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किसानों का हमसफर
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सत्यमेव जयते



Application of VP2 & VP4 mAb-based Indirect ELISA for Potency and Stability Assessment of Indian Trivalent FMD Vaccine Formulations

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FMD Vaccine Potency

Gold Standards: (Infrastructure Intensive)

- Cattle Challenge (PD₅₀ Method)
- Cattle Challenge (PGP Method)

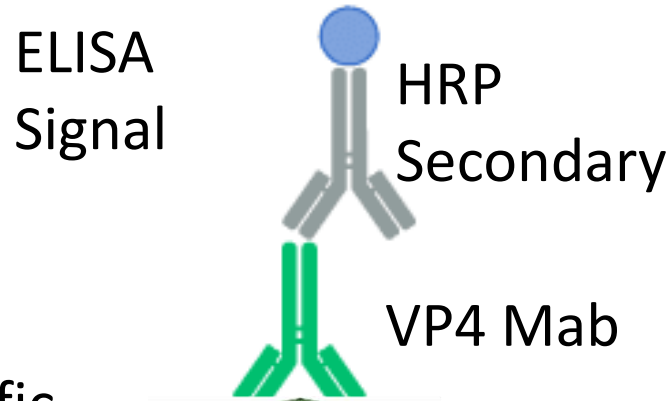
Alternate Methods

(Cumbersome, time consuming, infrastructure required)

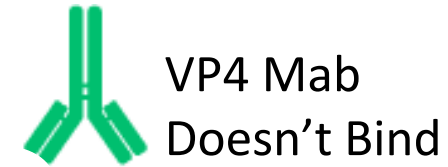
Cattle Serology using VNT

Cattle Serology using LPBE

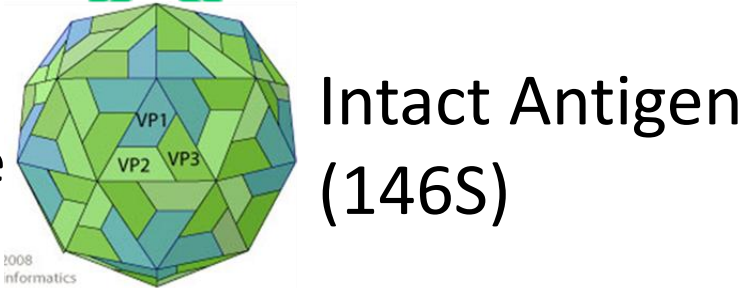
Vaccine Integrity ELISA - VP4 Mab Quantify Intact Antigen



No Signal

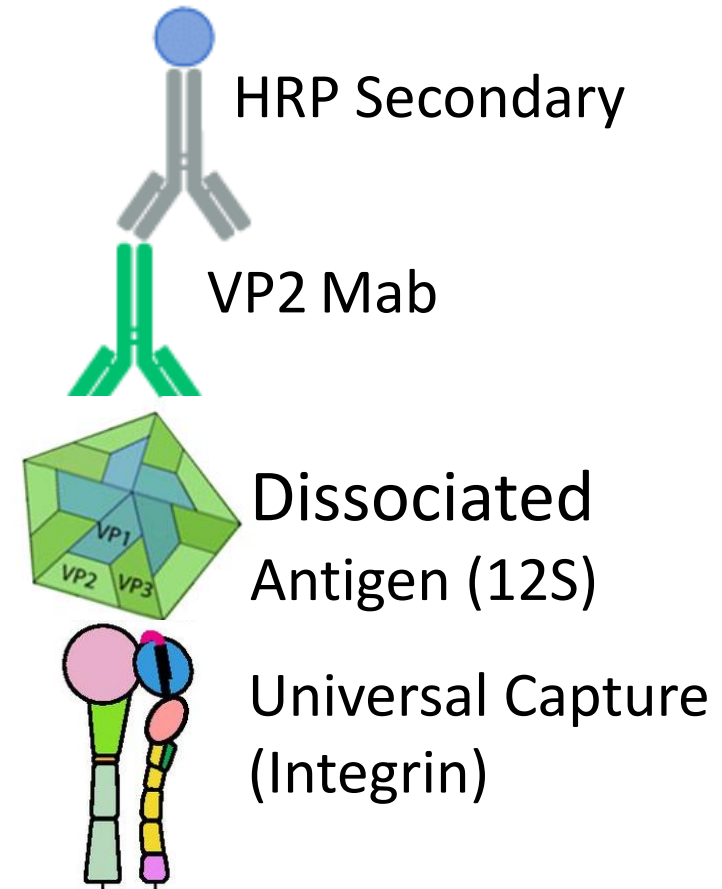
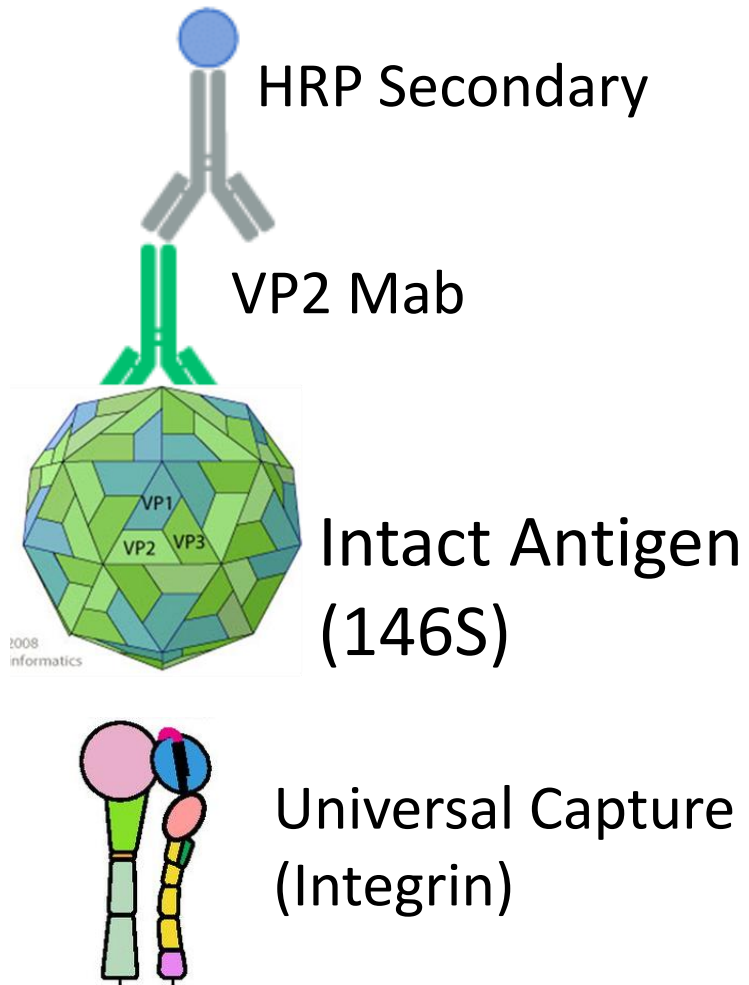


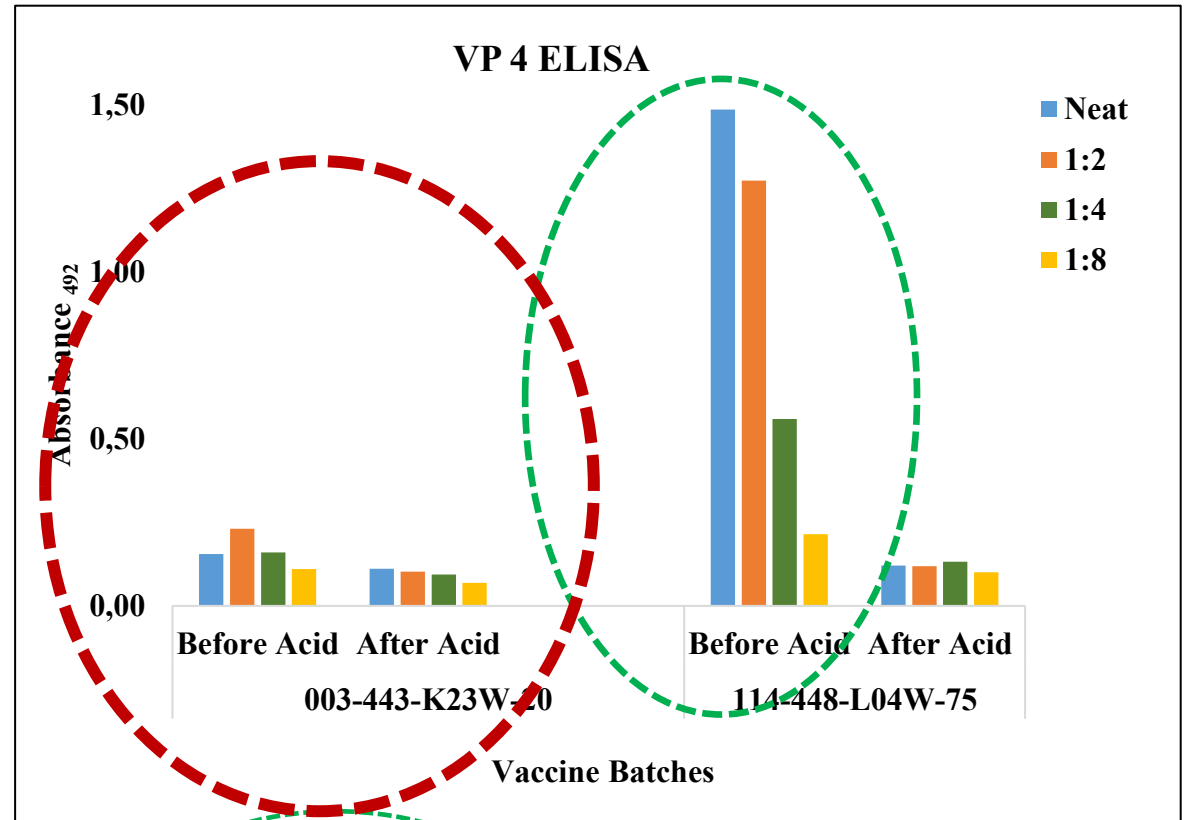
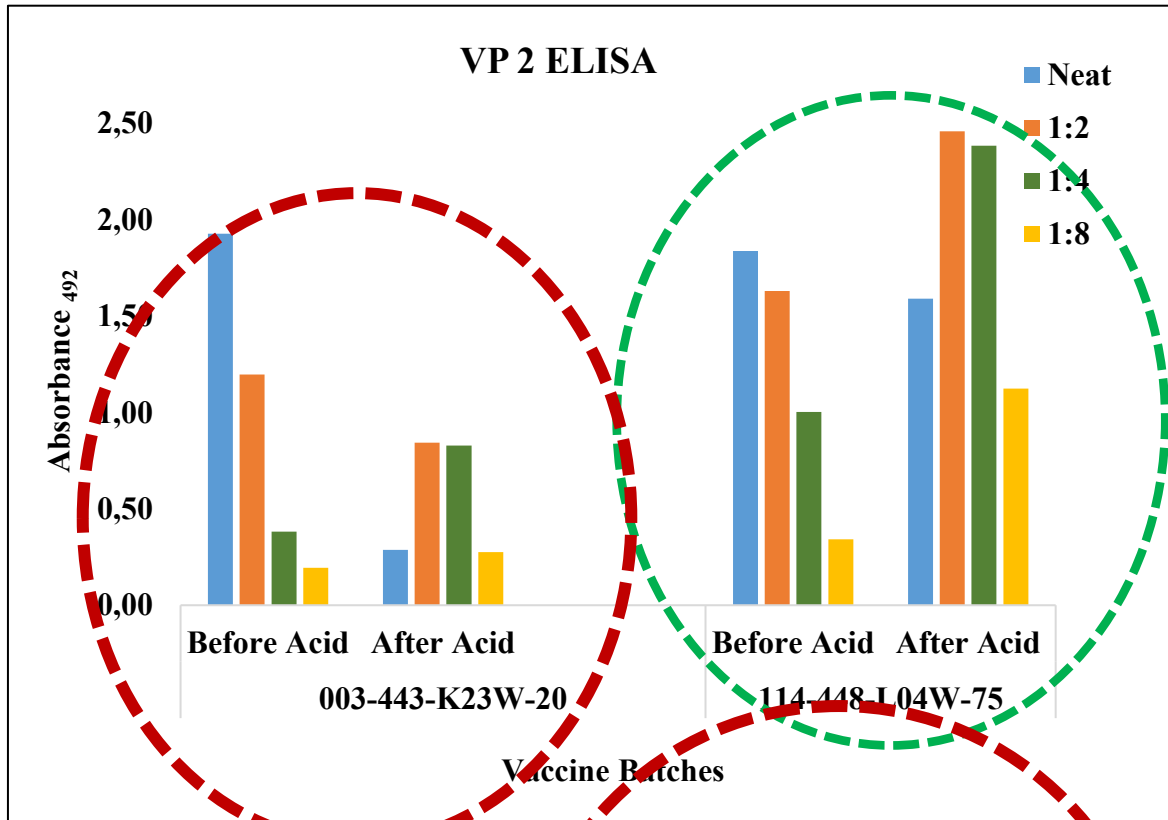
Serotype specific versions of the test will require serotype specific capture reagents



Recognition of intact 146S particles requires crosslinker treatment to enable the VP4 epitope to be accessible to the antibody.

Vaccine Integrity ELISA - VP2 Mab: Quantify Total Antigen





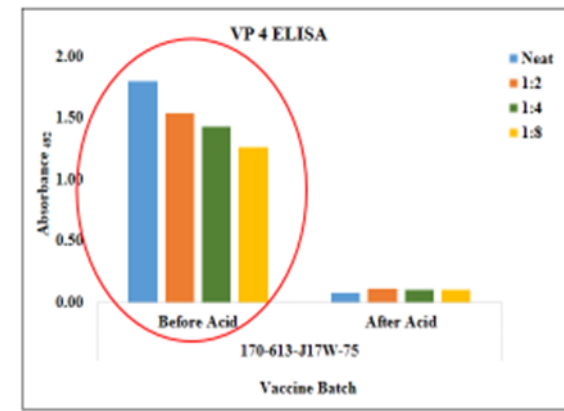
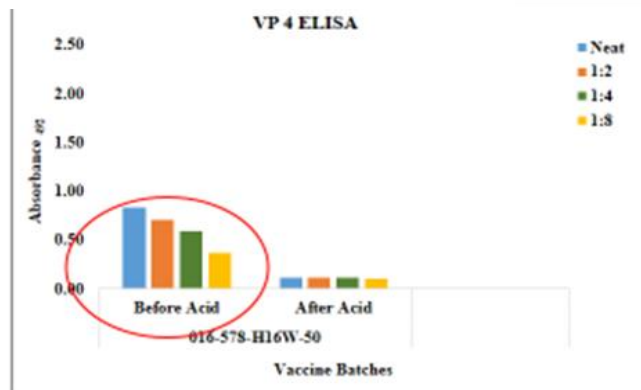
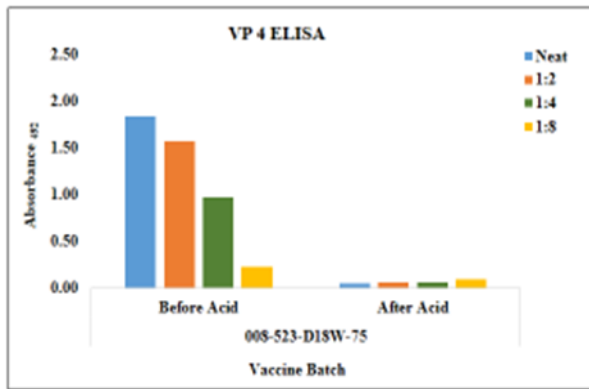
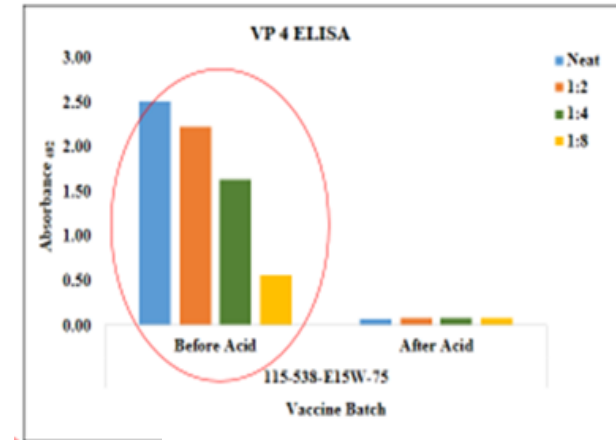
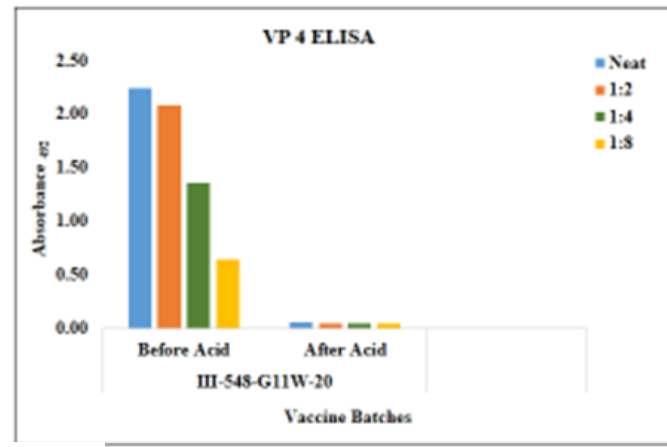
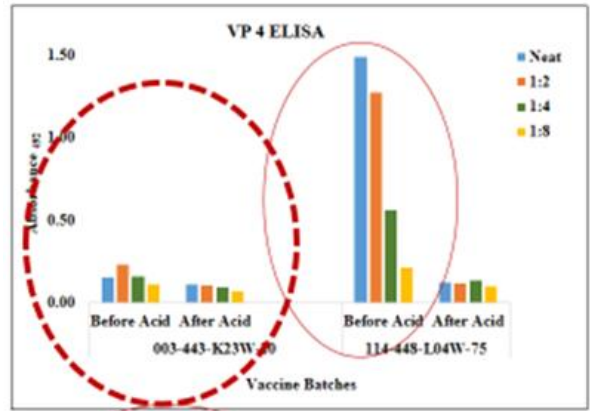
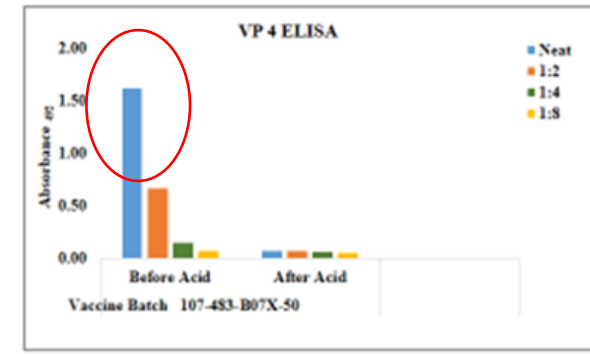
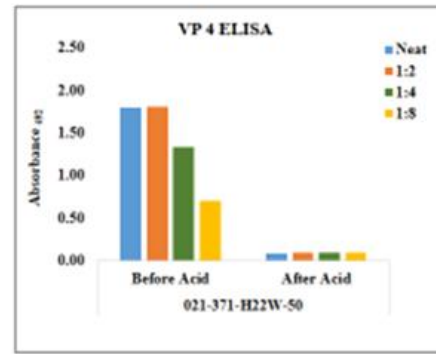
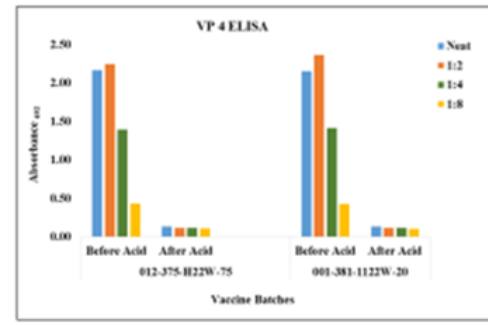
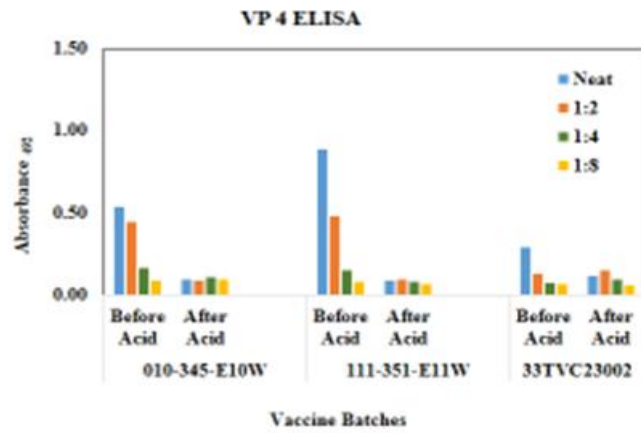
003-443-K23W-20

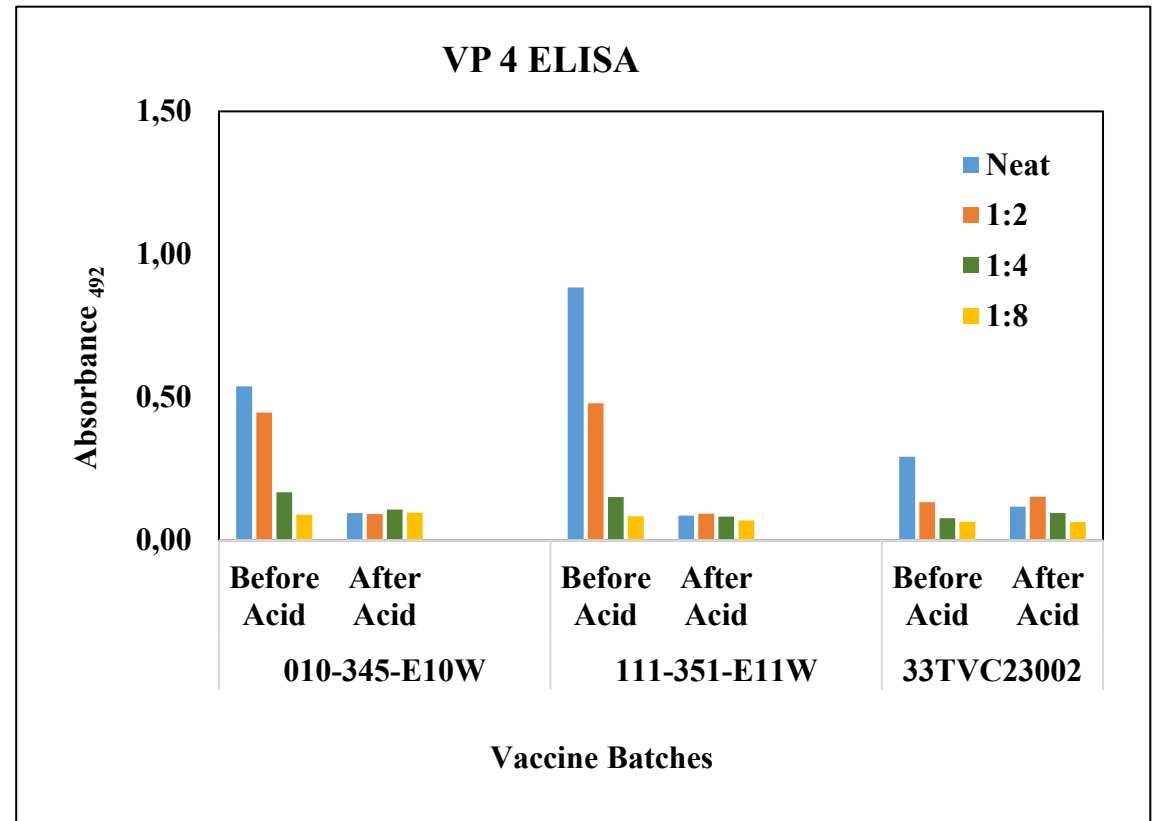
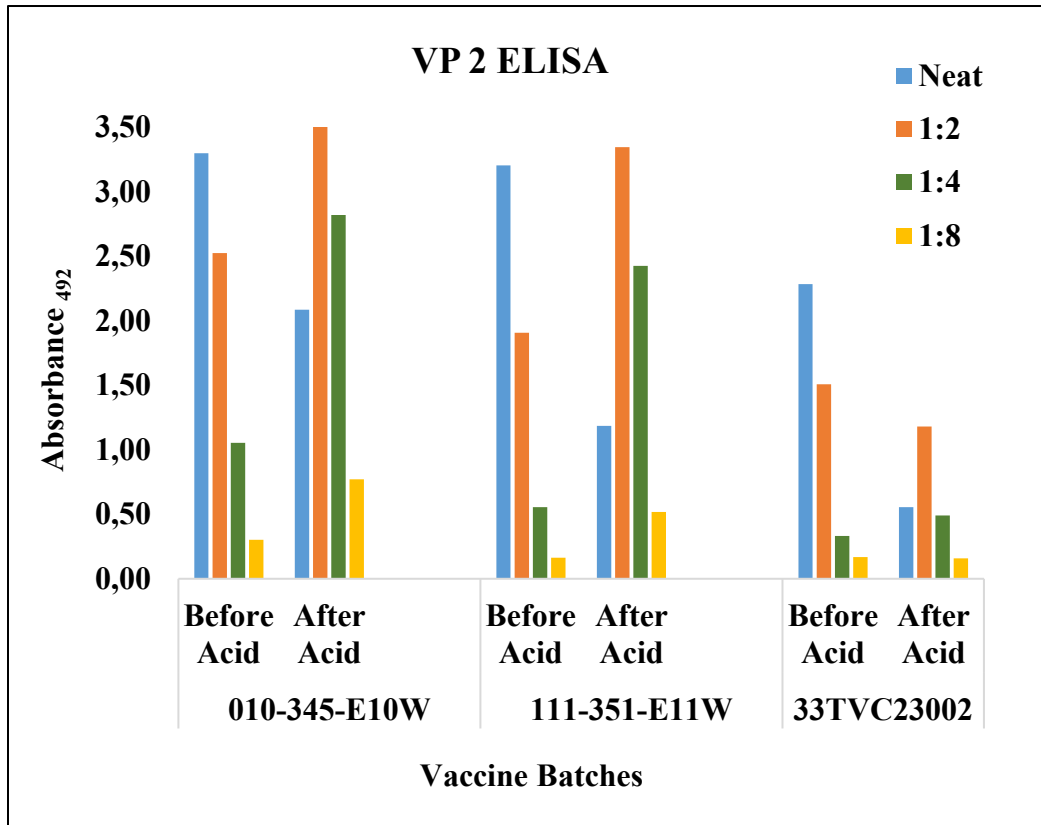
	Cattle	G Pig
O	21	47
A	18	29
Asia 1	56	79

Date of testing: 07-12-2023

114-448-L04W-75

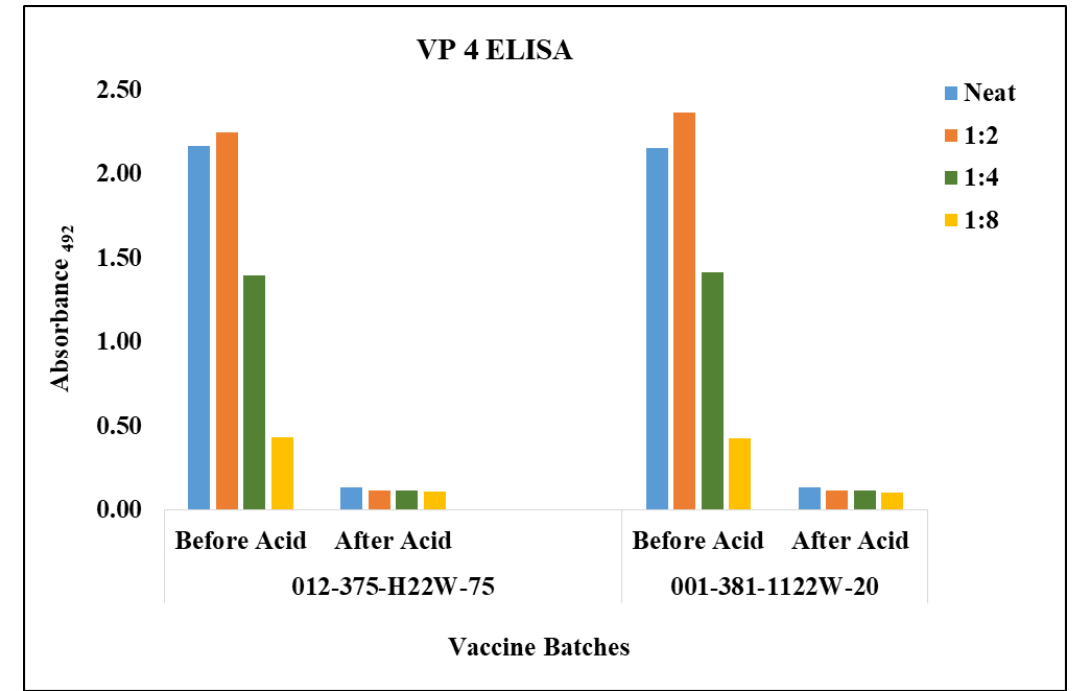
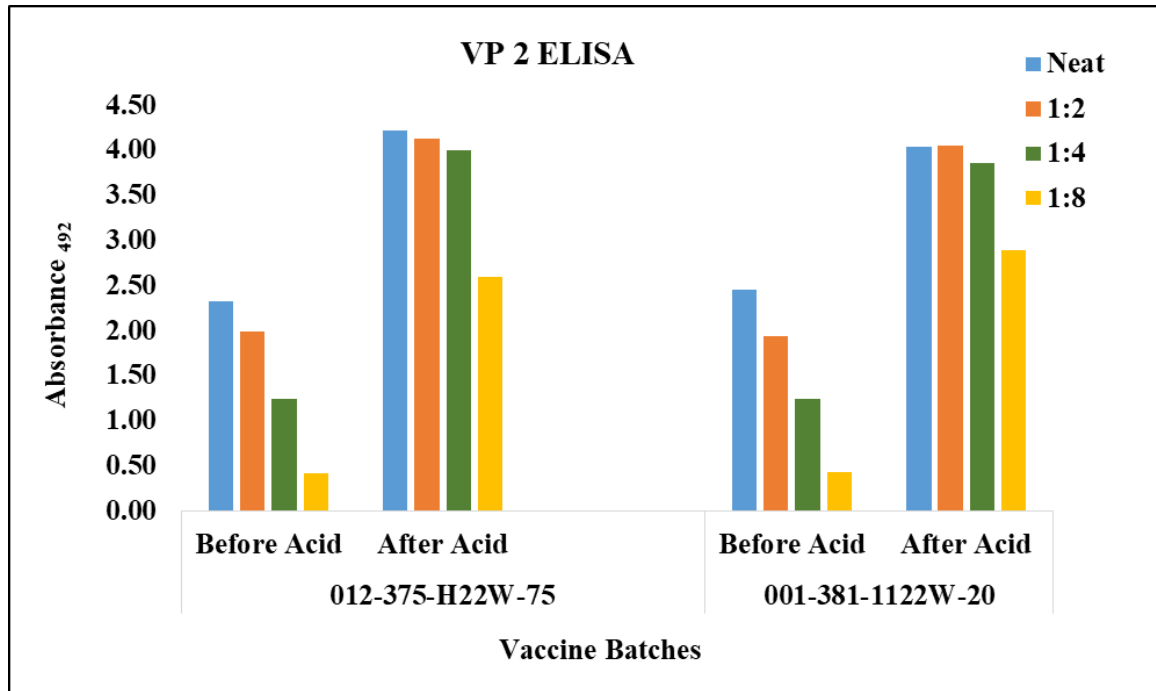
	Cattle	G Pig
O	83	82
A	128	61
Asia 1	98	50





	Cattle	G Pig	Cattle	G Pig	Cattle	G Pig
O	18	9	16	8	32	8
A	31	14	13	8	19	9
Asia 1	41	9	40	8	56	10

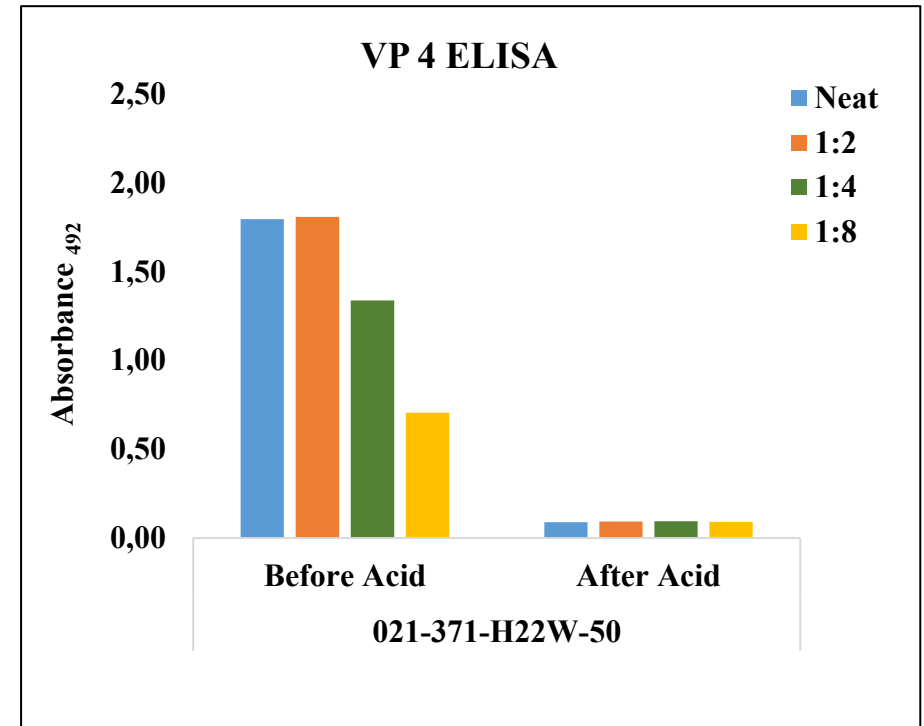
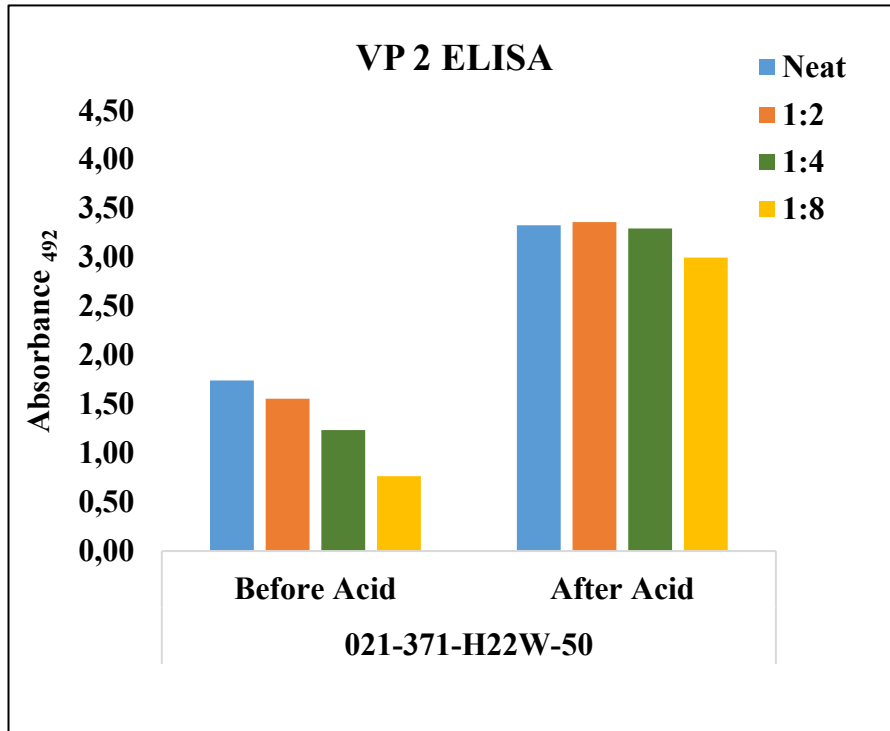
Date of testing: 07-06-2023 & 22-06-2023



Vaccine Batch: **012-375-H22W-75** **001-381-1122W-20**

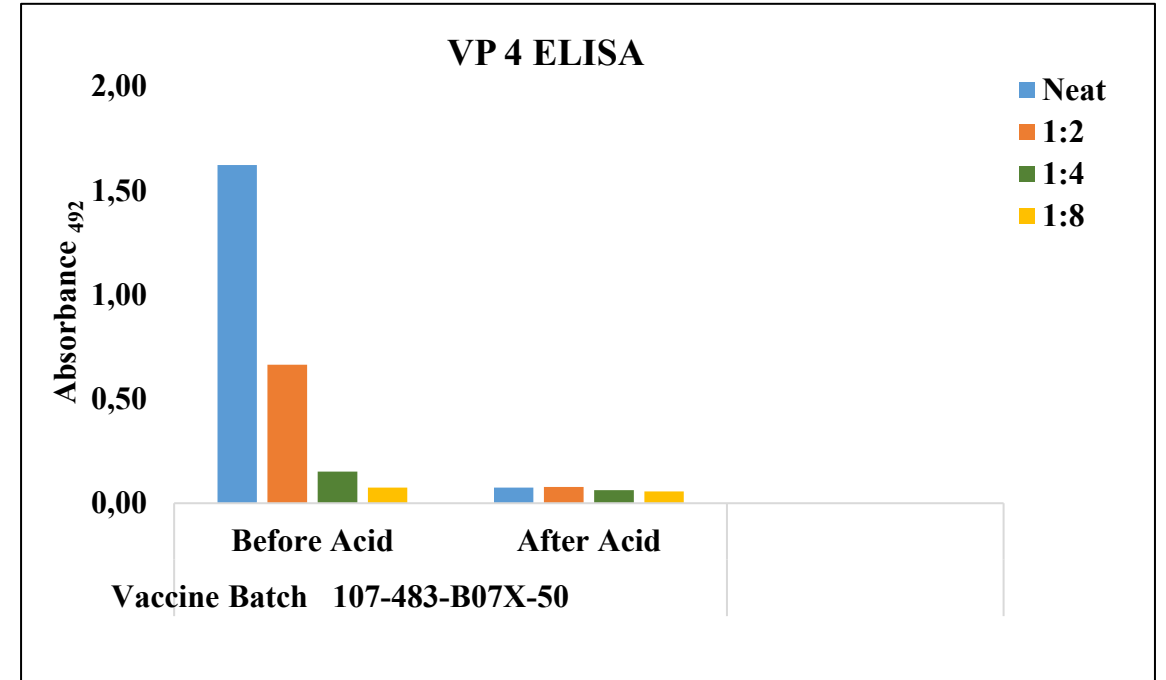
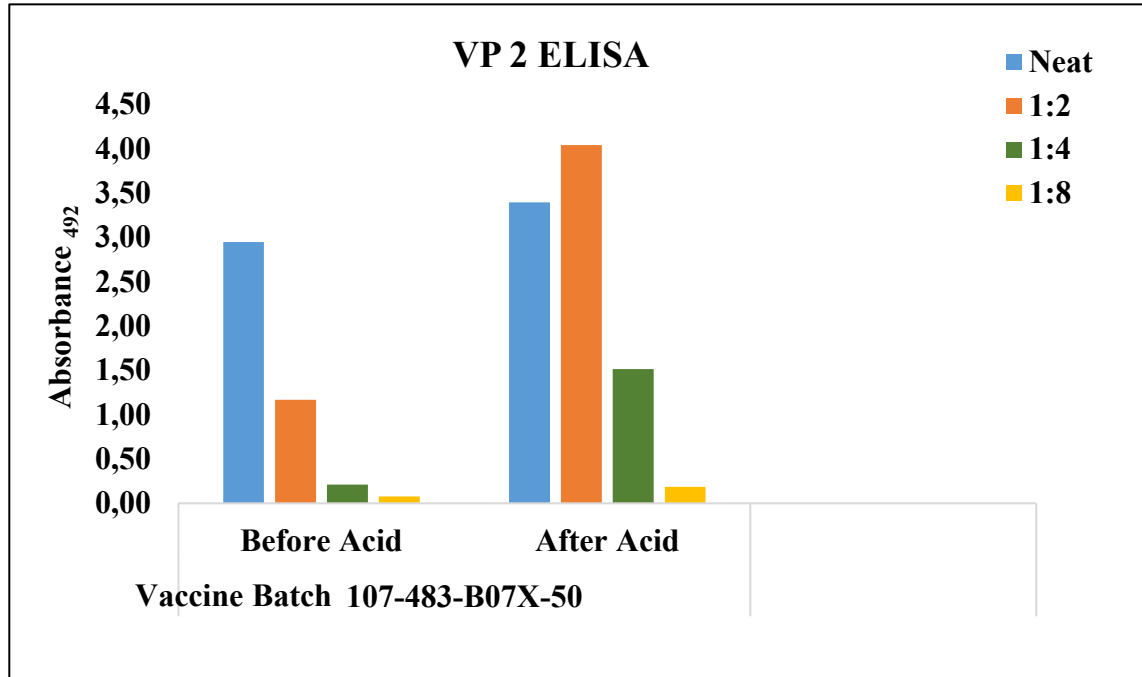
	Cattle	G Pig	Cattle	G Pig
O	83	31	67	25
A	123	95	76	95
Asia 1	118	99	91	113

Date of testing: 05-10-2023 & 13-10-2023 (AFSA 2-4 December, 2025 about one month after immunization in cattle or guinea pigs)



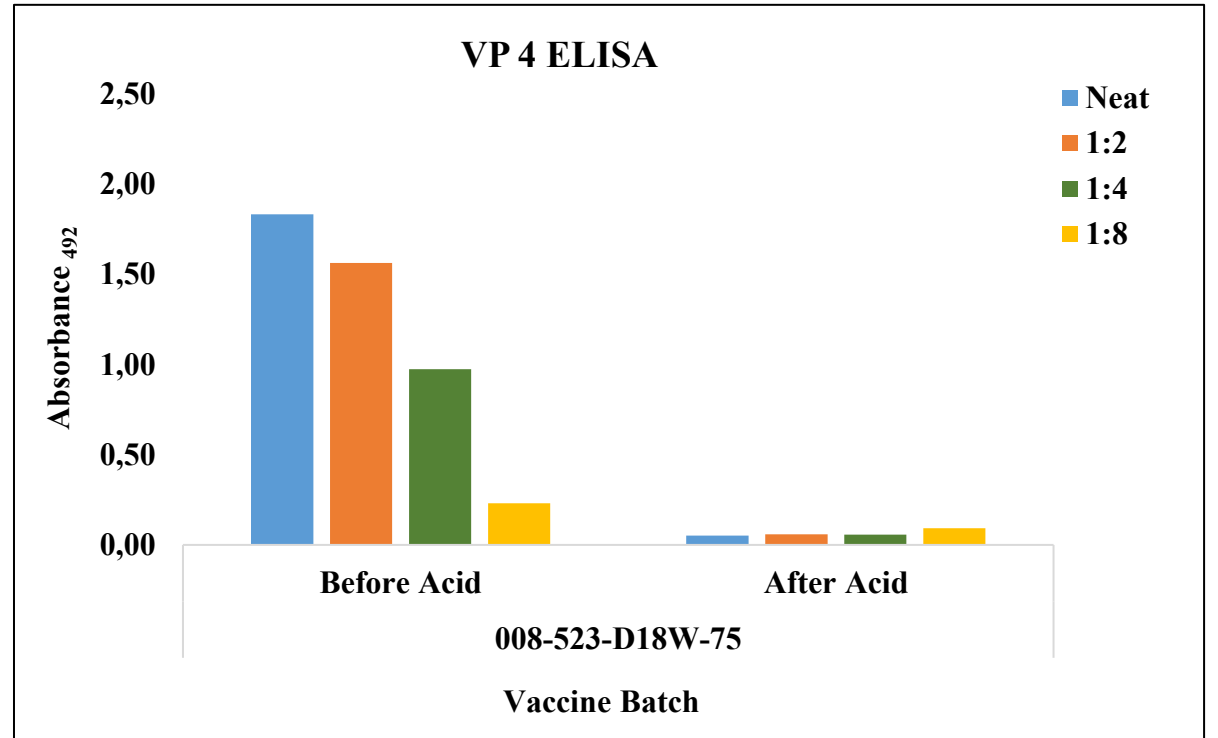
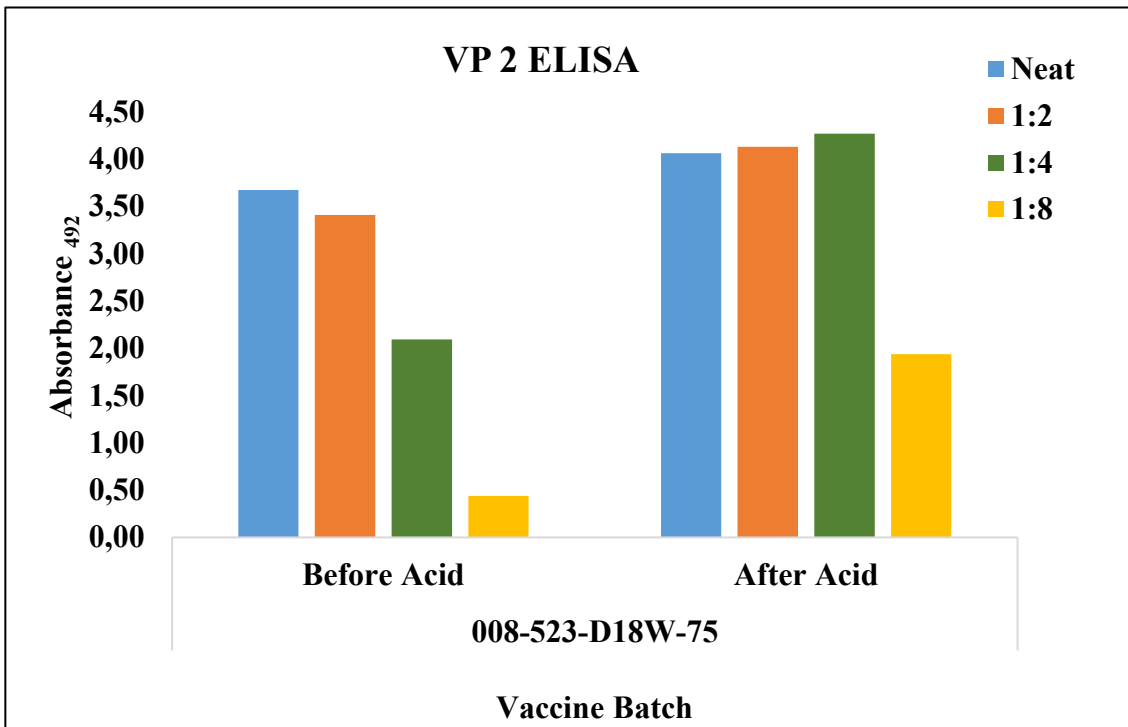
Vaccine Batch: 021-371-H22W-50

	Cattle	G Pig
O	76	12
A	54	34
Asia 1	53	43



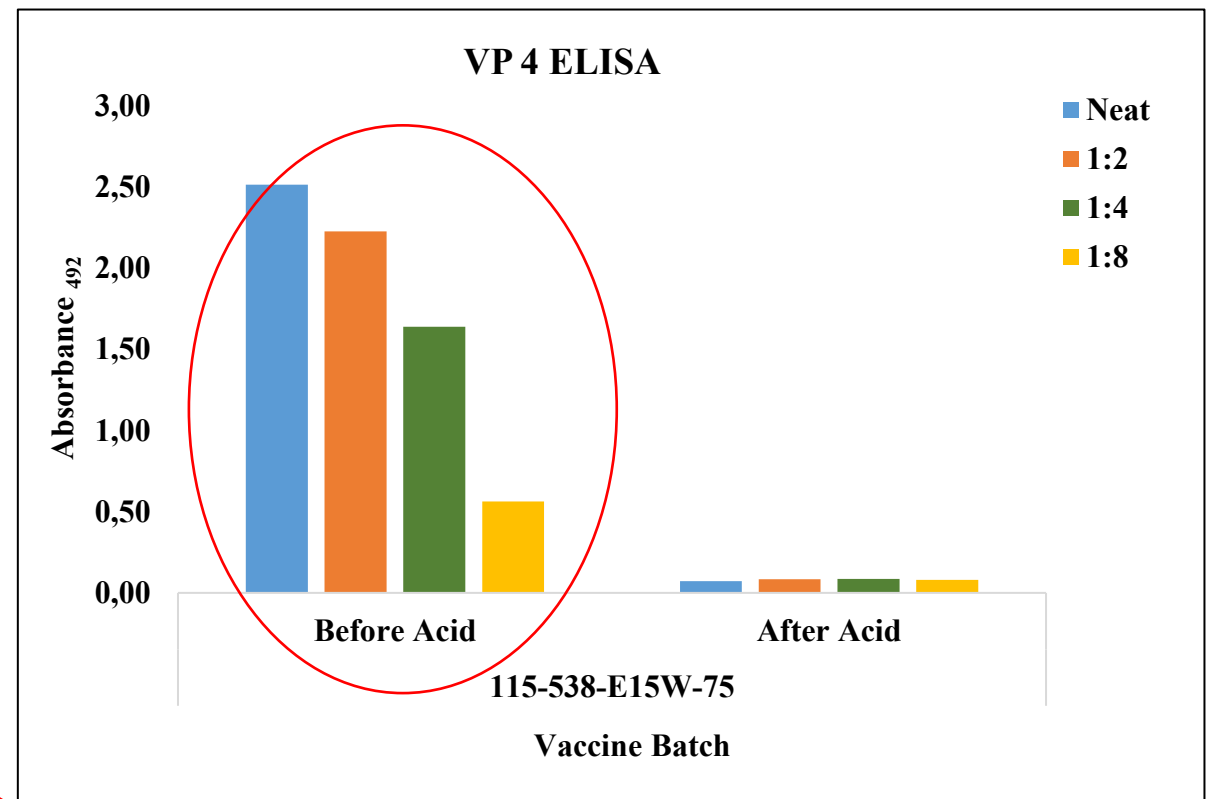
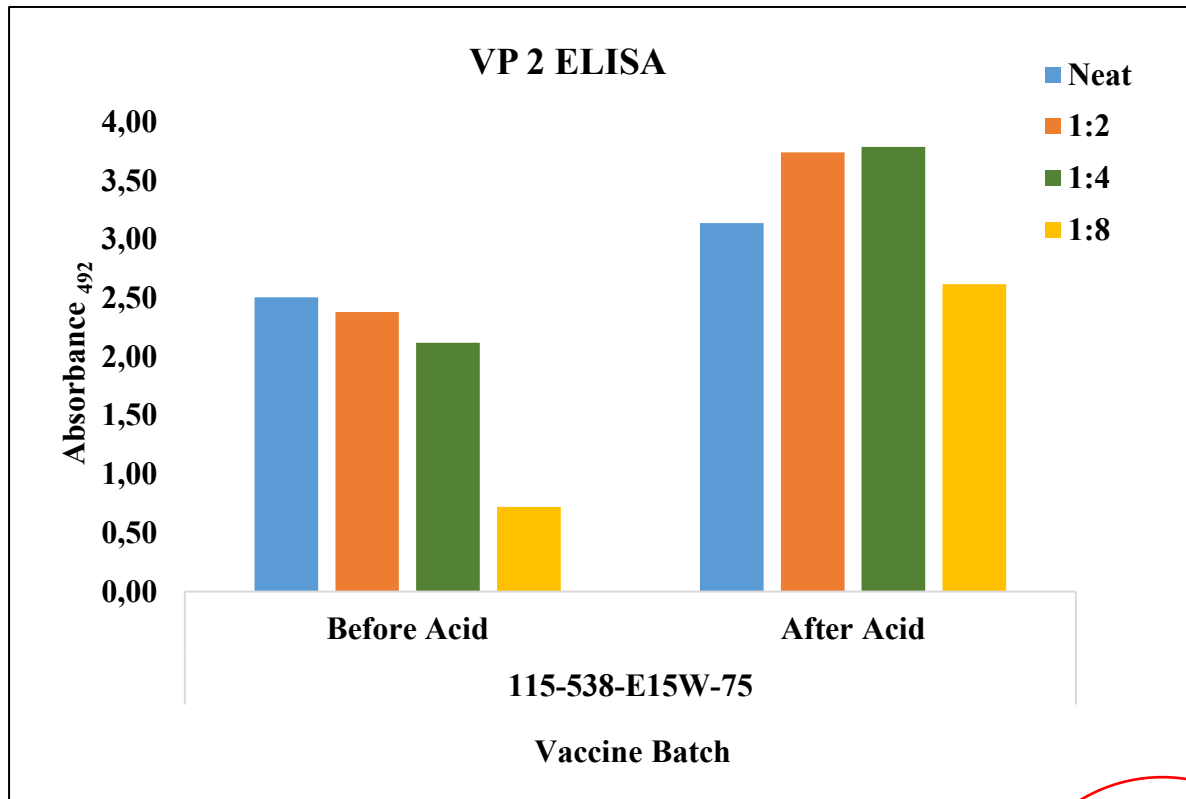
	Cattle	G Pig
O	49	33
A	76	79
Asia 1	76	56

Date of testing: 13.02.2024



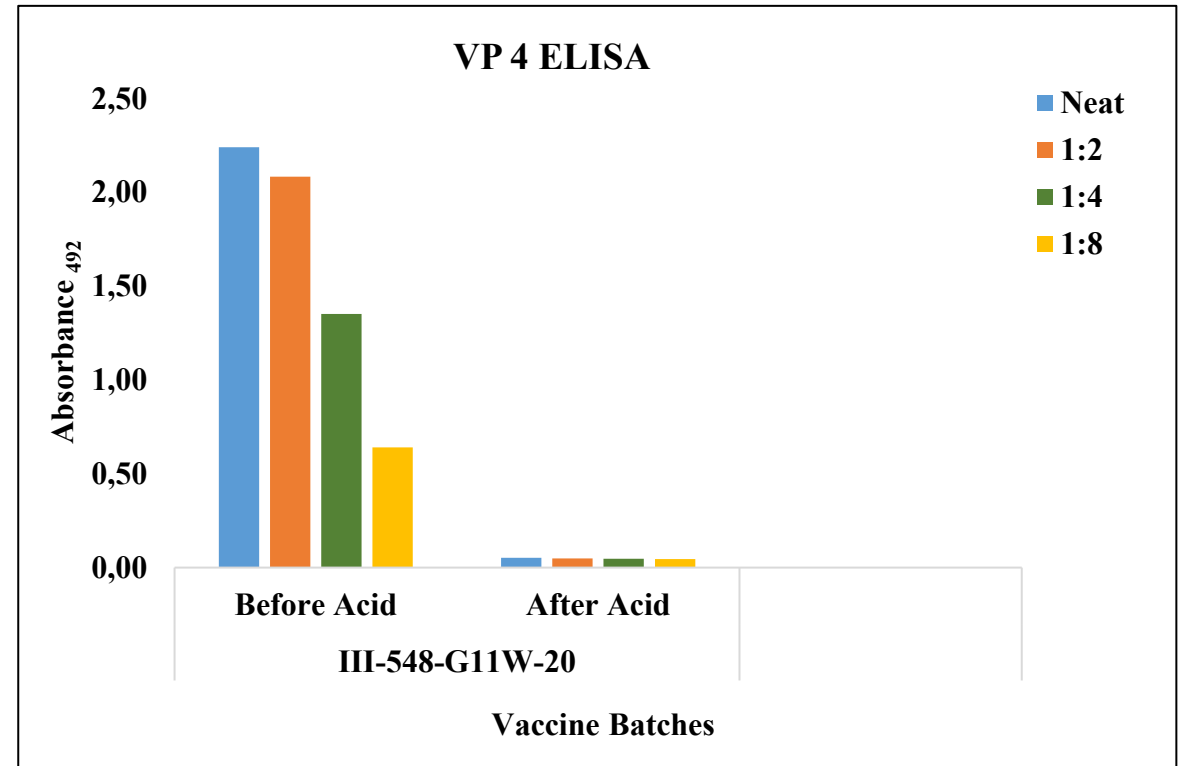
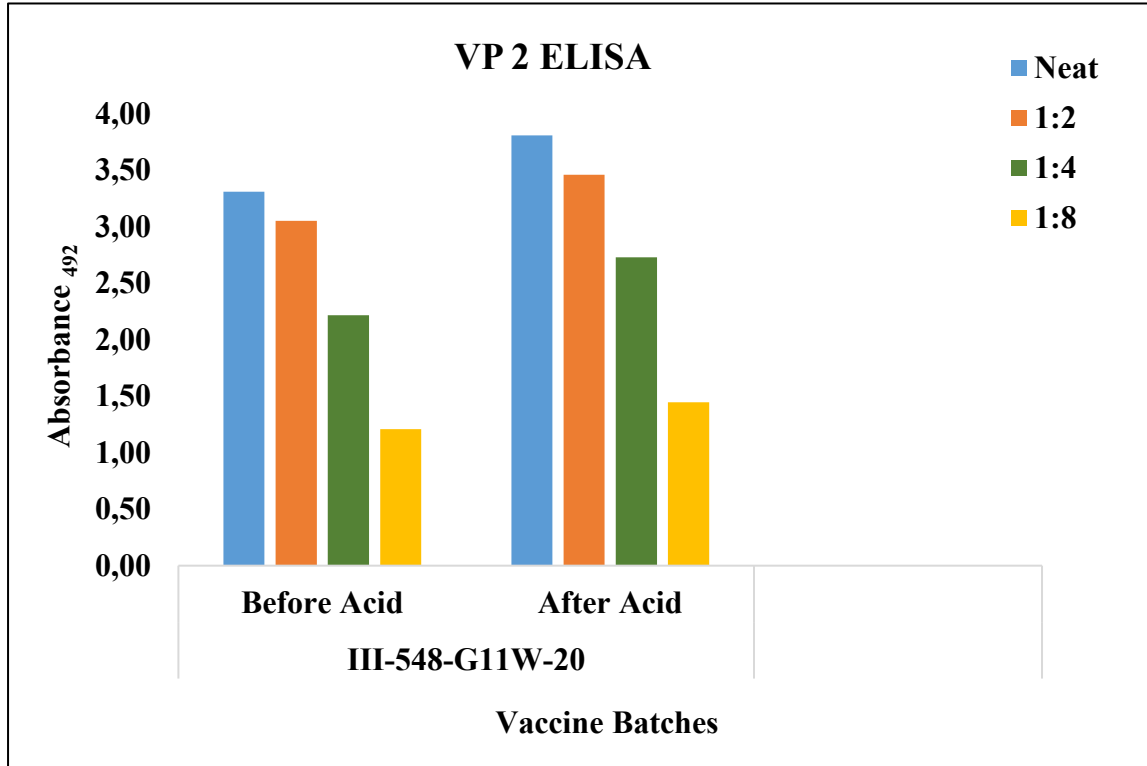
	Cattle	G Pig
O	245	112
A	99	206
Asia 1	146	206

Date of testing: 30.04.2024



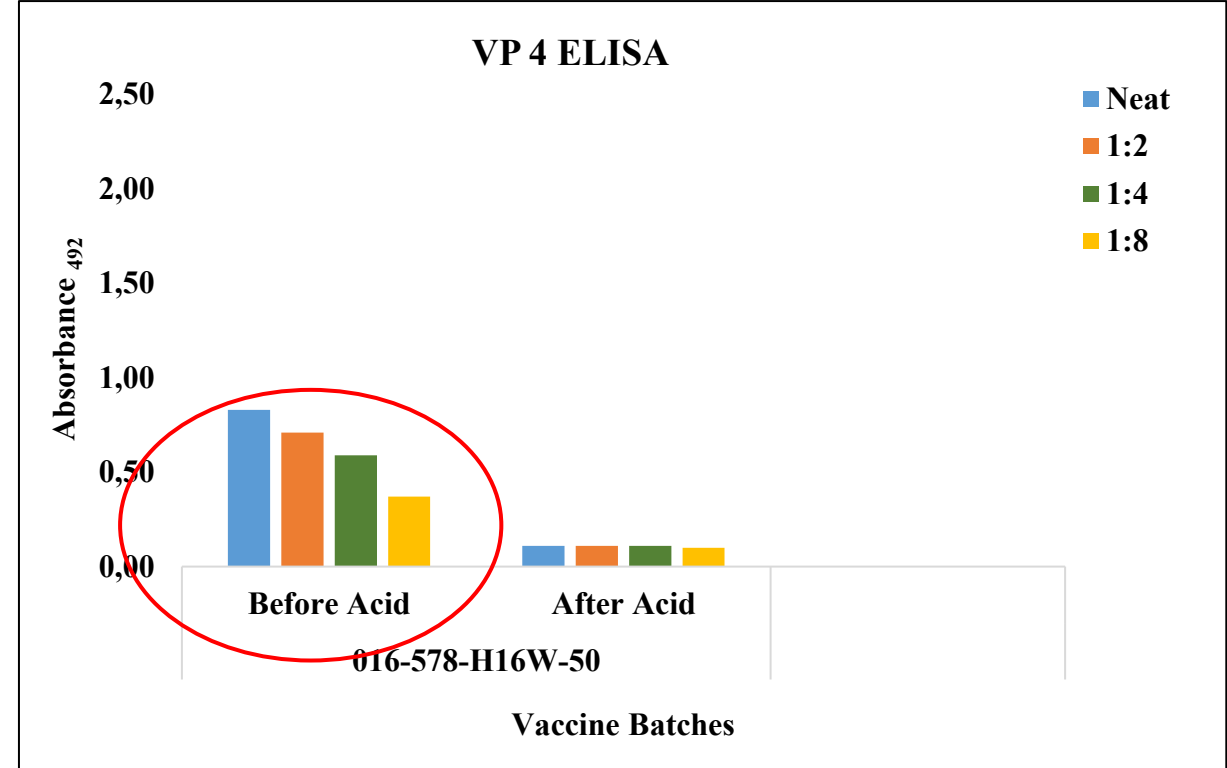
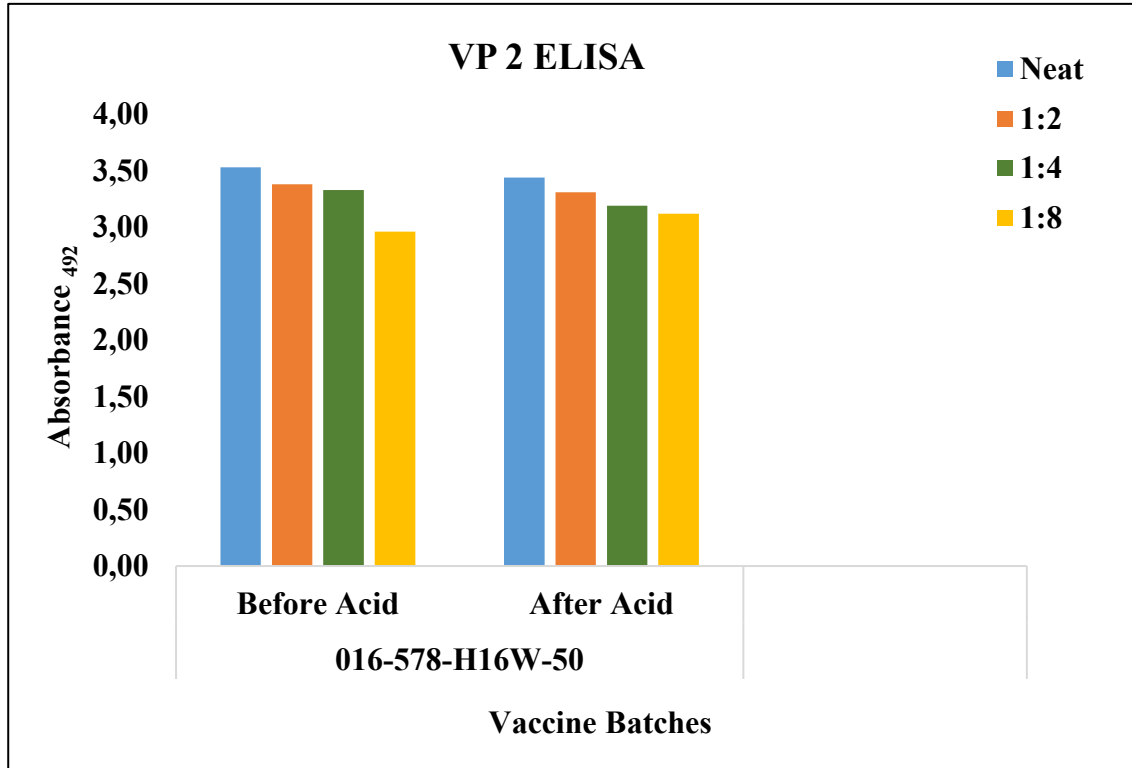
	Cattle	G Pig
O	94	75
A	66	98
Asia 1	51	67

Date of testing: 28.05.2024



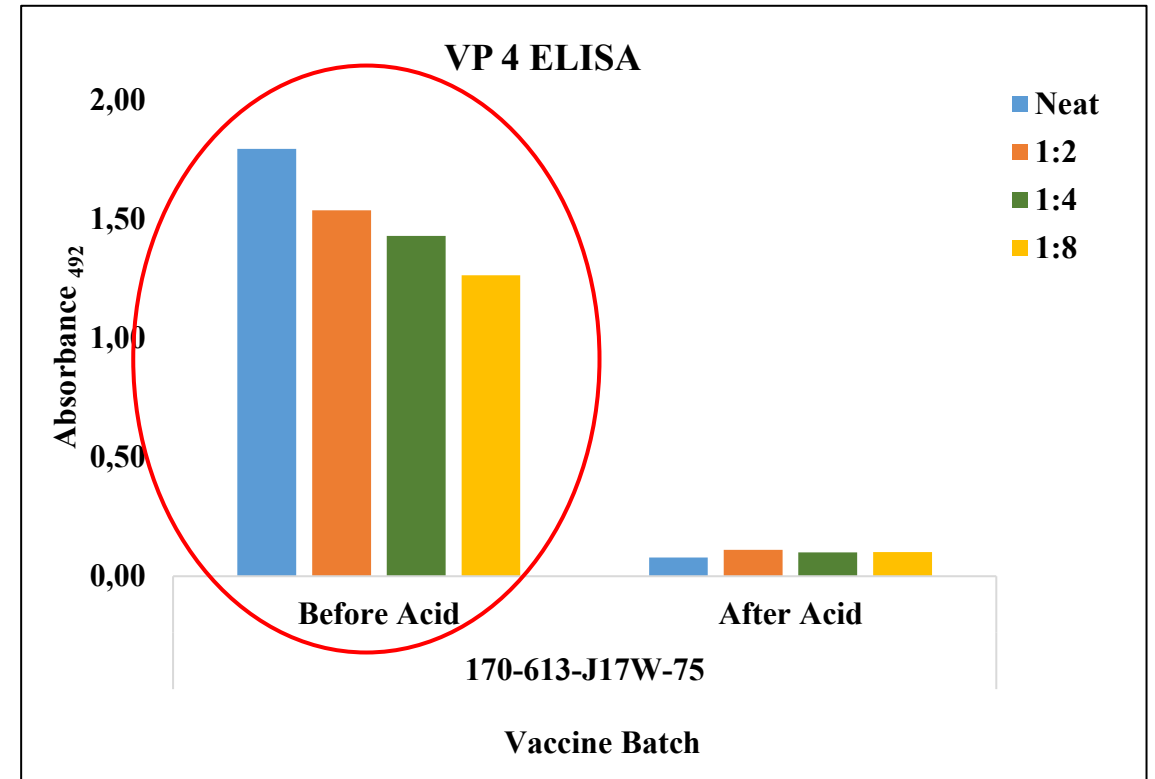
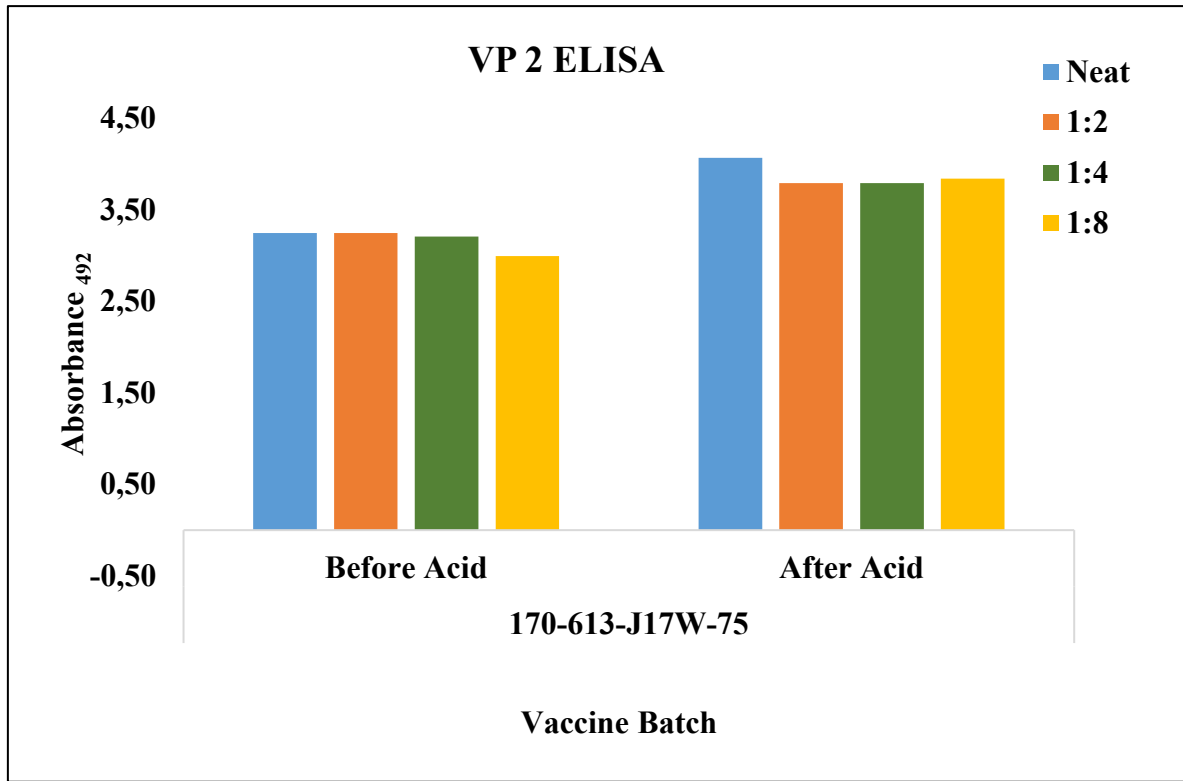
O: 139.48
A: 86.61
Asia 1: 95.25

Date of testing: 16.07.2024



O: 36.03
A: 22.42
Asia 1: 35.58

Date of testing: 03.09.2024

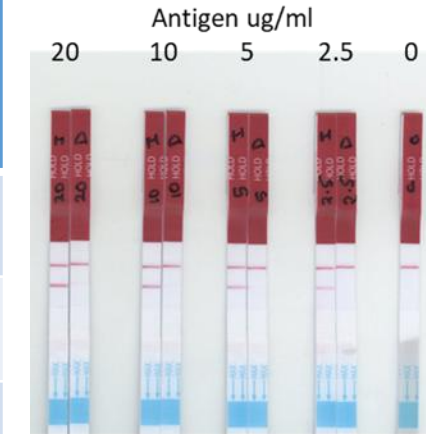


O: 53.36
A: 33.16
Asia 1: 53.33

Date of testing: 29.10.24

For Trivalent (O, A & Asia1) Vaccine Formulation

		VP4 & VP2 mAb based		
		ELISA		
		Good	Poor	Total
Bovine Serology (based on SN ₅₀)	Good	9	0	9
	Poor	0	5	5
	Total	9	5	14



- **Almost similar is the experience of IVRI Bengaluru (more than 90%)**
- **Self life of vaccine proved as at least 8 months at NIFMD**
- **In combination with our internal tools we can reach to 100% accuracy (48 hrs Vs 48 days), why DAHD should be hesitant to use this?.**
- **100% vaccine batches required for prediction before actual use.**

1. Simplification of testing procedures for intensified testing

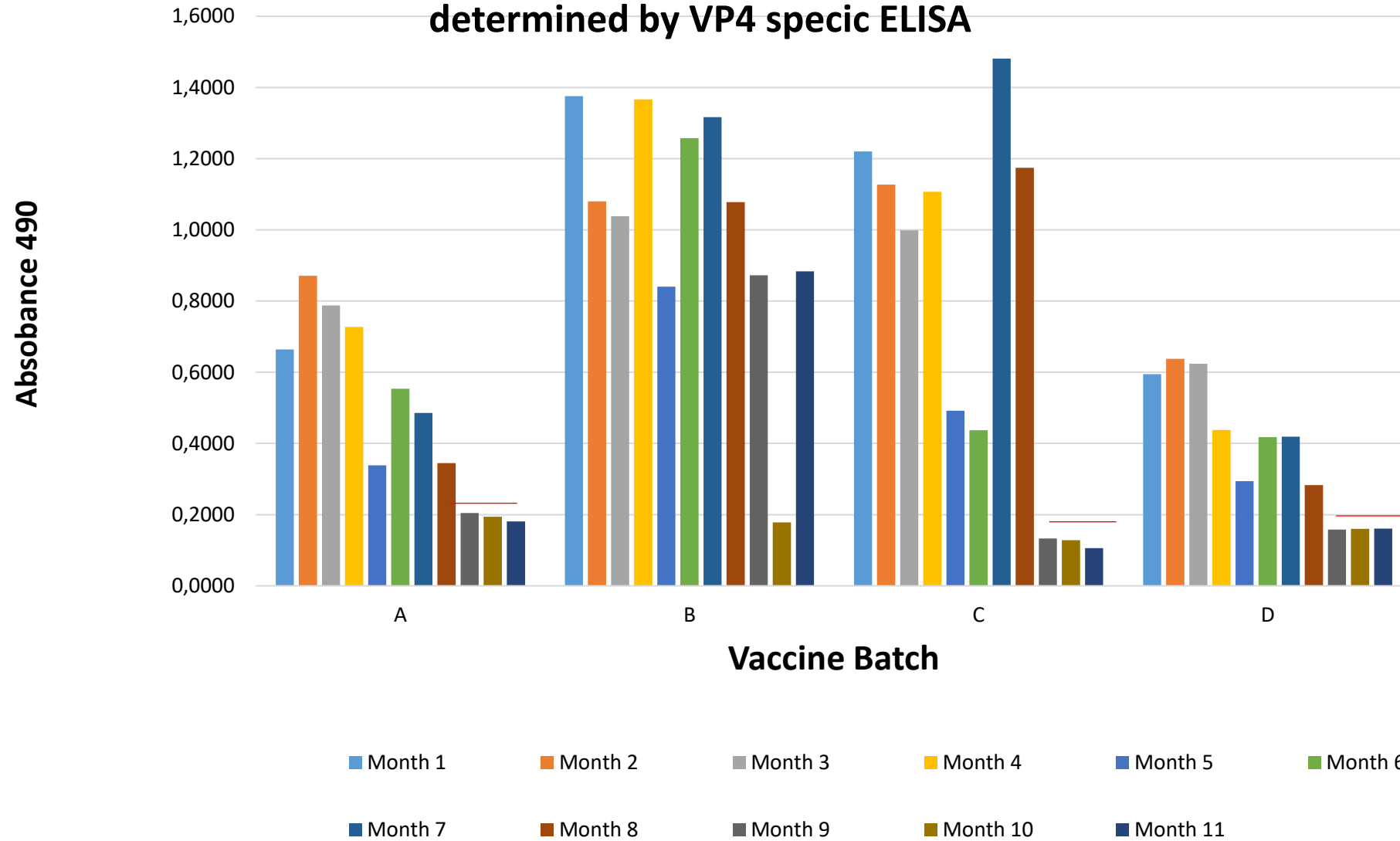
- Faster/quicker
- Accurate
- Every batch in place of 1/5 batches
- Self life
- Compromise in cold chain etc.
- Testing of vaccine received from field

2. If possible for up-scaling high quality vaccine -Vaccine quality is the responsibility of Industry

3. As we progress, standards will change-

- Stringent Purity,
- Stringent Self life,
- High Potency vaccines in general and also for
- Emergency vaccines banks.

Degradation pattern of FMDV Intact virus particle (146S) in 4 batches of trivalent vaccine formulations after storage at 4±2 0C as determined by VP4 specic ELISA



Observations:

- In VP4 ELISA, the low-medium range OD is indicative of sub-optimum quantity of intact antigen. This observation largely correlated with antibody titre in cattle. All Cattle QC passed batches revealed an absorbance of > 1.2 at 1:2 dilution in VP4 ELISA before acid treatment. Exception: only one batch where despite vaccine passing QC in cattle, revealed OD of 0.7. All cattle QC failed batches invariably revealed OD ranging up to 0.7.
- Indicative but not definitive for pass/fail judgement particularly for borderline cattle QC passed batches. However, could be used as in process control assay during vaccine manufacturing and storage.

Summary

- The serotype independent VP4 (Intact antigen) and VP2 (total antigen) ELISAs are able to recognise all 3 Indian vaccine strains (O, A, Asia 1).
- The ELISA data for intact antigen in vaccine batches tested correlates well with cattle serology data.
- This tool can be used for various other purposes for vaccine manufacturing, vaccine self life determination etc.
- We are currently working with UK partners on serotype specific versions of the tests to enable quantitation of individual vaccine components.



Thank You