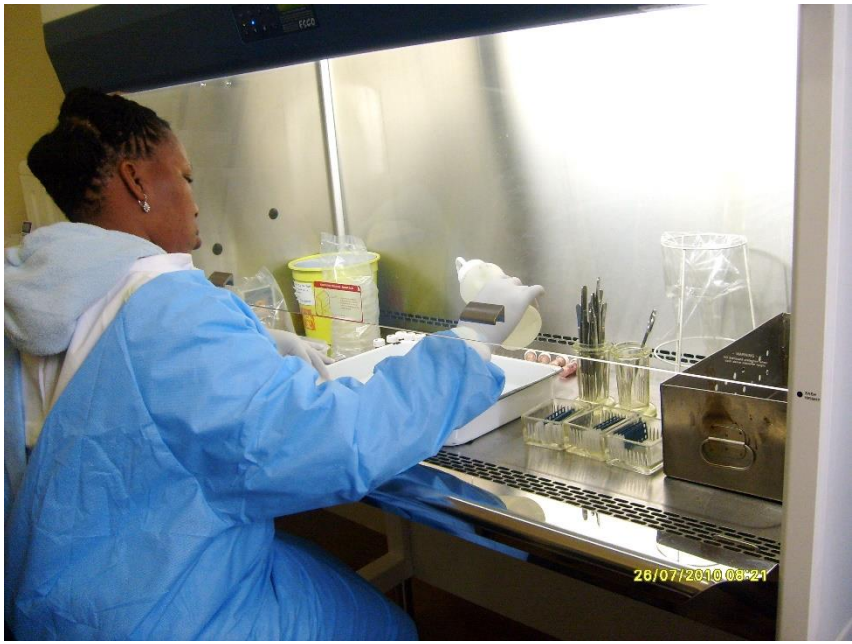
- Allerton (Pietermaritzburg)
- the National Health Laboratory Service (NHLS)
- Immunohistochemistry (IHC) is conducted at Pathology (Faculty of Veterinary Sciences, UP)

ARC • LNR
Excellence in Research and Development

Specimen submissions

ARC • LNR
Excellence in Research and Development

Specimen processing and rabies testing on central nervous tissues …



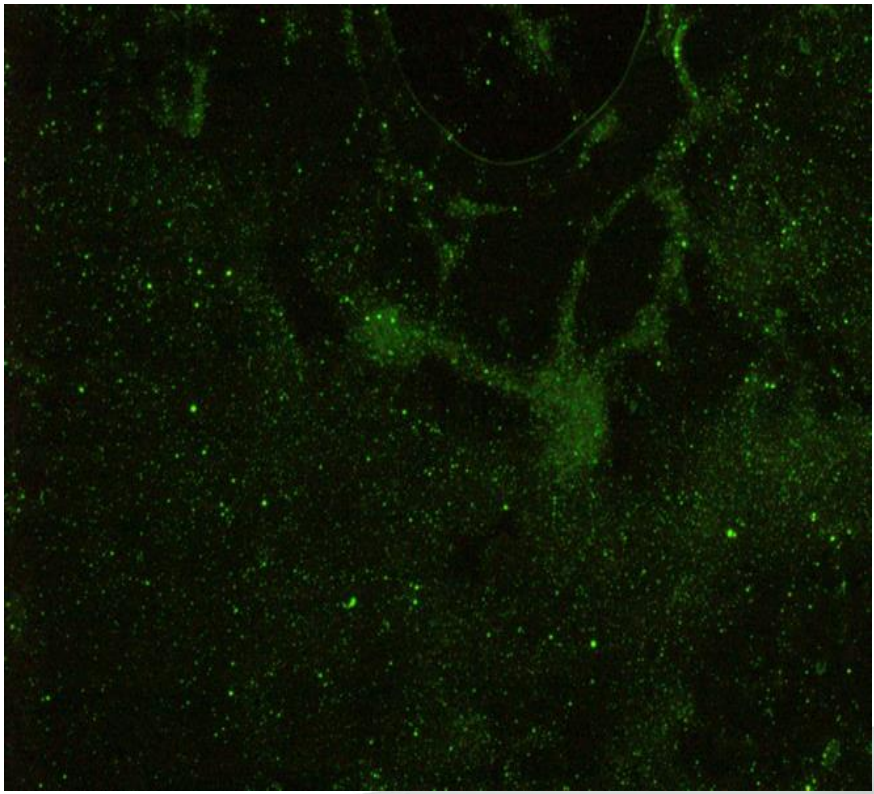
ARC • LNR
Excellence in Research and Development

The FAT and dRIT are the first line test methods approved by both the OIE/WHO for lyssavirus antigen in brain and central nervous tissues.

- Brain-impression smears fixed in cold acetone (formalin masks the RABV antigen).
- Stained with a polyclonal conjugate (Mok + ERA)
- Viewing slides under a fluorescent microscope
- Quickest and most reliable method for both diagnostic and research purposes
- The sensitivity of the FAT depends on the quality of the specimen and the degree of autolysis of the brain tissue.

Dean, D.L., Abelseth, M.K. & Atanasiu, P. (1996). The fluorescent antibody test. In: Laboratory Techniques in Rabies (F.X. Meslin, M.M. Kaplan & H. Koprowski, eds). Geneva, WHO, 88-95.

Rabies diagnosis with the FAT/dRIT gives an all-or-nothing result ……..

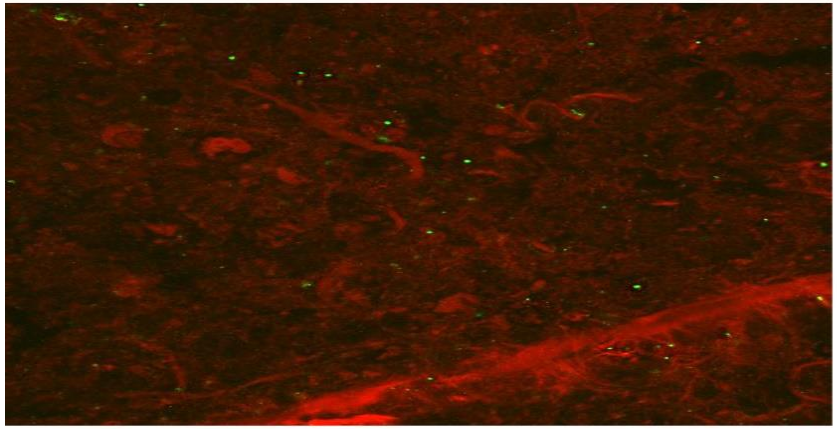


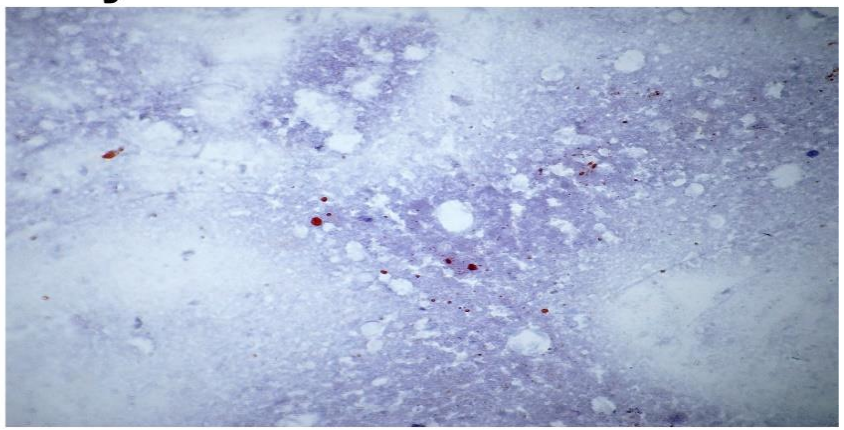
ARC • LNR
Excellence in Research and Development

FAT

Immunoreactivity: + + +

dRIT

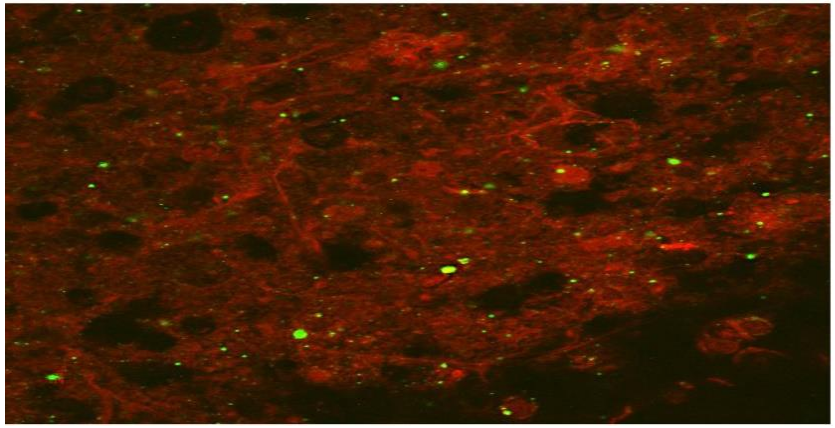


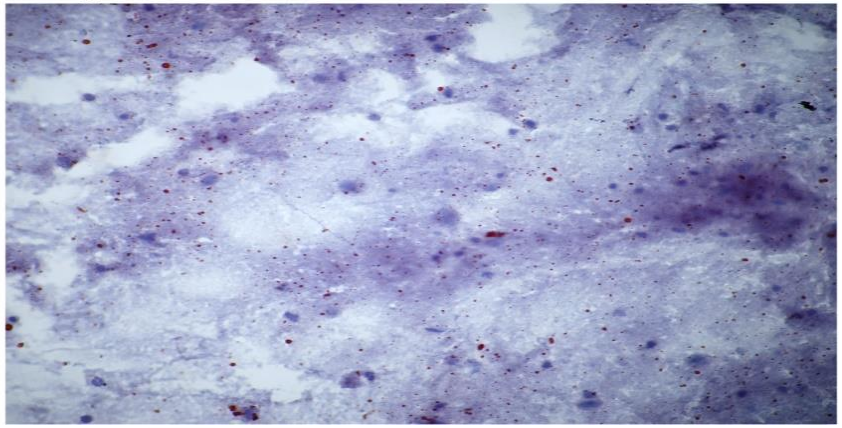


FAT

Immunoreactivity: + + + +

dRIT

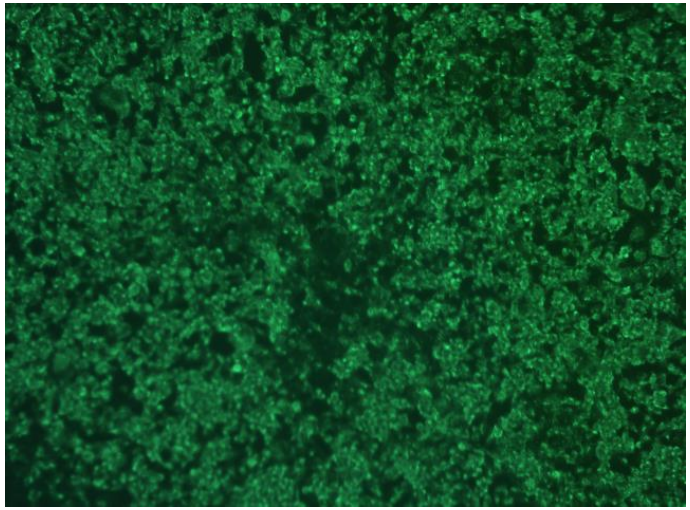




ARC • LNR

Excellence in Research and Development

Biological tests for rabies diagnosis …..

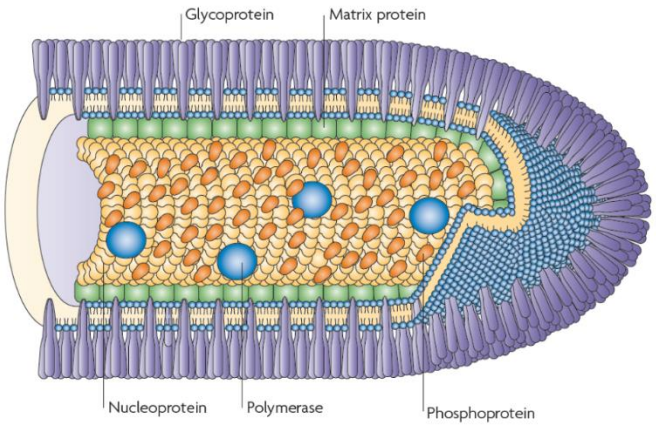


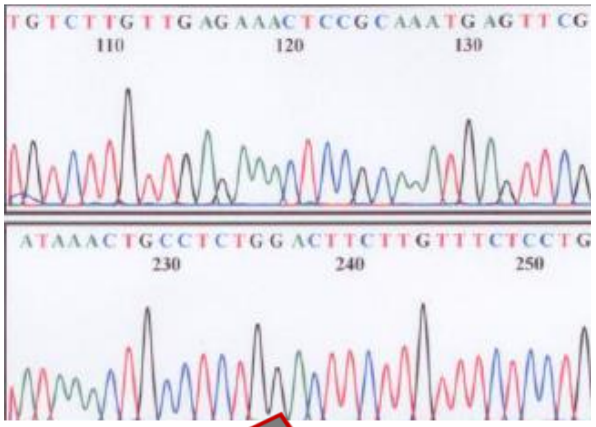
- Suckling mice
- 10% brain tissue suspension in cell culture media/PBS, pH 7.2
- Intra-cranial challenge
- Mice monitored for 28 days [Seo's thesis].
- RTCIT has replaced MIT in many laboratories.

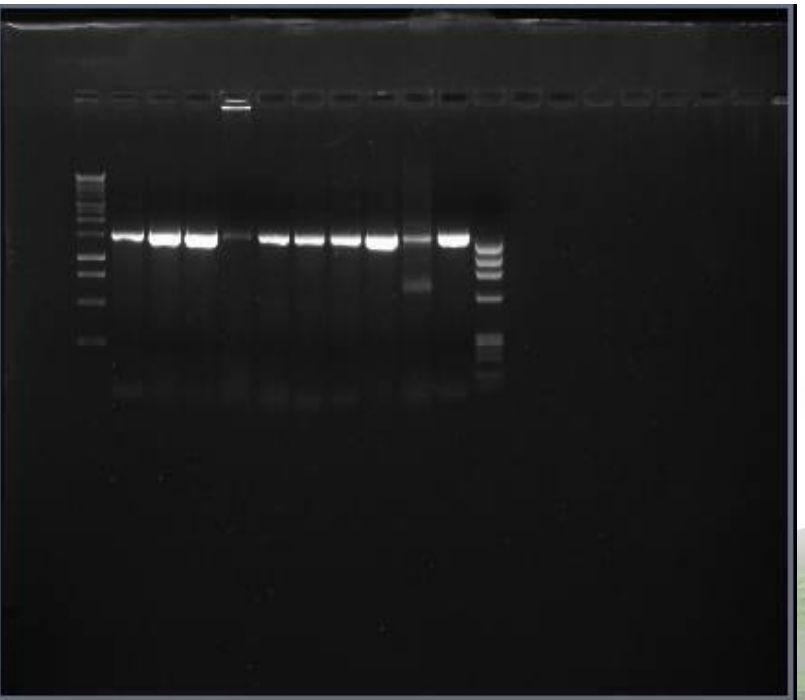
Rabies tissue culture isolation (RTCIT) on neuroblastoma cells (ATCC)

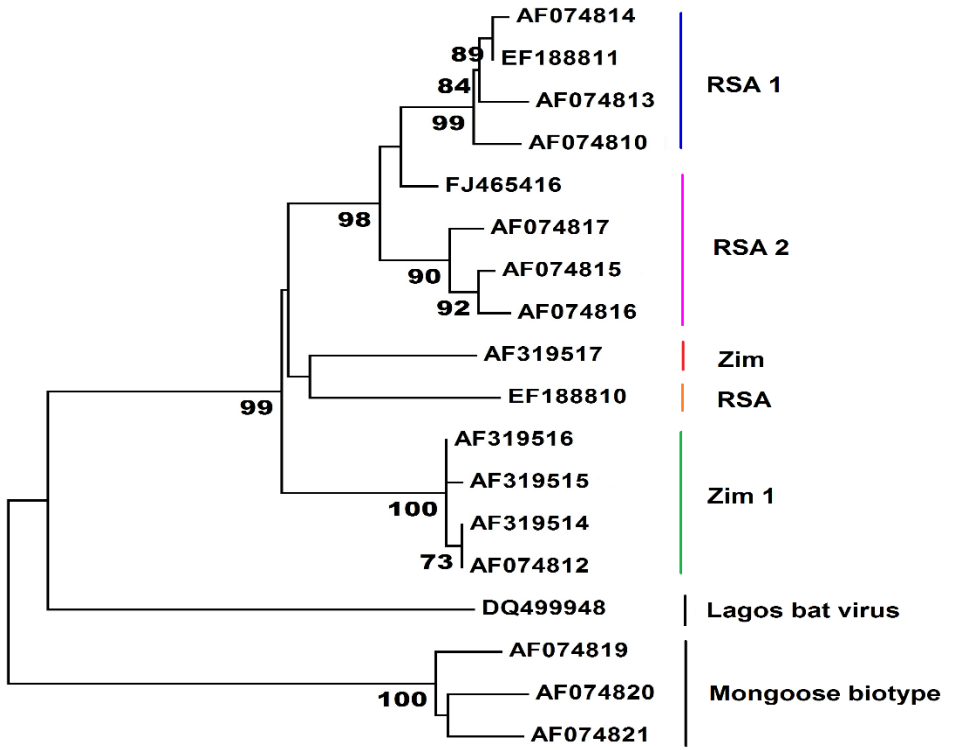
ARC • LNR
Excellence in Research and Development

Determine origin of outbreaks/relationships

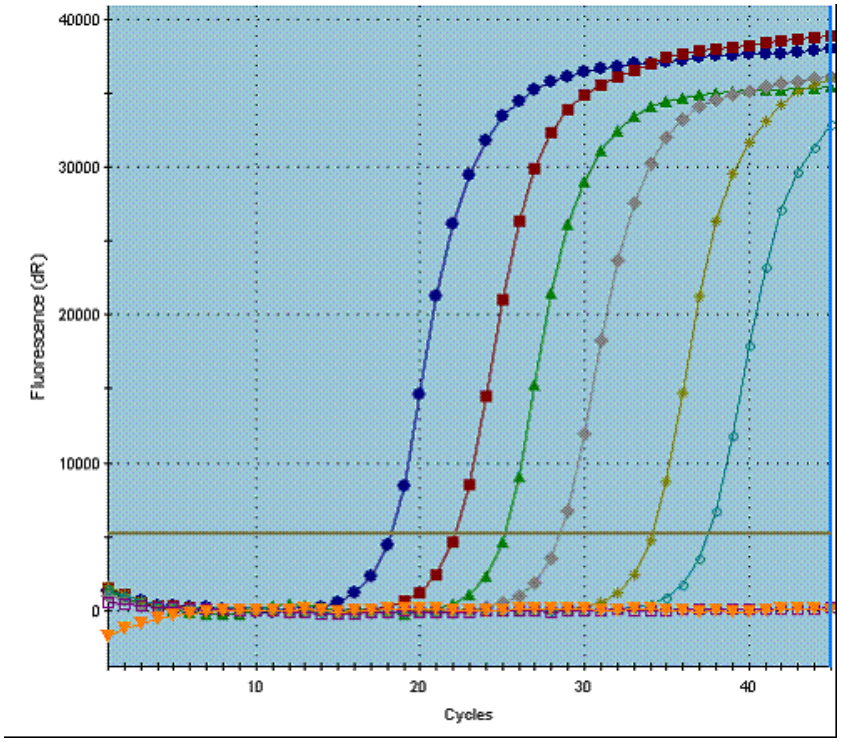








Molecular (PCR) now recommended for confirmatory diagnosis of rabies .....



Rabies diagnosis in South Africa (Onderstepoort)- 2018 1

Origin	Negative	Positive	Total
GP	113	3*	116
FS	39	12	51
LP	52	37	89
MP	97	24	121
NC	13	2	15
NW	58	46	104
EC	5	5	10
WC	2	0	2
KZN	10	6	16
Tanzania	0	2	2
Namibia	10	19	29
Malawi	0	1	1
	399	157 [39.3%]	556

ARC • LNR
Excellence in Research and Development

Rabies diagnosis in South Africa (Onderstepoort)- 2018 2

	GP	NW	EC	FS	LP	MP	NC	WC	KZN	MALAWI	NAMIBIA	TANZANIA	
CANINE	54	26	4	12	54	60	5	0	7	1	8	2	233
FELINE	17	9	1	2	5	14	0	0	5	0	2	0	55
LIVESTOCK	18	43	1	13	10	16	1	1	0	0	8	0	111
WILDLIFE	26	26	4	24	21	33	9	2	3	0	11	0	159
UNKNOWN	1	0	0	0	0	0	0	0	2	0	0	0	3
UNSUITABLE	0	0	0	0	0	0	1	0	0	0	0	0	1
	116	104	10	51	90	123	16	3	17	1	29	2	562

Canine – 41.5%
Feline - 9,7%
Livestock – 19.8%
Wildlife - 28,3%

ARC • LNR
Excellence in Research and Development

Problems encountered in rabies testing ……..

Sample numbers	Direct fluorescent antibody test		dRIT	RT-PCR (lys-550B)	Real time qPCR (all pos except 1)
	Nig	RSA			
1-23	+	+	+	+	+
265	-	-	+	+	+
271	-	-	+	+	+
273	-	-	+	+	+
276	-	-	+	+	+
277	-	-	+	+	+
278	-	-	+	+	+

Importance of rabies diagnosis to animal surveillance

- **Confirm diagnosis of suspect or clinical cases (includes confirmation of positive screening test).**
- Contribute to the demonstration of freedom from infection in a defined population (country/zone/compartment/herd) (prevalence apparently zero): “Free” with and/or without vaccination,
- **Re-establishment of freedom after outbreaks**
- Certify freedom from infection or presence of the agent in individual animals or products for trade/movement purposes.
- **Contribute to the eradication of disease or elimination of infection from defined populations.**
- **Estimate prevalence of infection or exposure to facilitate risk analysis (surveys, herd health status, disease control measures).**
- Determine immune status of individual animals or populations (post-vaccination).

Summary - test methods available for the diagnosis of rabies and their purpose

Method	Population freedom from infection	Contribution to eradication policies	Confirmation of clinical cases	Prevalence of infection - surveillance	Immune status in individual animals or populations post vaccination
DFA	+++	+++	+++	+++	n/a
dRIT	+++	+++	+++	+++	n/a
Cell culture (virus isolation)	+	+++	+++	+++	n/a
MIT (virus isolation)	n/a	+++	+++	+	n/a
Conventional RT-PCR (RNA detection)	+++	+	+++	+++	n/a
Real-time PCR (RNA detection)	+++	+++	+++	+++	n/a

Test	Target	Turnaround time	Advantages	Disadvantages
Fluorescent antibody test (FAT)	Virus antigen	2-3 hours	• Rapid • High sensitivity and specificity	• Expensive conjugate • Requires an ultraviolet light microscope • Can be difficult to interpret results
Direct rapid immunohistochemical test (dRIT)	Virus antigen	2-3 hours	• Rapid • Requires only a light microscope	• Can be difficult to interpret results
Rabies tissues isolation test	Live virus	4-6 days	• Enables propagation of virus for characterisation	• Requires specialist facilities and operator expertise • Long turnaround time
Mouse inoculation test (MIT)	Live virus	Up to 20 days	• Enables propagation of virus for characterisation	• Requires specialist facilities and operator expertise • Very long turnaround time • Ethical issues owing to the use of in vivo models
Conventional RT-PCR	Viral nucleic acid	3-4 hours	• Rapid • High sensitivity and specificity • Amplicons can be used for genetic characterisation	• Requires specialist equipment • Potential for contamination
Real-time PCR	Viral nucleic acid	2-3 hours	• Rapid • High sensitivity and specificity • One-tube systems reduce contamination risks	• Requires specialist equipment • Potential for contamination • Amplicons not useful for genetic characterisation

Thank you for listening

ARC • LNR
Excellence in Research and Development