

RIFT VALLEY FEVER

Diagnostic Tests

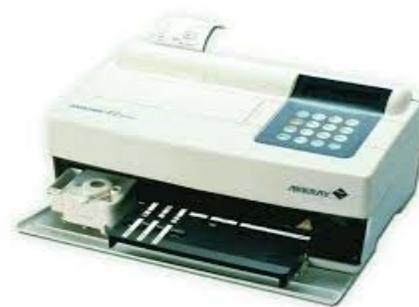
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Role of OIE Reference Laboratories

(Part II)

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DIAGNOSTIC TESTS



- ❑ RVF remains an **important** human and livestock disease, with huge implications for animal/human health and livestock trade.
- ❑ The **quest** for highly sensitive and specific, affordable and rapid diagnostic tools with DIVA capacity & improved vaccines **continues**.
- ❑ **Availability of methods with applications suitable for various purposes is crucial.**

- ❑ There are **commercially available** RVF IgM and IgG antibody ELISA kits.
- ❑ Improved antigen **capture ELISAs** to augment tests used for the diagnosis of recent infection have been developed.
- ❑ **New technologies** to produce RVFV replicon particles (RRPs) that can be safely used in **virus neutralization tests** without bio-containment facilities have been developed.

- ❑ Numerous real-time RT-PCR methods have been developed for rapid detection of RVFV, with detection limits of 10 to 100 genome copies.
- ❑ Reverse transcription loop-mediated isothermal amplification (RT-LAMP) techniques are now available, with comparable detection limits to the real time RT-PCR.

Diagnostic Tests Challenges



1. Purpose for testing

(OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals - 2014)

- i. Population freedom from infection (non-vaccinated animals)
(ELISA; VNT)**
- ii. Individual animal freedom from infection prior to movement
(VNT >ELISA)**
- iii. Contribution to eradication policies
(ELISA;VNT)**
- iv. Confirmation of clinical cases
(VI;RT-PCR > Ag detection; Histopathology; ELISA; VNT)**
- v. Prevalence of infection – surveillance
(ELISA > VNT)**
- vi. Immune status in individual animals or populations post-vaccination
(ELISA; VNT)**

2. Facilities

i. Biosafety

ii. Biosecurity

3. Specimens/samples

i. Type & Condition

ii. History

4. Time

Turnaround time

5. Availability & Costs

- Possibility and ease of procurement of reagents and/kits
- Cost per sample
- Cost of setting up the method in the laboratory (including maintenance)

6. Validation

- Application for a species of interest
- Availability of samples;
- High numbers of samples
- Continuous validation: participating in proficiency test scheme and inter-laboratory test exercises

7. DIVA

- Ability to differentiate infected from vaccinated animals

OIE Reference Laboratories



Terms of Reference (OIE Reference Laboratories)

1. **Use, promote** and disseminate **diagnostic methods** validated according to OIE Standards
2. **Recommend** the prescribed and alternative **tests** or vaccines as OIE Standards
3. Develop reference material in accordance with OIE requirements, and implement and promote the application of OIE Standards
4. **Store and distribute** to national laboratories biological reference products and any other **reagents used in the diagnosis** and control of the designated pathogens or diseases;
5. **Develop, standardise and validate** according to OIE Standards new **procedures for diagnosis** and control of the designated pathogens or diseases;
6. **Provide diagnostic testing facilities**, and, where appropriate, scientific and technical advice on disease control measures to OIE Member Countries;
7. Carry out and/or coordinate scientific and technical studies in collaboration with other laboratories, centres or organisations

Terms of Reference (OIE Reference Laboratories)

8. Collect, process, analyse, publish and disseminate epizootiological data relevant to the designated pathogens or diseases

9. Provide scientific and technical training for personnel from OIE Member Countries

10. Maintain a system of quality assurance, biosafety and biosecurity relevant for the pathogen and the disease concerned

11. Organise and participate in scientific meetings on behalf of the OIE

12. Establish and maintain a network with other OIE Reference Laboratories designated for the same pathogen or disease and organise regular inter-laboratory proficiency testing to ensure comparability of results

13. Organise inter-laboratory proficiency testing with laboratories other than OIE Reference Laboratories for the same pathogens and diseases to ensure equivalence of results;

14. Place expert consultants at the disposal of the OIE.



Ref. Lab for RVF

Onderstepoort Veterinary Institute



Excellence in Research and Development

Diagnositics



VIROLOGY: Serology & Virus Isolations



BIOTECHNOLOGY: PCR Labs



Upgrade to MP96



Lab 1: Reagent and master mix preparation



Lab 2.1 Sample processing, nucleic acid extraction



Lab 2.2 The MagNaPure-automated DNA/RNA extraction - 32 samples



Lab 2.3 The nested PCR room



Lab 3: Thermocycling room



Lab 4: Agarose gel electrophoresis and gel documentation

ToR	Activity	Challenges Addressed
1	Diagnostics: Perform tests using OIE listed serological, antigen identification and molecular methods, and virus isolation	Testing for all 5 purposes listed by the OIE can be conducted.
2.	Diagnostics: Recommend the use of OIE listed diagnostic tests	Testing for all 5 purposes listed by the OIE can be conducted.
6.	Diagnostics	Provide testing for other countries where there are no facilities/capacity
9.	Training: Diagnostic Techniques	Diagnostic capacity in other laboratories can be established when resources are availed and staff members are trained

ToR

Activity

Challenges Addressed

- | | | |
|-----------|---|---|
| 10. | <ul style="list-style-type: none">i. Subscribed to ISO17025 QA Systemii. Adhere to Occupational Health & Safety Regulationsiii. Adhere to Animal Diseases Act, Act 35 of 1984 | <ul style="list-style-type: none">▪ Enforce the use of validated tests▪ Provision of a safe working environment for diagnostic testing; reagent production; and research |
| 12. & 13. | Organisation & participation in interlaboratory test excercises with other laboratories | Ensure continuous diagnostic test validation |

Research



ToR

Activity

Challenges Addressed

3. & 4. Research: Development of a LSD-RVF-PPR vaccine construct. Spin off - Reference antisera was produced and stored

Diagnostic reagents available for distribution

- 5. Research: Development and validation of:**
- i. RVF indirect IgG ELISA (Kortekaas et al., 2013)**
 - ii. RVF capture IgM ELISA (Williams et al 2011)**
 - iii. RVF double antigen ELISA (Ellis et al., 2014)**

Cheaper testing costs per sample; Detection of both IgM & IgG; Reduced turn-around time;

ToR

Activity

Challenges Addressed

7.

Collaborative Research:

1. Validation of a new strip/rapid test for RVF using positive and negative polyclonal RVFV antibody sheep sera (GALVMed)

- Rapid test –possible pen side use.
- Reduced turn – around time

2. Development of a multiplex fluorescent microsphere immunoassay (FMIA), or Luminex assay, for the analysis of RVFV infection, vaccination, and immunological protection from disease (US group).

- Testing for various purposes listed in The OIE Manual can be conducted.

Discussion

- ❑ Antibody detecting **pen side tests** have yielded unsatisfactory results, and research to optimise them continues.
- ❑ The **type of neutralisation test** to use and associated cut-off values has not been decided or a standardisation exercise involving a group of laboratories taken place.
- ❑ Differentiation of infected from vaccinated animals (**DIVA**) in enzootic countries is still not possible. Development of diagnostic tools using vaccine strains such as clone 13 has the potential to provide the highly desired diagnostic tools with such capacity.

- ❑ Scarcity of statistically sound numbers of well characterised/known positive and negative specimens from diverse species and geographical regions impact negatively on assay optimisation and validation.

Test cut-off values determine DSe and DSp, and these are affected by the number and type of samples used.

- ❑ There are no structured proficiency test schemes for RVF for continuous validation of existing assays.

In conclusion:

- ❑ Great strides have been made in the development and improvement of RVF diagnostic tests, but **continued research is needed** to address the shortcomings of the current tests.
- ❑ **Reference laboratories have a role to play in all endeavours aimed at development, optimisation and validation of RVF diagnostic tests**

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