GF-TADs

GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

Africa

Food and Agriculture Organization of the United Nations

World Organisation for Animal Health
Founded as OIE

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CHALLENGES OF VALIDATING AFRICAN HORSE SICKNESS LABORATORY ASSAYS

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INTRODUCTION

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- Validation is a process that determines the fitness of a properly developed, optimised and standardized assay for fitness for an intended purpose
- The tests are applied on individual animals or populations for reasons such as: documenting freedom from disease in a country or region; preventing spread of disease through trade; eradicating an infection from a region or country; confirming diagnosis of clinical cases; estimating infection prevalence to facilitate risk analysis; identifying infected animals towards implementation of control measures; and classifying animals for herd health or immune status post-vaccination
- The results generated using these assays influence decisions affecting food safety and security (livestock), and trade. The tests used must therefore be as accurate as possible

- The validation process may however not be easy due to lack of samples from target species (numbers), certain sample types (matrices), enough quantities (volume), presence of desired analyte in the samples (positives), and reference assays and standard methods of comparison *etc*

- This presentation is aimed at:
  i). highlighting the challenges experienced with validating the tissue culture based diagnostic methods at ARC-OVR using African horse sickness as an example
  ii). soliciting samples from the SADC and African region in general for mitigation of the challenges alluded to above

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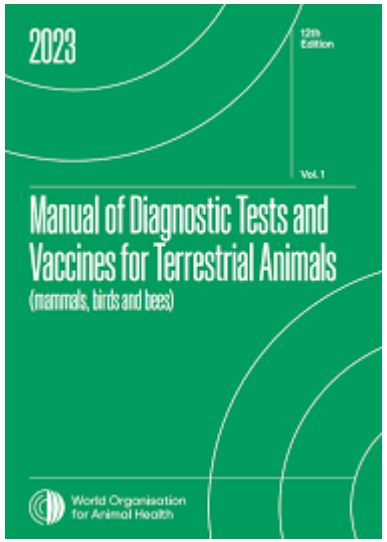
METHODS



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WOAH assay validation pathway

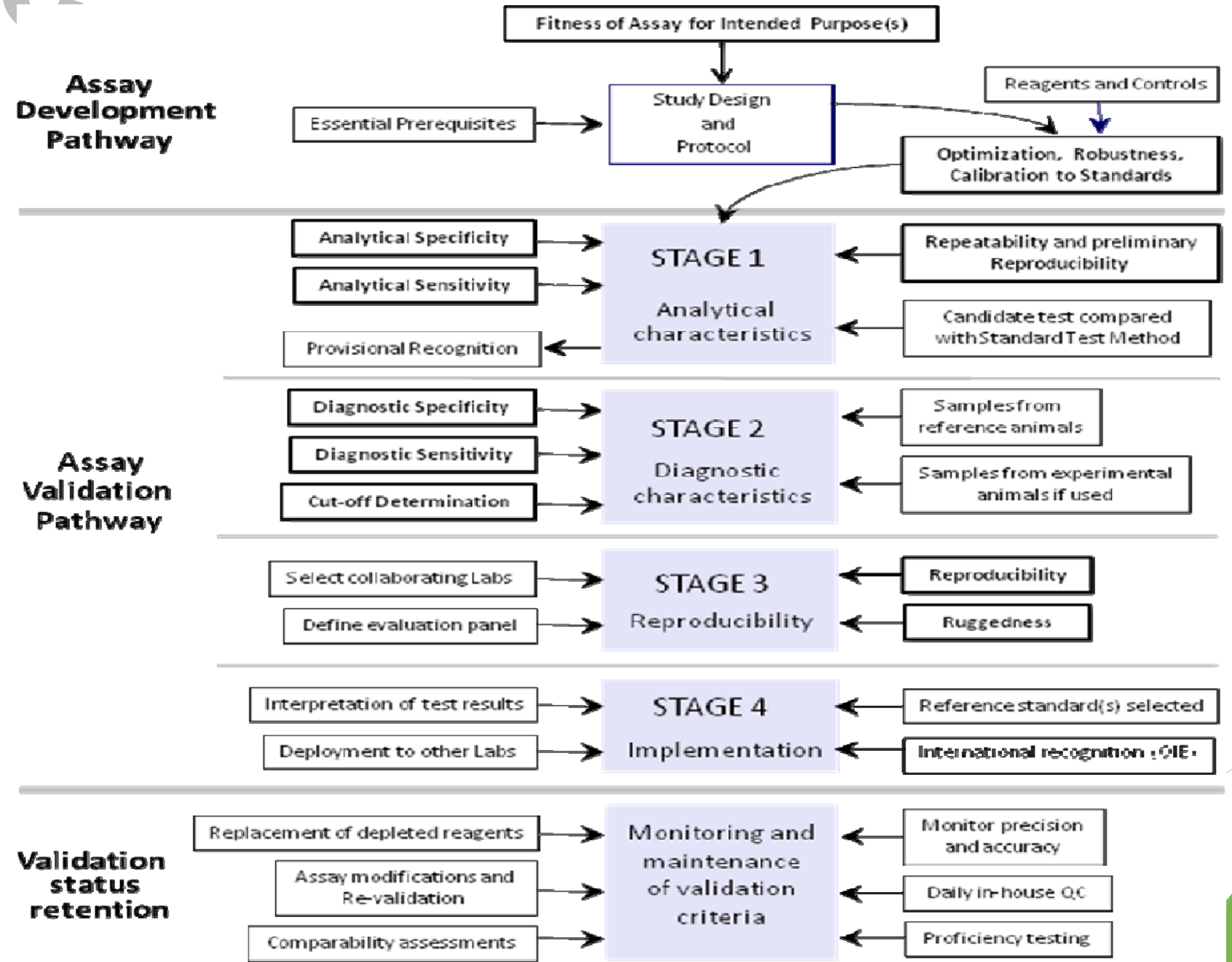

Fig. 1 WOAH diagnostic test validation pathway from the Terrestrial Manual

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Table 1. *Theoretical number of samples from animals of known infection status required for establishing diagnostic sensitivity (DSe) and specificity (DSp) estimates with known confidence*

Estimated DSe or DSp	**2%** error allowed in estimate of DSe and DSp						**5%** error allowed in estimate of DSe and DSp					
	Confidence						Confidence					
	75%	80%	85%	90%	95%	99%	75%	80%	85%	90%	95%	99%
90%	257	369	475	610	864	1493	41	59	76	98	138	239
92%	210	302	389	466	707	1221	34	48	62	75	113	195
94%	161	232	298	382	542	935	26	37	48	61	87	150
95%	136	196	251	372	456	788	22	31	40	60	73	126
96%	110	158	203	260	369	637	18	25	32	42	59	102
97%	83	119	154	197	279	483	13	19	25	32	45	77
98%	56	80	103	133	188	325	9	13	16	21	30	52
99%	28	41	52	67	95	164	4	7	8	11	15	26

Percent error allowed in the estimate of DSe or DSp = 2% in the left panel and 5% in the right panel. For the number of samples required for 1%, 3%, and 4% allowable error in the estimate of DSe and DSp, multiply the number of samples in the left panel of the table by a factor of 4.0, 0.44, and 0.25, respectively.

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CHALLENGES

- The validation pathway as outlined in Fig.1 above is easier for assays such as antibody ELISA and PCR

  - The tests are group specific
  - There is an availability of field samples (naturally infected)
  - There is an availability of vaccination campaign/vaccine trial samples
  - There are other already validated and similar tests to use for comparison that are commercially available
  - There are proficiency test schemes (PTS) for standard method comparison purposes
  - Antibodies and RNA can usually still be found in samples after a month of infection

- Virus isolation methods pose a challenge

  - The tests are expensive and time consuming
  - There is usually a shortage of samples since people usually request PCR for disease confirmation (rapid and cheaper)
  - The samples submitted for PCR are usually blood, thus no tissues are available for virus isolation (owners refuse postmortems)
  - Viraemia in the blood is transient and usually missed (Fig. 2)
  - There are no other similar and validated tests to use for comparison (Tissue culture standardisation almost impossible)
  - There are no PTS for standard method comparisons

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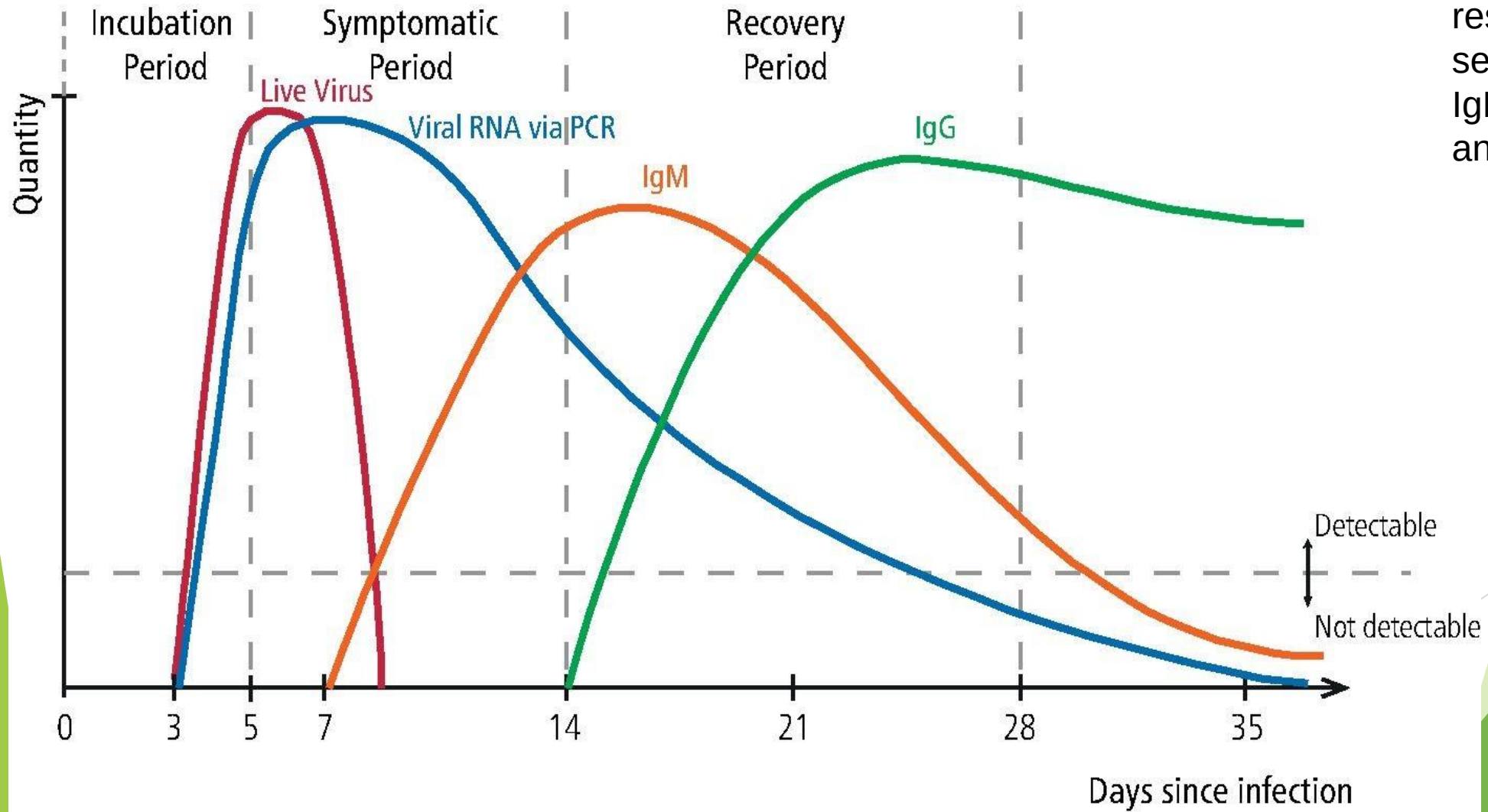

Fig. 2 Example of a virus infection cycle and host humoural immune responses, indicating the sequence of virus, RNA, IgM and IgG appearance and disappearance

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- Even though any isolate can be confirmed using a validated real time RT-PCR test and genome sequencing performed, regulatory authorities still want all tests used for controlled and notifiable animal diseases to be validated using the WOAH guidelines

- Serotyping using neutralization methods poses additional challenges

  - The tests are expensive, labour intensive and time consuming
  - There are 9 AHS virus (AHSV) serotypes
  - South Africa is endemic for AHS and there are hardly field sera positive for a single AHSV serotype (Fig. 3)
  - There are no validated and serotype specific tests to use for typing serum
  - There are no validated and serotype specific tests to use for typing virus. Molecular tests (virus typing) are not yet validated.

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Fig. 3 Map of South Africa indicating the AHS endemic areas in yellow

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- Despite the endemicity of the disease in the country and scarcity of antisera positive for antibodies against one serotype, regulatory authorities want the neutralization test to be validated per serotype using the WOAH guidelines

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POSSIBLE SOLUTIONS & CONCLUSION

African Solutions to African Problems

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- Countries with suspect outbreak cases are urged to submit samples for disease confirmation early. This will increase chances of successful virus isolations if present, and add to the number of samples to use in the validation

- African countries to actively participate in interlaboratory comparison (ILC) exercises for all tests, where countries take turns in providing test panels. This will add to the number of samples required for the validation, and provide reproducibility data

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- Countries with one or few AHSV serotypes circulating (endemic/sporadic) are requested to share sera samples for serotype specific validation of the neutralization test
- With enough samples from the African region used in validation albeit modified due to challenges of standardizing tissue culture based tests, acceptable validation reports can be produced and a motivation provided for acceptance

- In conclusion

Validation and eventual accreditation of an array of available test methods at an African Reference Laboratory will enable other laboratories in the region to do the same. This will be achieved through provision of technical support and resources throughout the validation and accreditation processes. Africa's laboratory capacity will be strengthened and positively contribute towards the progressive control of transboundary animal diseases.

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For Your Attention

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