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Activity report from the Australian Centre for Disease Preparedness

Wilna Vosloo

CSIRO

Overview

- Report on DIVA validation in sheep
- PrioCHECK® NSP ELISA short and long protocol
- Summary of regional activities

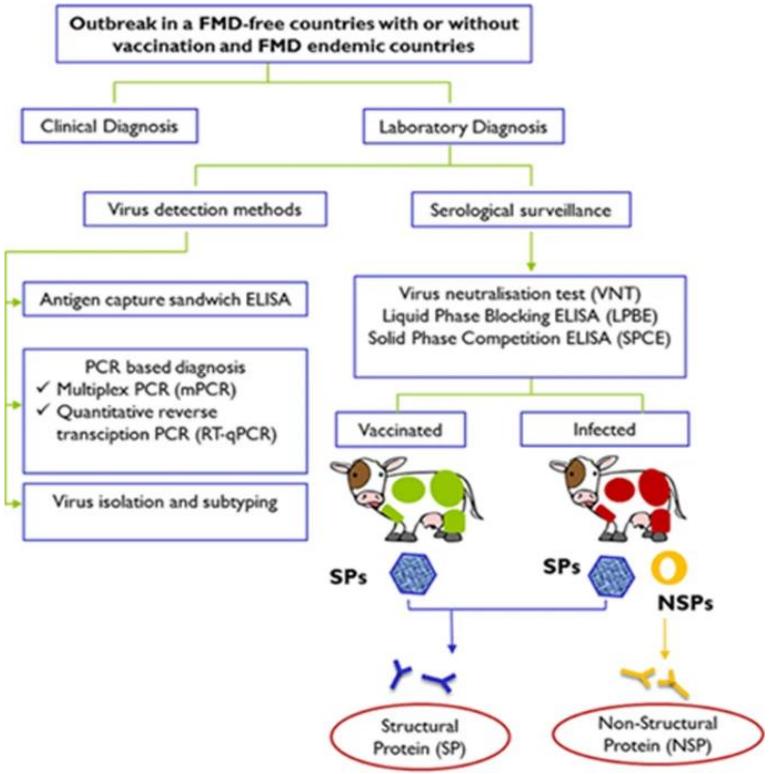
Australia's National Science Agency

FMD DIVA validation for sheep

Nagendra Singanallur

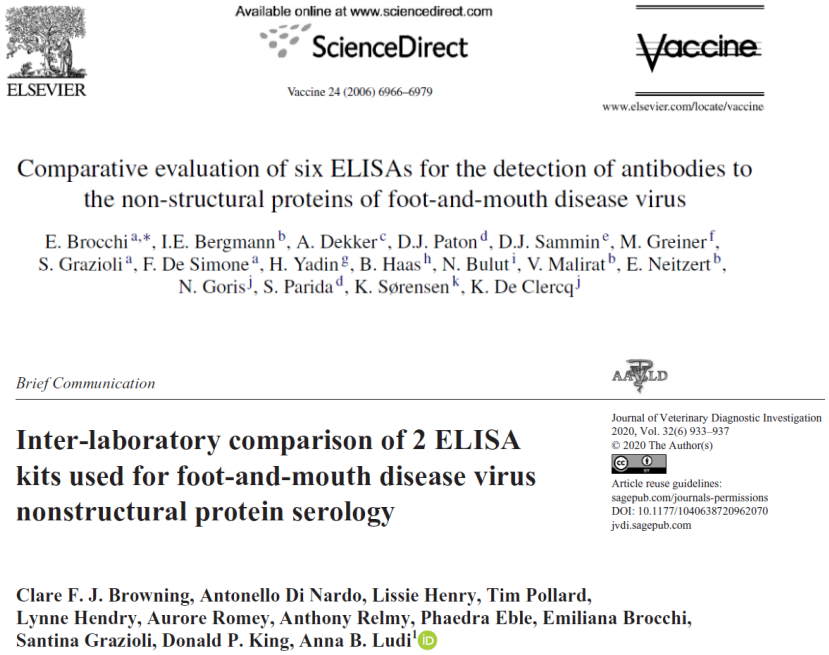
Introduction

- Serological assays can detect antibodies in the absence of overt clinical signs.
- It is essential for surveillance, post-vaccination monitoring and vaccine-matching studies.
- Detection of antibodies to structural and non-structural FMD virus proteins can differentiate infected from vaccinated animals (DIVA), which will be crucial if vaccination is used to control the disease.
- NSP antibody detection will be crucial to '*proof of freedom*' from disease.



Source: Wong et al 2020

NSP antibody assays



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Vaccine 24 (2006) 6966–6979

Vaccine

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Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus

E. Brocchi[a,*], I.E. Bergmann[b], A. Dekker[c], D.J. Paton[d], D.J. Sammin[e], M. Greiner[f], S. Grazioli[a], F. De Simone[a], H. Yadin[g], B. Haas[h], N. Bulut[i], V. Malirat[b], E. Neitzert[b], N. Goris[j], S. Parida[d], K. Sørensen[k], K. De Clercq[j]

Brief Communication

AAVLD

Inter-laboratory comparison of 2 ELISA kits used for foot-and-mouth disease virus nonstructural protein serology

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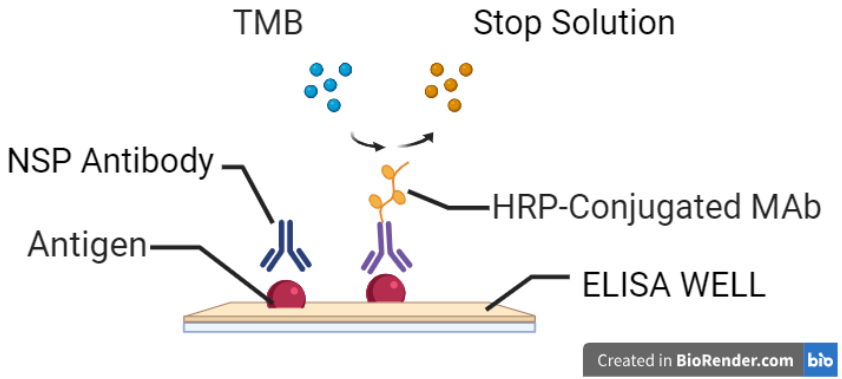
Clare F. J. Browning, Antonello Di Nardo, Lissie Henry, Tim Pollard, Lynne Hendry, Aurore Romey, Anthony Relmy, Phaedra Eble, Emiliana Brocchi, Santina Grazioli, Donald P. King, Anna B. Ludi[1]

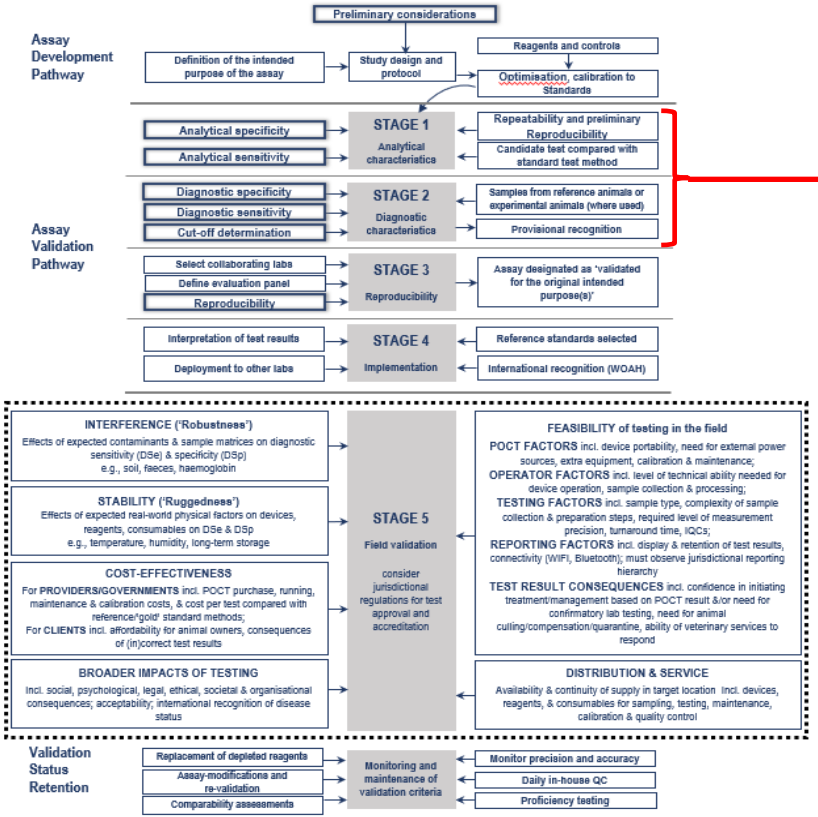
- Problem:
  - Two commercial cELISA kits, which do not contain components derived from an infectious virus, are the accredited tests for routine diagnostic use at ACDP.
  - However, the diagnostic test parameters are based mostly on data are from use with cattle sera.
  - Interlaboratory comparisons were conducted using cattle samples (Brocchi et al. 2006).
  - A systematic validation of these commercial kits for use in sheep is identified as a gap.

Objectives

- The main aim of this study was to fill this gap with the following objectives:
  - Evidence-based validation and comparison of two commercial kits: PrioCHECK™ FMDV NS and IDScreen® FMD NSP Competition for FMD DIVA in sheep.



WOAH General Assay Development and Validation Pathways*



Criteria for Assay Development and Validation

- i) Definition of the intended purpose(s) — Manufacturer
- ii) Optimisation — Manufacturer
- iii) Standardisation — Manufacturer
- iv) Repeatability — Frequentist approach
- v) Analytical sensitivity — Frequentist approach
- vi) Analytical specificity — Frequentist approach
- vii) Thresholds (cut-offs) — Manufacturer
- viii) Diagnostic sensitivity — Latent class approach
- ix) Diagnostic specificity — Latent class approach
- x) Reproducibility — Frequentist approach
- xi) Fitness for the intended purpose(s) — Manufacturer

*WOAH Terrestrial Manual Chapter 1.1.6

Sera tested

Origin	Status	Not infected	Infected	Total
Egypt	Non vaccinated	0	0	0
	Vaccinated	26	2	28
Armenia	Non vaccinated	14	21	35
	Vaccinated	5	12	17
Libya	Non vaccinated	182	52	234
	Vaccinated	349	145	494
Total		576	232	808

Summary

- The median relative diagnostic sensitivity (DSe) and diagnostic specificity (DSp):
  - PrioCHECK™ kit: DSe 93.1% (85.2-97.6 CI*) and DSp 97.6% (91.3-99.5 CI)
  - IDScreen® kit: DSe 96.4% (87.3-99.8 CI) and DSp 95.1 (88.8-98.6 CI)
- The earliest detection of antibodies against NSPs
  - Around day 7 post-infection in unvaccinated and infected sheep
  - Around day 11 post-challenge in vaccinated sheep
- The overall measure of agreement was ‘almost perfect agreement’ with weighted kappa (k) = 0.91 (0.87-0.95 95%CI)
- The diagnostic test accuracy between the kits was 96.92 per cent (95.79-97.81 per cent 95% CI)
- Both kits are reliable for use in sheep (Cronbach’s α = >0.99)

*CI: Posterior credibility intervals

Acknowledgements

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  - Wilna Vosloo
- GFRA Partners
  - Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Brescia, Italy (IZSLER)
    - Santina Grazioli, Marita Scaramuzza
  - Wageningen BioVeterinary Research, Lelystad, The Netherlands (WVBR)
    - Aldo Dekker, Phaedra Eble, Cynthia Baars-Lorist

PrioCHECK® FMDV NS ELISA

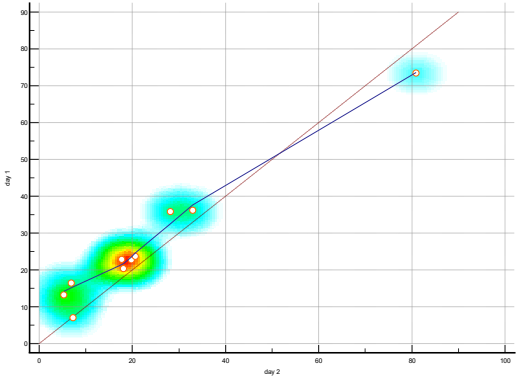
- At ACDP test accredited only for overnight protocol
- Comparing overnight and short protocol to expand accreditation

Method	ELISA Buffer (µL)	Sample (µL)	Incubation (Hrs)
Single day protocol	80	50	2
Overnight protocol	80	20	16-18

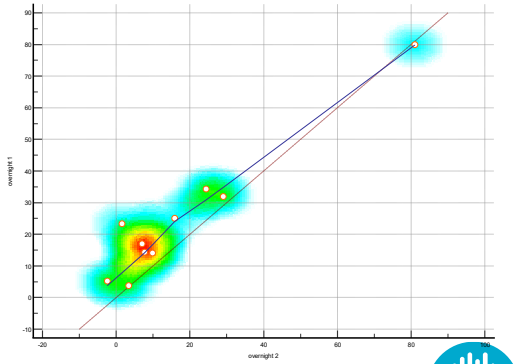
- Bovine, porcine and caprine (pos and neg) tested by 2 operators independently at ACDP
- PT round in Australia via LEADDR network (9 participants)

PrioCHECK® FMDV NS ELISA

- Good agreement between results
- Measurement of uncertainty
  - Short protocol:
    - Positive result (PI ≥ 50%) that is < 56% is not positive with 95% confidence
    - Negative result (PI < 50%) that is ≥ 44% is not negative with 95% confidence
  - Long protocol:
    - Positive result (PI ≥ 50%) that is < 53% is not positive with 95% confidence
    - Negative result (PI < 50%) that is ≥ 47% is not negative with 95% confidence



Scatter plot short



Scatter plot long

PrioCHECK® FMDV NS ELISA

- Single day protocol allows results in 4 hours
- Recommended for CAT 2 and 3 submissions at ACPD
  - All sera tested using the short protocol
  - All reactors will be tested with O/N protocol
  - Reactors on O/N protocol will be tested by SPCE for all serotypes

- Data supports findings from Browning et al 2020* (98% DSe and 99% DSp) for short protocol

*doi: 10.1177/1040638720962070

ACDP Regional Activities

- Indonesia
  - BICOLLAB
    - Collaborated with **DIC Wates** to undertake surveillance of FMD in the field across April-June 2024 to support the evaluation of LFDs for FMD for their performance characteristics under field conditions and standardise methods to recover FMDV genome from inactivated LFDs for confirmatory testing in the laboratory.
  - REDS
    - Establishing external QA (EQA) programs for FMD PCR and NSP ELISA with the Indonesian national reference laboratory for FMD, **PUSVETMA**, for the Indonesian veterinary laboratory network. The two main EQA programs are:
      - i) establishing a network quality control with results returned to PUSVETMA for collation to provide progressive monitoring of FMD PCR and ELISA assays across the network (commenced Jan 2024) and
      - ii) PT – scheduled for implementation in Oct/Nov 2024.

DFAT funding

ACDP Regional activities

- Papua New Guinea
  - LABCAP
    - FMD PCR testing, ELISA testing, Rapid Test Kit provision.
    - Training on all of the above, NAHFTL/NAQIA can run all these tests in-country.
    - Collaborative workshops on FMD sampling, surveillance and diagnostic testing in the field with DAFF/NAQIA/ACDP.

DAFF funding

Australian Centre for Disease Preparedness
Wilna Vosloo

+61 3 5227 5015
Wilna.Vosloo@csiro.au

https://research.csiro.au/fmd