Serological Tests for FMDV a WRL perspective

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Importance of understanding:

(1) the intendent use

(2) the limitations

Main Purpose of Serological Tests

Four main purposes defined in the OIE Terrestrial Manual

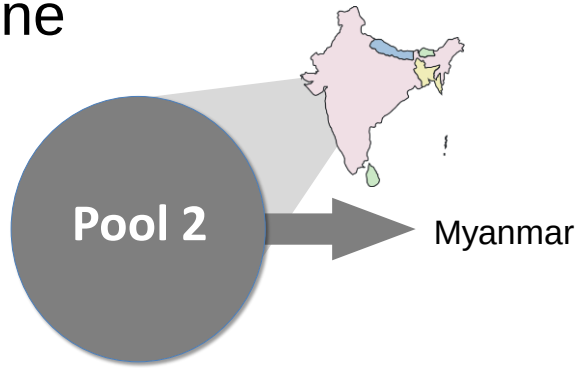
1. To certify individual animals prior to import or export (i.e. for trade)
2. To confirm suspected cases of FMD
3. To substantiate absence of infection
4. To demonstrate the efficacy of vaccination

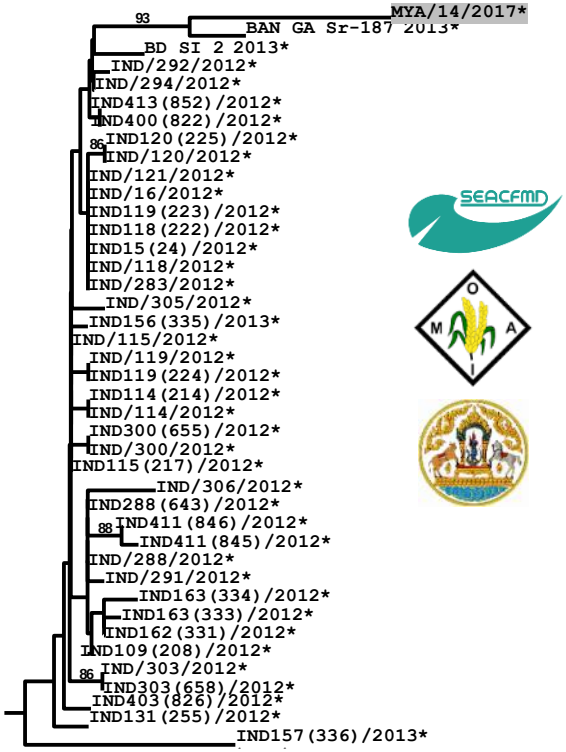
** key point – “cut-offs may be set at different threshold for herd-based sero-surveillance than is appropriate for certifying freedom from infection for individual animals for the purpose of international trade.”

*OIE Terrestrial Manual 2018, Chapter 3.1.8

SEA: outbreaks of Serotype Asia 1

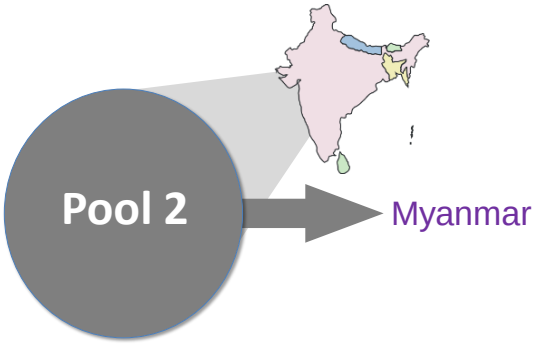
- Serotype Asia 1 not reported in Southeast Asia since 2006/7(?)
- February 2017: FMD outbreaks in cattle in Myanmar
- Sequence data from RRL-SEA in Pakchong, Thailand
- New introduction of the virus from Pool 2
- Asia 1 component had been removed from the vaccine

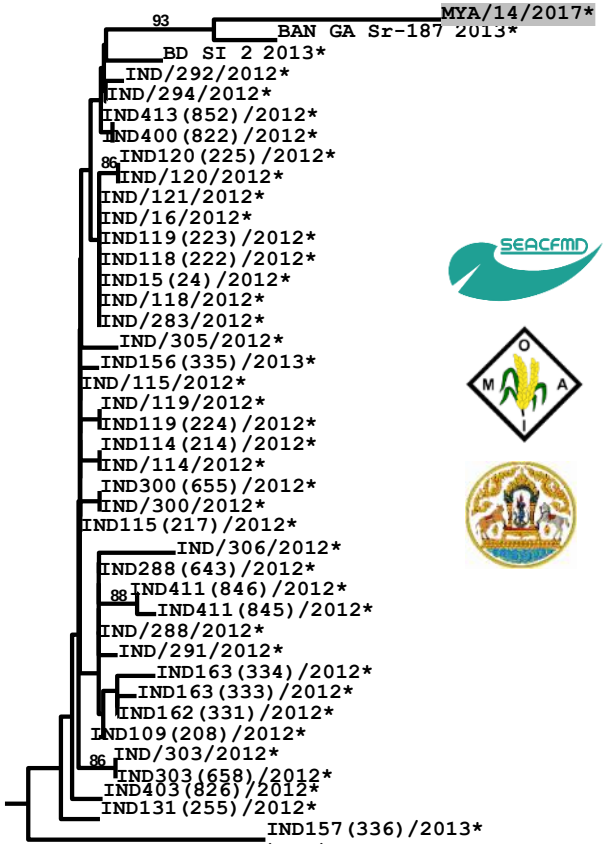




SEA: outbreaks of Serotype Asia 1

? Is these evidence that the Asia 1 serotype has spread more widely in Myanmar





Looking for evidence of Asia 1 infection in Myanmar

- Two recent serosurveys:

  1. 2016/2017 – cattle (NZ Project)
  2. 2018 – multiple species (OIE Project)

- Samples screened for NSP (PrioCheck) and Asia 1 specific antibodies (LPBE)

* Thank you to the Government of Myanmar, Andy McFadden, Yu Qiu and colleagues

Looking for evidence of Asia 1 infection in Myanmar

- Representative LPBE positive sera (**n = 204**) sent to WRLFMD for confirmation by **VNT** (Asia 1 Shamir virus)

RESULTS

- Very few Asia 1 antibody-positives

- *NZ data: 6 positives, 5 inconclusive, 40 negative*
- *OIE Data: 1 positive, 8 inconclusive, 143 negative (1 test failure)*

Serological Assays

- Non-structural protein ELISA (NSP)

Non-Structural Protein ELISA

- Liquid Phase Blocking ELISA (LPBE)
- Solid Phase Competition ELISA (SPCE)
- Virus Neutralisation Test (VNT)
- Vaccine Matching VNT

Structural Protein ELISA

Others (not covered):

- Complement Fixation Test
- Agar gel immunodiffusion
- Avidity ELISA
- Isotype ELISA

Purpose according to OIE Terrestrial Manual

	NSP Ab	SP Ab	VNT
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	-	+++	+++

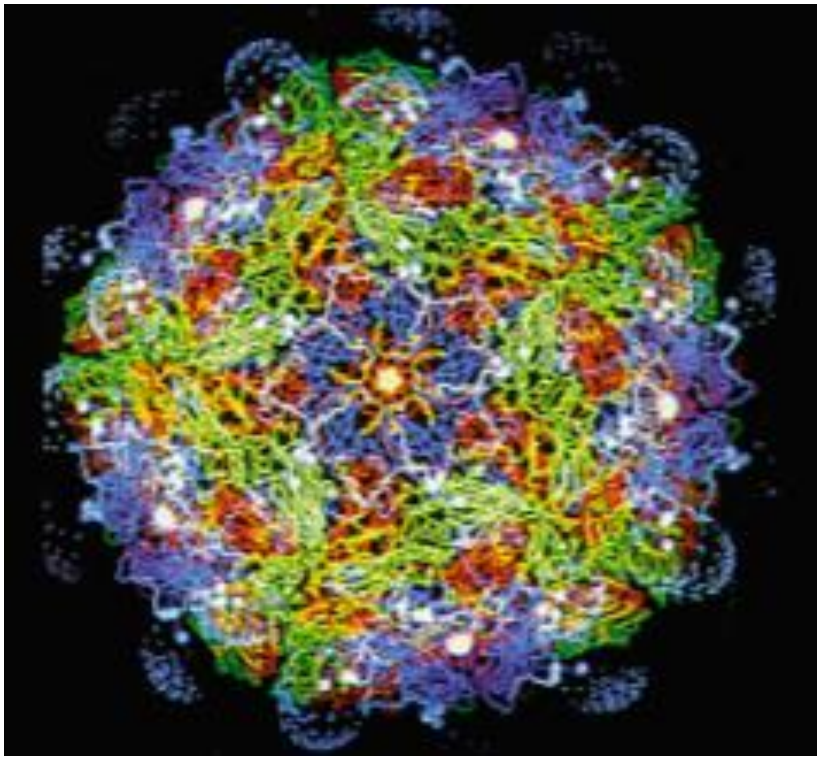
KEY:

+++ recommended, validated for the purposes shown

++ suitable method but may need further validation

- Not appropriate for this purpose

Structural Protein ELISA



Liquid Phase Blocking ELISA
How does it work?

A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus

I. Development and method of ELISA

C. Hamblin *, I.T.R. Barnett and R.S. Hedger

Department of Virus Diagnosis, Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 ONF, U.K.

(Received 13 March 1986, accepted 12 May 1986)

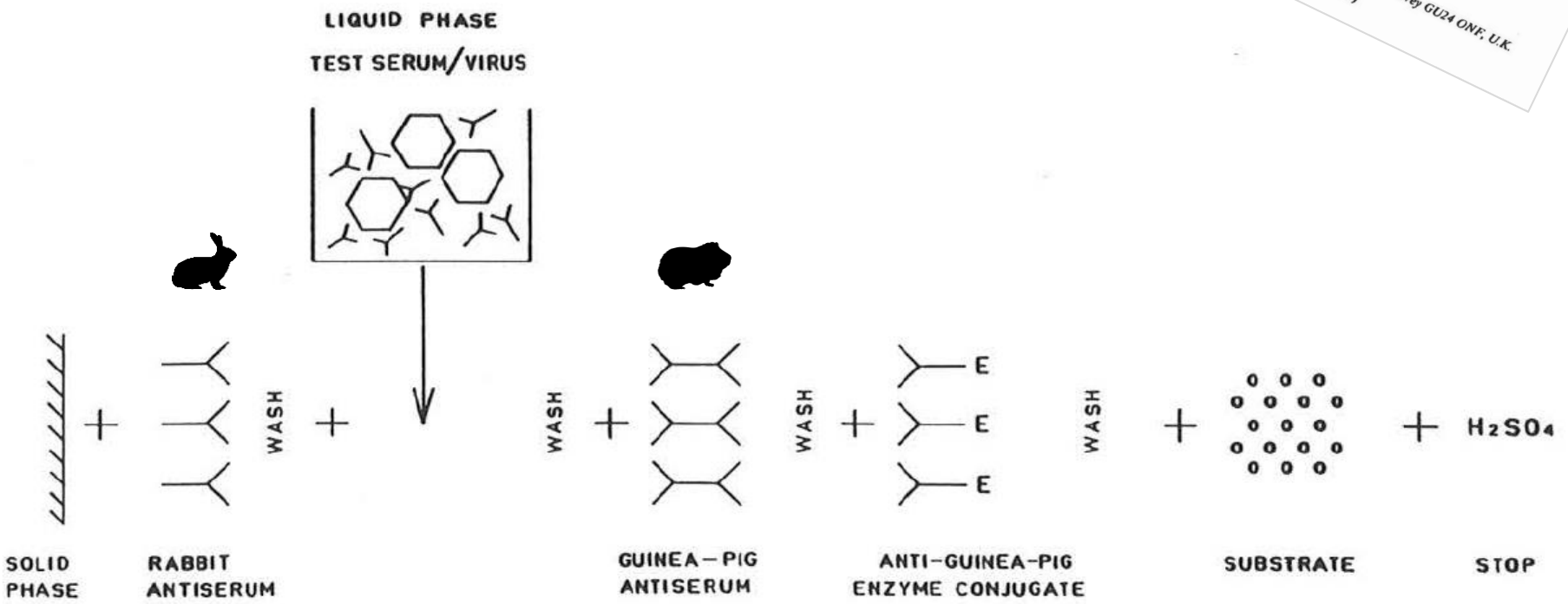
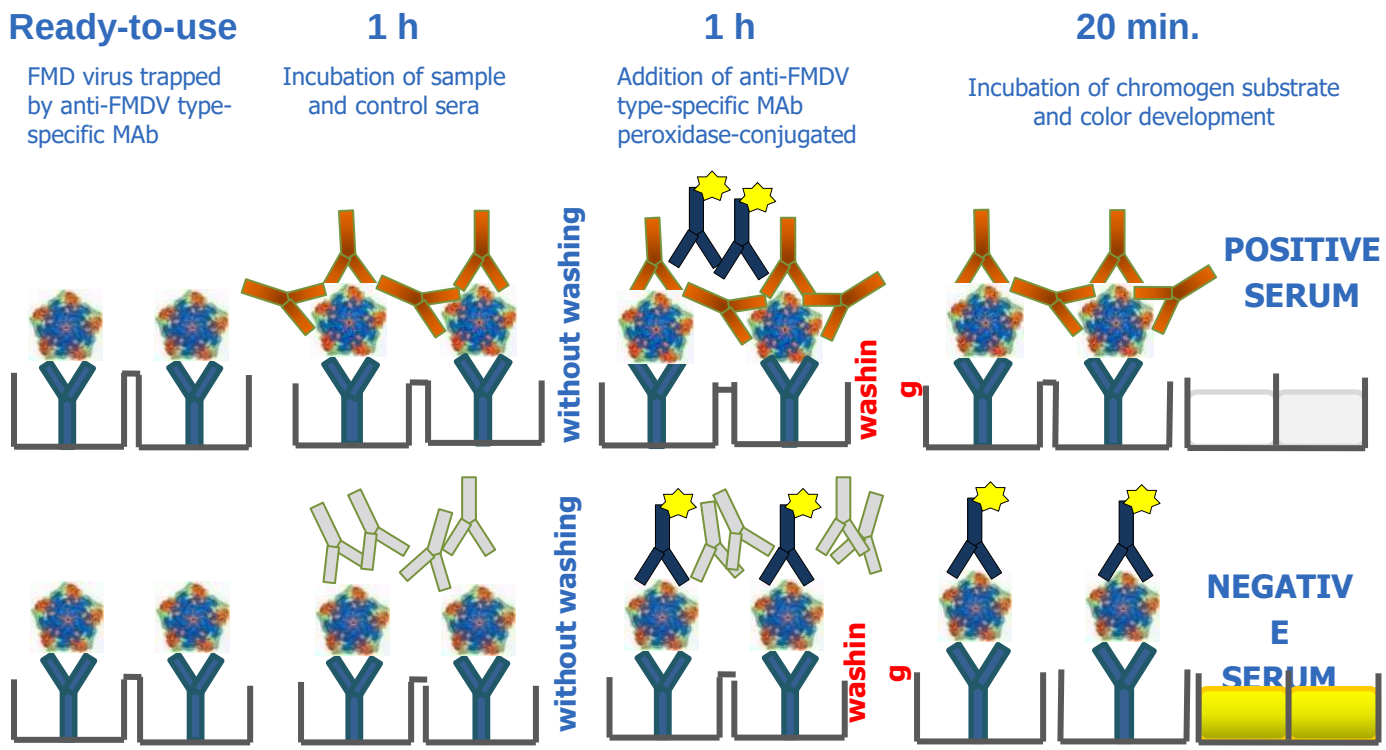


Fig. 1. Flow diagram of the liquid-phase blocking sandwich ELISA.

Solid Phase Competition ELISA
How does it work?



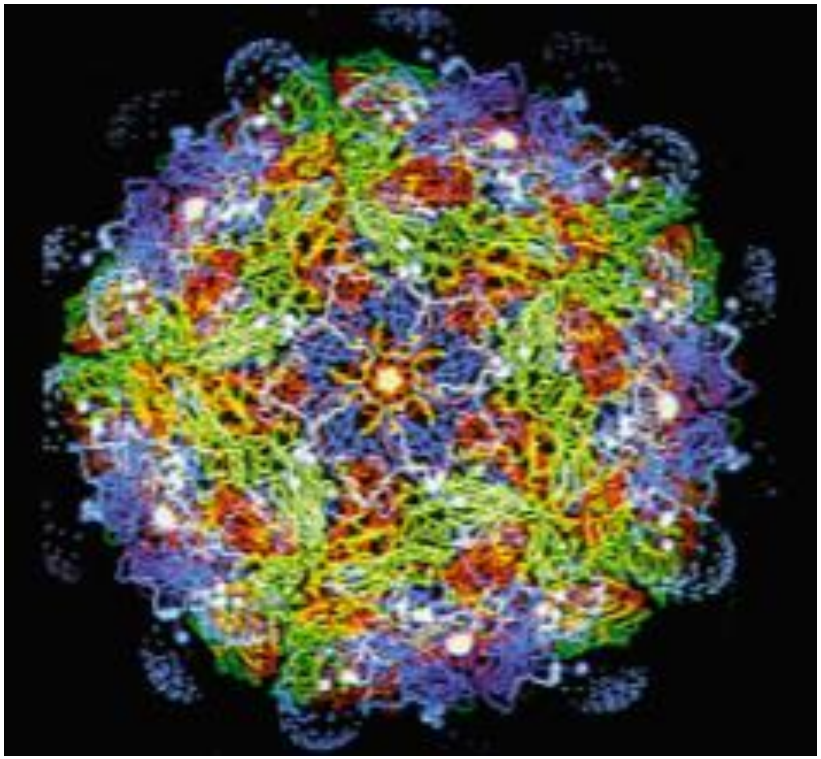
LPBE vs SPCE

	LPBE	SPCE
MAIN USES		
Import/Export Testing	+	+
Vaccine Matching	+	-
Post-Outbreak Surveillance	+	+
Post Vaccination Monitoring***	+	+
Availability	Individual reagents from RL	Commercial kits available
Optimization	yes	no
Validation	yes	yes

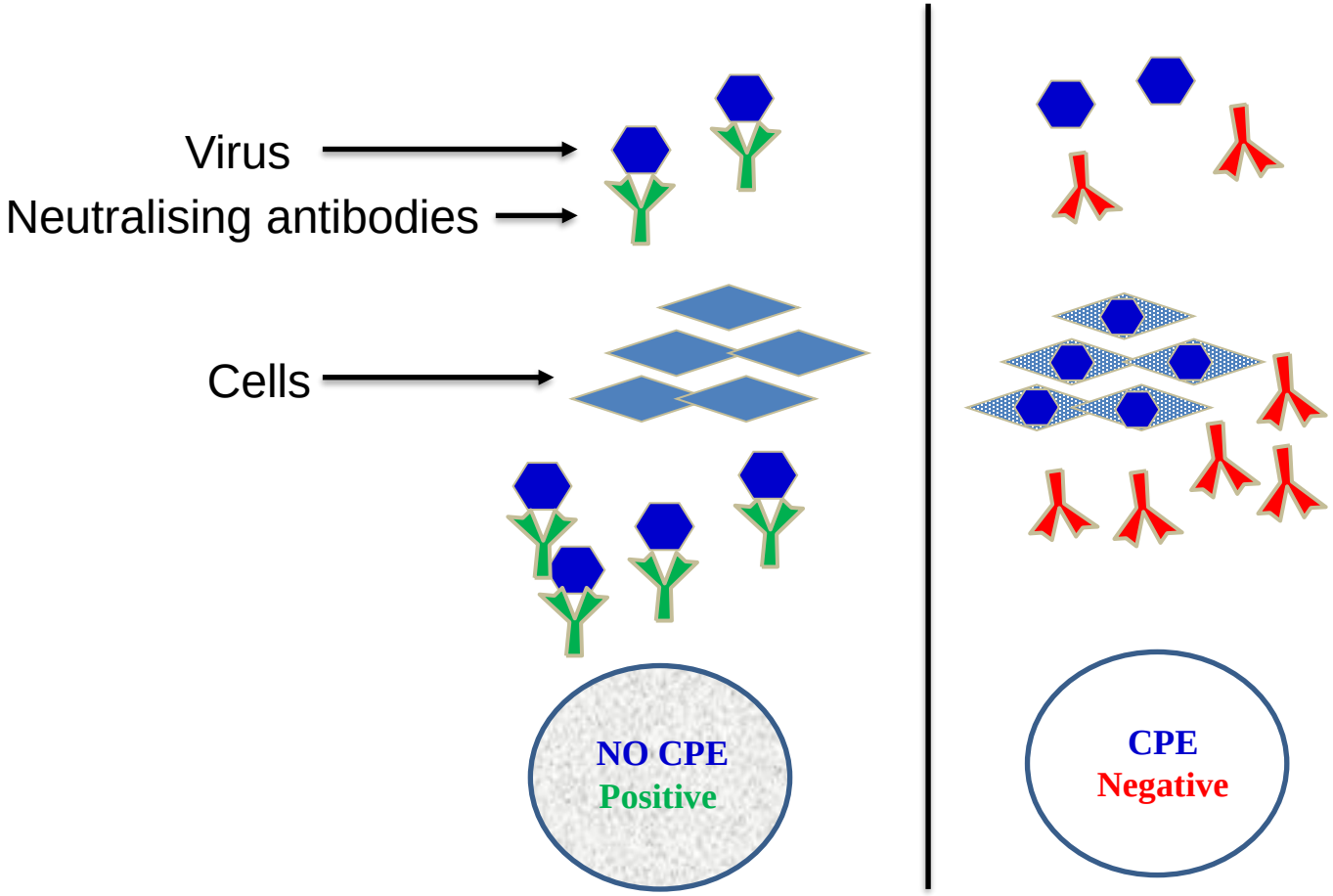
*** No data is available on how ELISA data correlates directly to protection (more on this later)

Measures:
Neutralising Antibodies

Virus Neutralisation Test



Virus Neutralization Test - How does it work?



VNT – Advantages / Disadvantages

Advantages	Disadvantages
Strain specific	Live virus
Homologous and Heterologous titres	Cell culture essential
Little to no cross-reactivity	More variable and poorer repeatability than ELISA

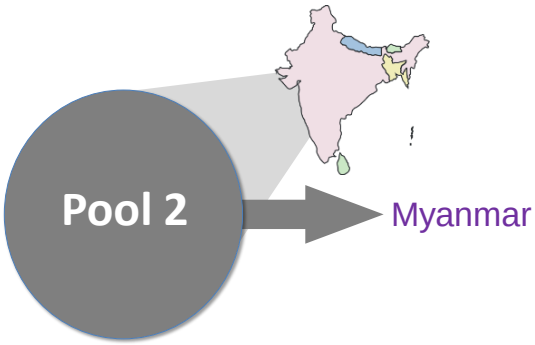
MAIN USES	
Import/Export Testing	+
Vaccine Matching	+
Post-Outbreak Surveillance	+
Post Vaccination Monitoring***	+

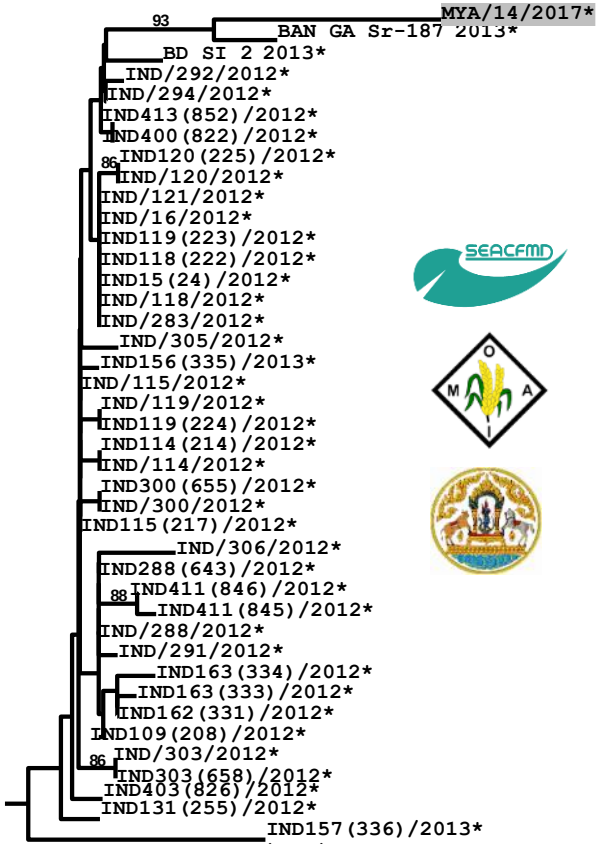
*** Data is available on how VNT titres correlate to protection (more on this later)

SEA: outbreaks of Serotype Asia 1

?

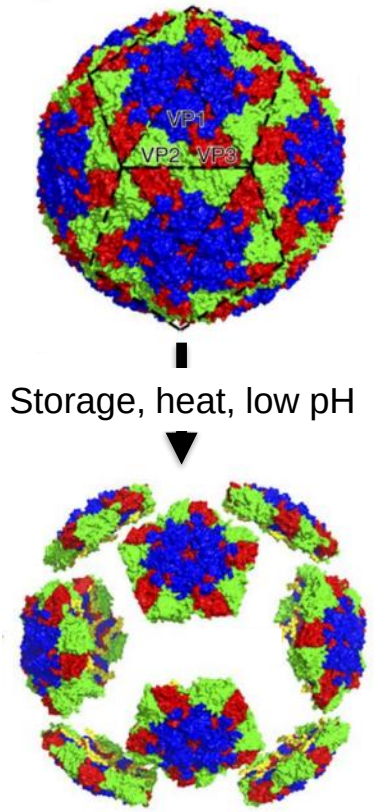
Is there evidence that the Asia 1 serotype has spread more widely in Myanmar





Understanding discrepancy between LPBE & VNT

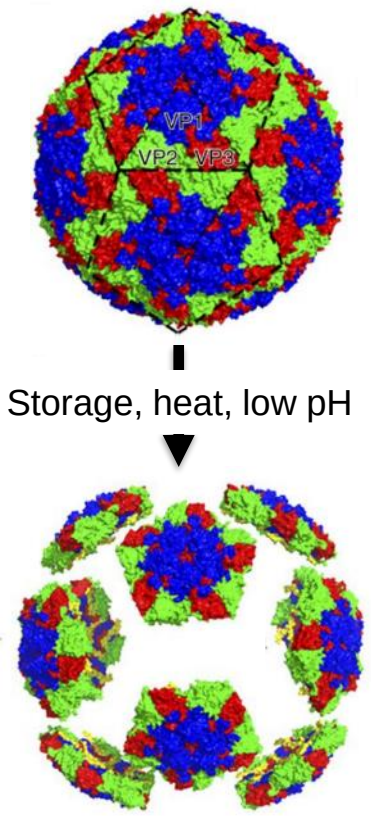
- Structural protein antibodies measured by LPBE and VNT target different epitopes
- VNT only detects neutralising antibodies (surface exposed at defined sites)
- LPBE detects a wider-range of epitopes and cannot distinguish between external and internal epitopes



How does this affect the diagnostic test results??

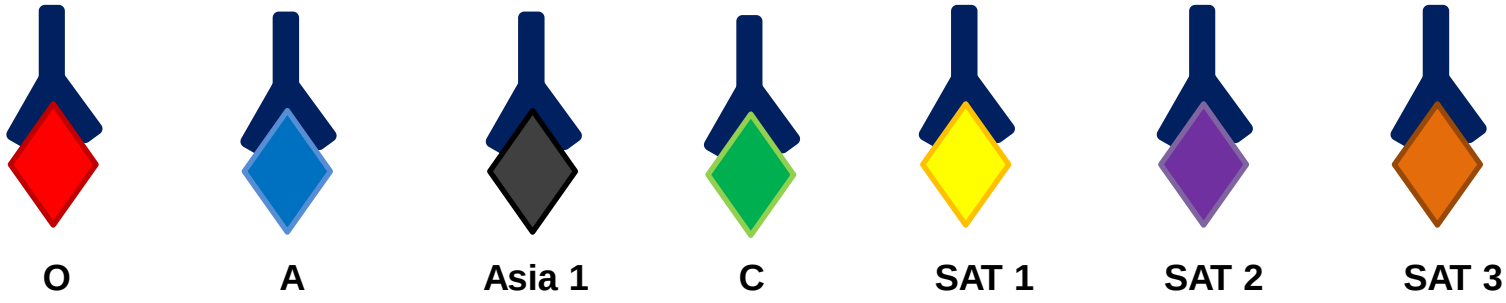
Understanding discrepancy between LPBE & VNT

- FMDV capsids are easily degraded
- Host polyclonal responses are directed at antigenic sites (contribute to neutralising responses), other surface “binding” epitopes and **epitopes exposed after capsid degradation**
- Serotypic determinants are on the outside of the capsid, while many internal epitopes are shared between serotypes



Is this why we see Serotypic Cross-reactivity?

‘The ability of an antibody to react with similar antigenic sites on different proteins’

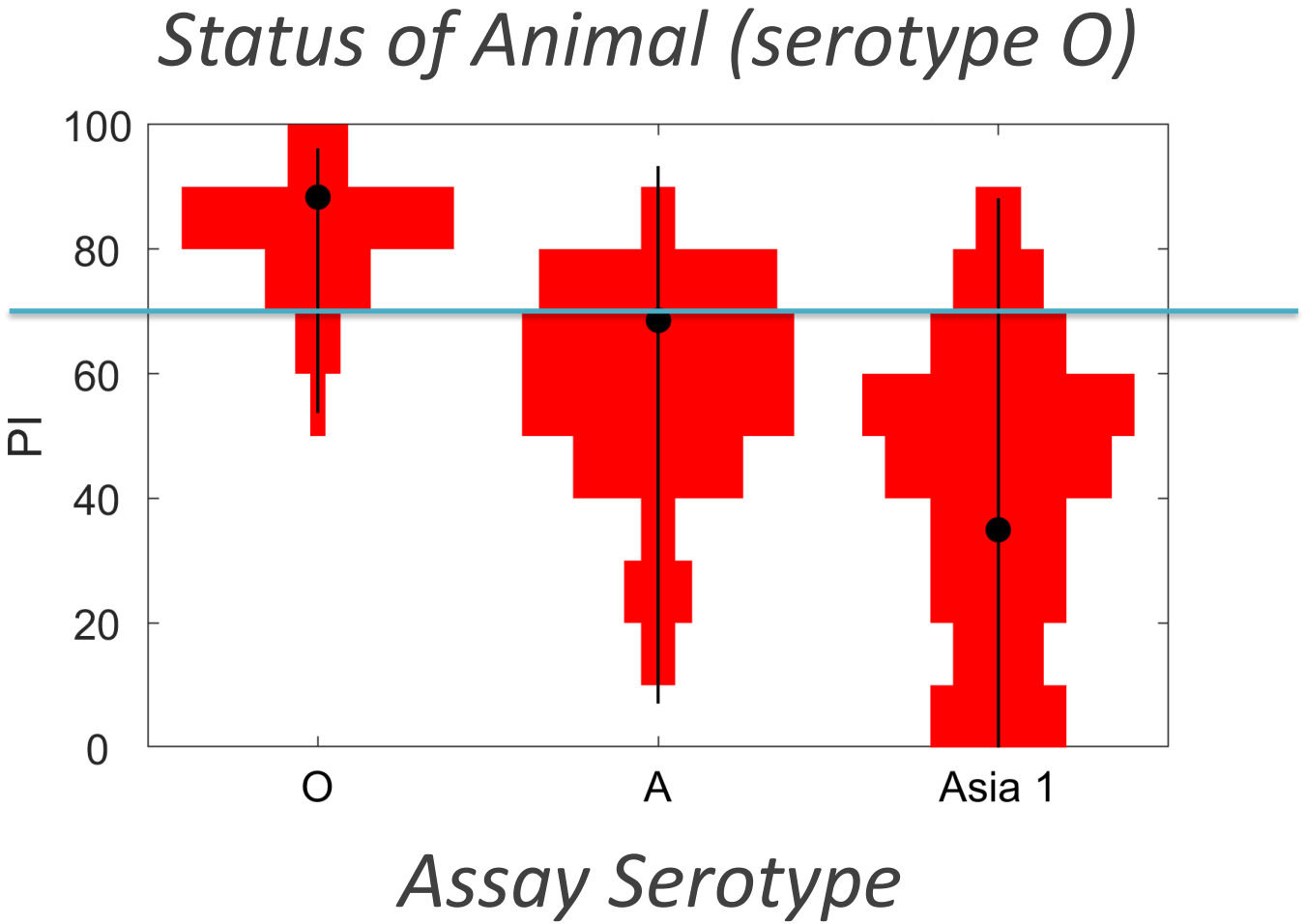


ELISA set-up

Liquid phase ELISA	TPI LPBE	In-house	Polyclonal	O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3
Competition ELISA	TPI SPCE			O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3
	IZSLER SPCE	Commercial	Monoclonal	O, A, Asia 1, SAT 1 and SAT 2
	IDVet			O, A and Asia 1
	PrioCHECK			O, A and Asia 1

9125 individual tests carried out!

Results (infected cattle)



Status of Animal ⟹ O A As 1

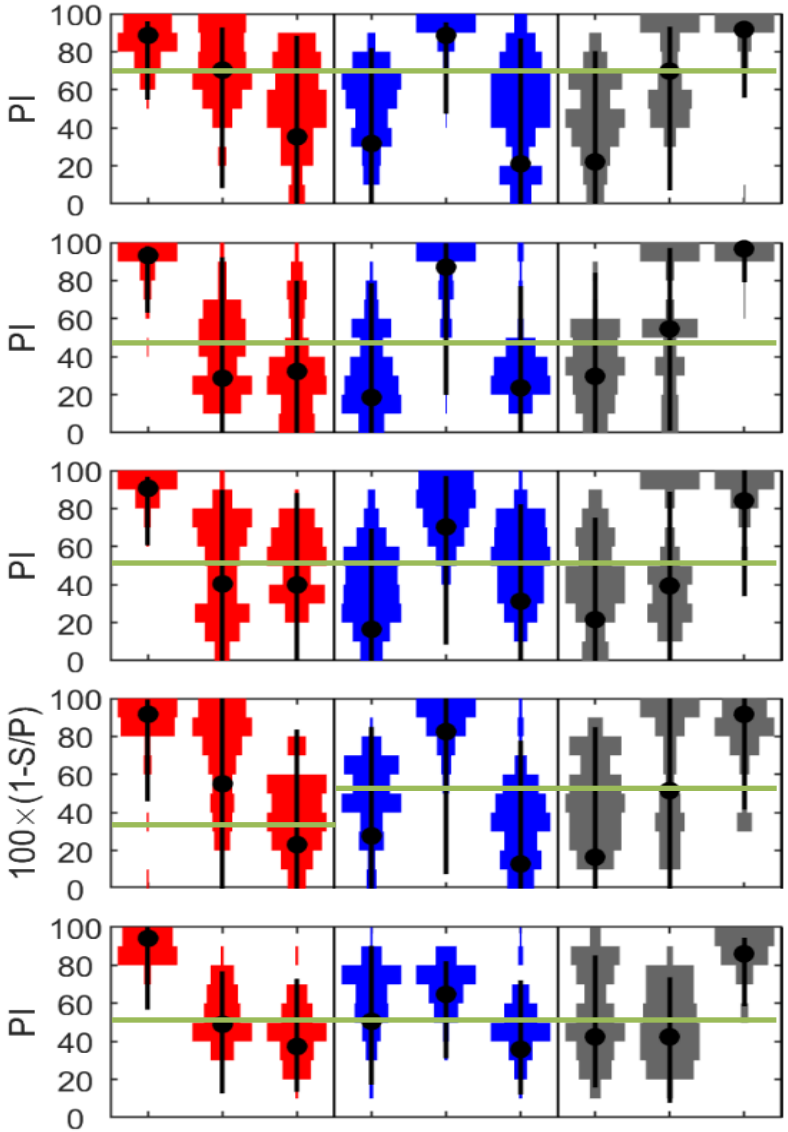
IZSLER

LPBE

SPCE

IDVet

PrioCHECK



Assay Serotype ⟹ O, A, Asia 1

Highlights importance of serological validation

Specificity
- Test a negative cohort of sera – no vaccination, no infection
- Preferably from a region free from FMDV without vaccination

Sensitivity
- Experimentally infected/vaccinated animals of known serotype
- Monovalent infection/vaccination

Experimental Design
- Compare to another assay
- Run assays side by side under same conditions
- Insure staff are trained and competent to carry out validation
- Set acceptance criteria **before** testing takes place

Acknowledgements

- Support for the WRLFMD and research projects
- Collaborating FMD Reference Laboratories and field teams
- Partners within the OIE/FAO FMD Lab Network