

ELISA FOR DETECTION OF ANTIBODIES AGAINST AVIAN INFLUENZA VIRUS

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ELISA TYPE

- Indirect ELISA
- Plates coated with purified Type A (H1N1) viral antigen:
 - Pelleted and mostly Matrix & Nucleocapsid fractions withdrawn from sucrose gradient
- Rabbit anti-Chicken IgY conjugate

Theoretical detection

- All subtypes of Type A influenza
- 2 weeks post infection/vaccination (no IgM detection)

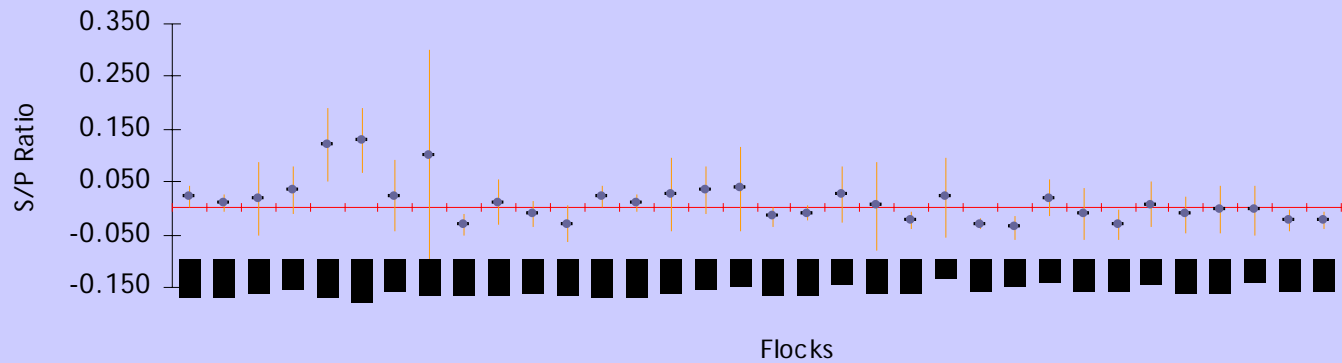
CIVTEST AVI Influenza - CROSS-RECOGNITION of sub-types:

The samples tested here were from SPF birds immunised at VLA (Weybridge). Because these were derived from individual birds the variance of the S/P is of no significance as it is within the normal bird to bird variance.

Serum	O.D.	S/P
Pos. Ctrl	1.324	
Pos. Ctrl	1.387	
Neg. Ctrl	0.113	
Neg. Ctrl	0.095	
H1N1	1.568	1.170
H2N2	1.255	0.920
H2N3	1.263	0.926
H3N2	1.251	0.917
H3N2	1.170	0.852
H3N6	1.397	1.033
H3N8	1.490	1.107
H3N8	1.460	1.083
H4N3	1.186	0.865
H4N8	1.303	0.958
H4N8	0.814	0.567
H5N2	1.749	1.314
H6N8	1.476	1.096
H7N1	1.608	1.202
H7N1	1.431	1.060
H8N4	0.993	0.710
H9N2	1.539	1.147
H9N2	1.599	1.195
H9N2	1.464	1.087
H10N7	1.436	1.064
H11N6	1.303	0.958
H12N5	1.239	0.907
H13N6	1.017	0.730
H14N6	1.509	1.123
H15N6	1.430	1.060

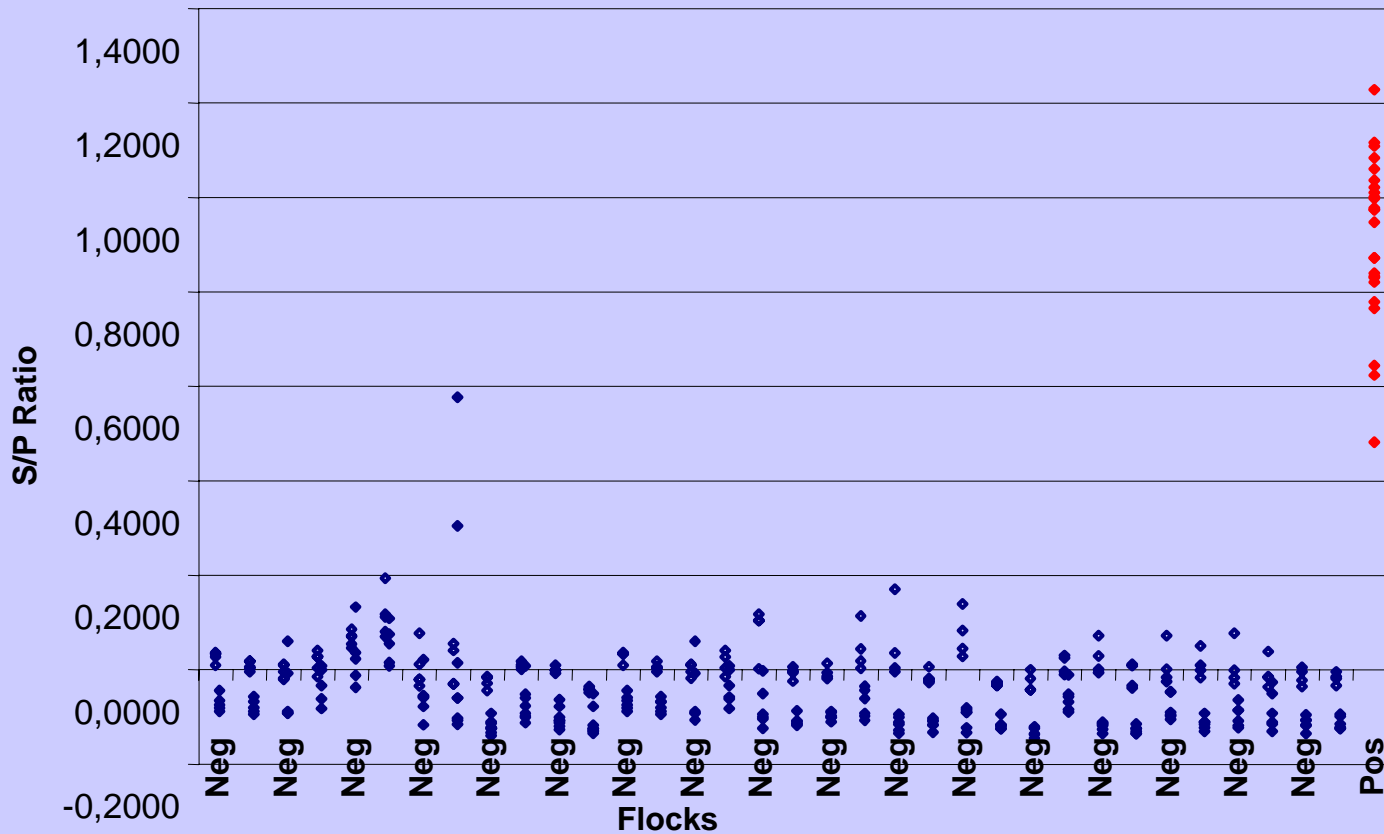
CIVTEST AVI Influenza - Specificity:

Honduras Flocks Negative to Influenza - mean S/P +/- 1SD Bars



CIVTEST AVI Influenza - negative and positive flocks.

Honduras - One Positive (vaccinated H5N2) flock versus 34 negative flocks



CIVTEST AVI Influenza - Summary for SENSITIVITY & SPECIFICITY

$$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP}) \times 100\% = 346 / (346 + 2) \times 100$$

99.43%

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}) \times 100\% = 25 / 25 \times 100$$

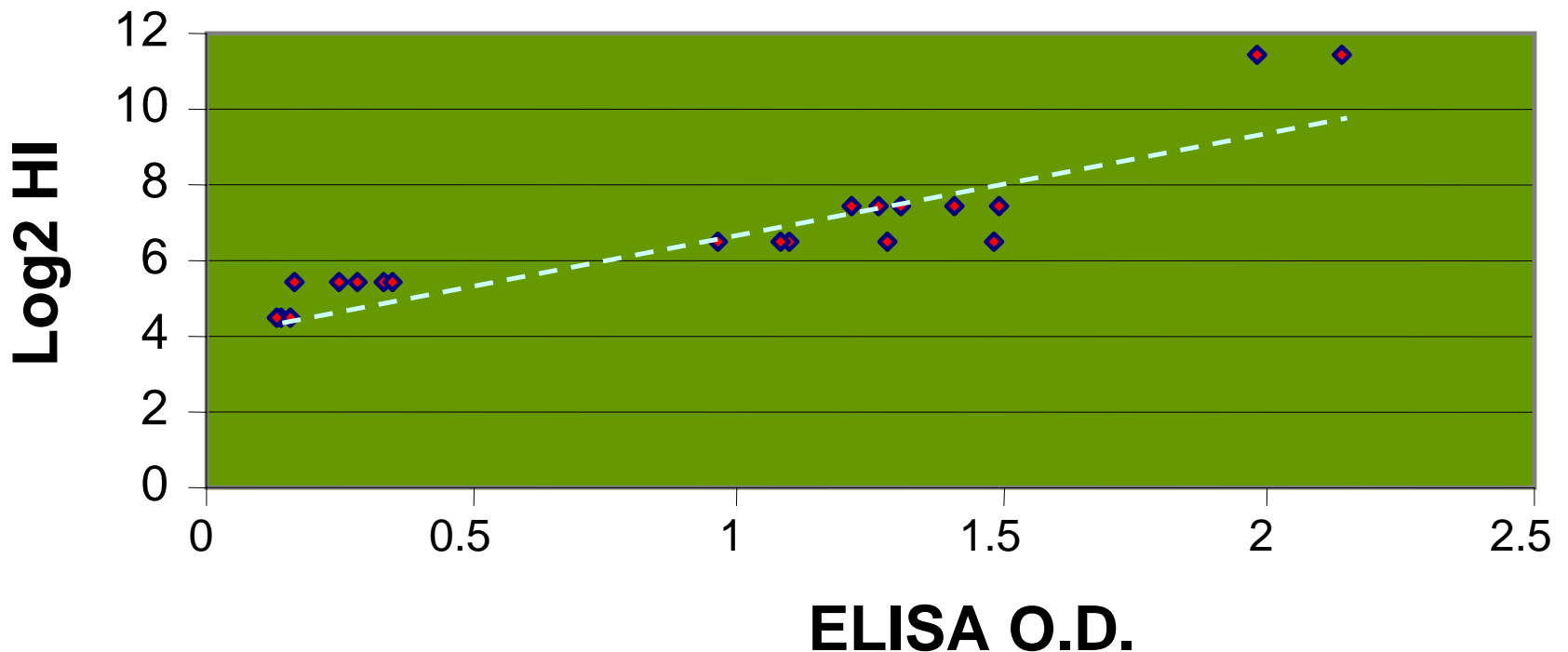
100%

CIVTEST AVI Influenza - Correlation of ELISA vs. HI with H5N2

(infection and IHA with H5N2 serotype)

$$y = 2.6864x + 4.0058$$

$$R^2 = 0.8051$$

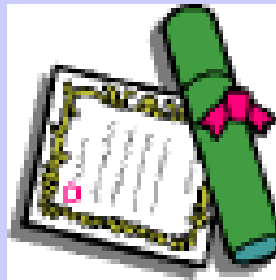


CIVTEST AVI Influenza - Conclusion

- The results of these tests demonstrate a high Sensitivity and Specificity of this technique.
- This technique allows the detection of positive animals whichever serotype is involved.
- The results indicate a reasonable correlation versus the reference technique, HI.

CIVTEST AVI Influenza - Validation

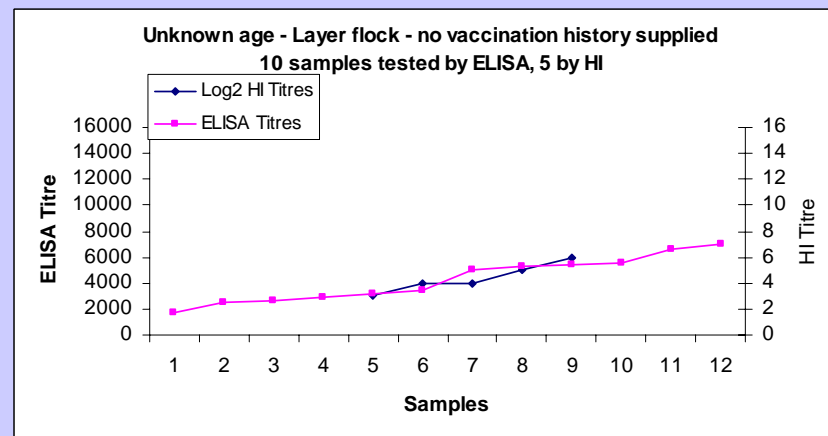
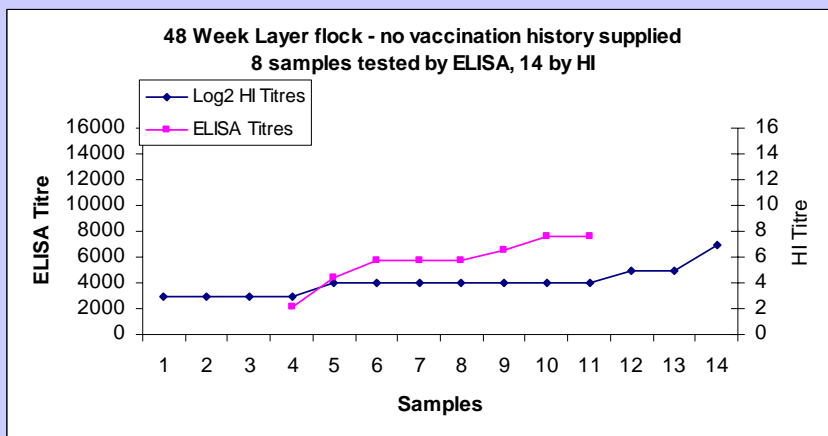
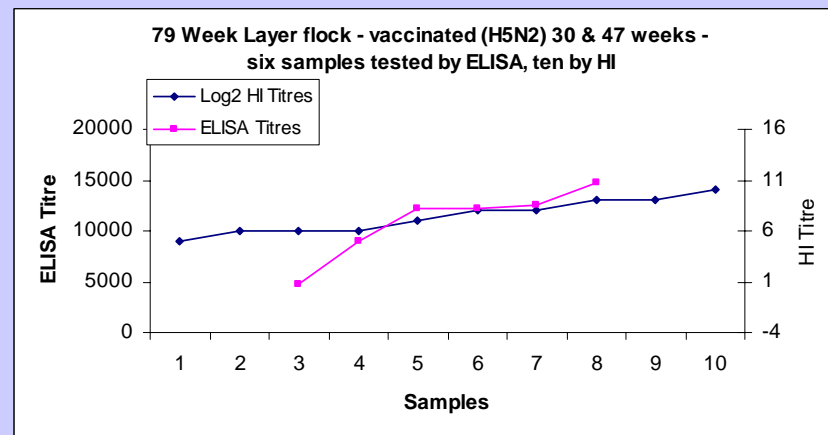
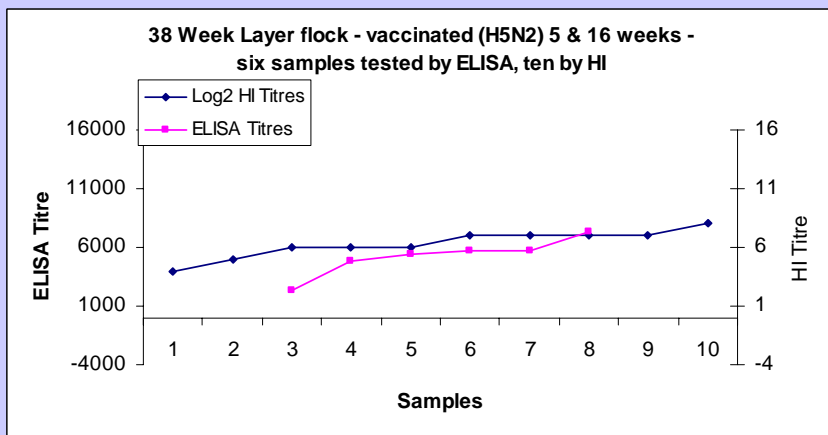
Since may 2002 CIVTEST AVI Influenza has been approved by the Spanish “Laboratorio de Algete”, the official lab from MAPA charged register reagents for diagnosis



CIVTEST AVI Influenza - Acknowledge

**We are very grateful to Ruth Manvell
(Veterinary Laboratory Agency, UK) for
her valued assistance**

Some Indonesian Data – positive flocks



Some Indonesian Data – Negative flock

