



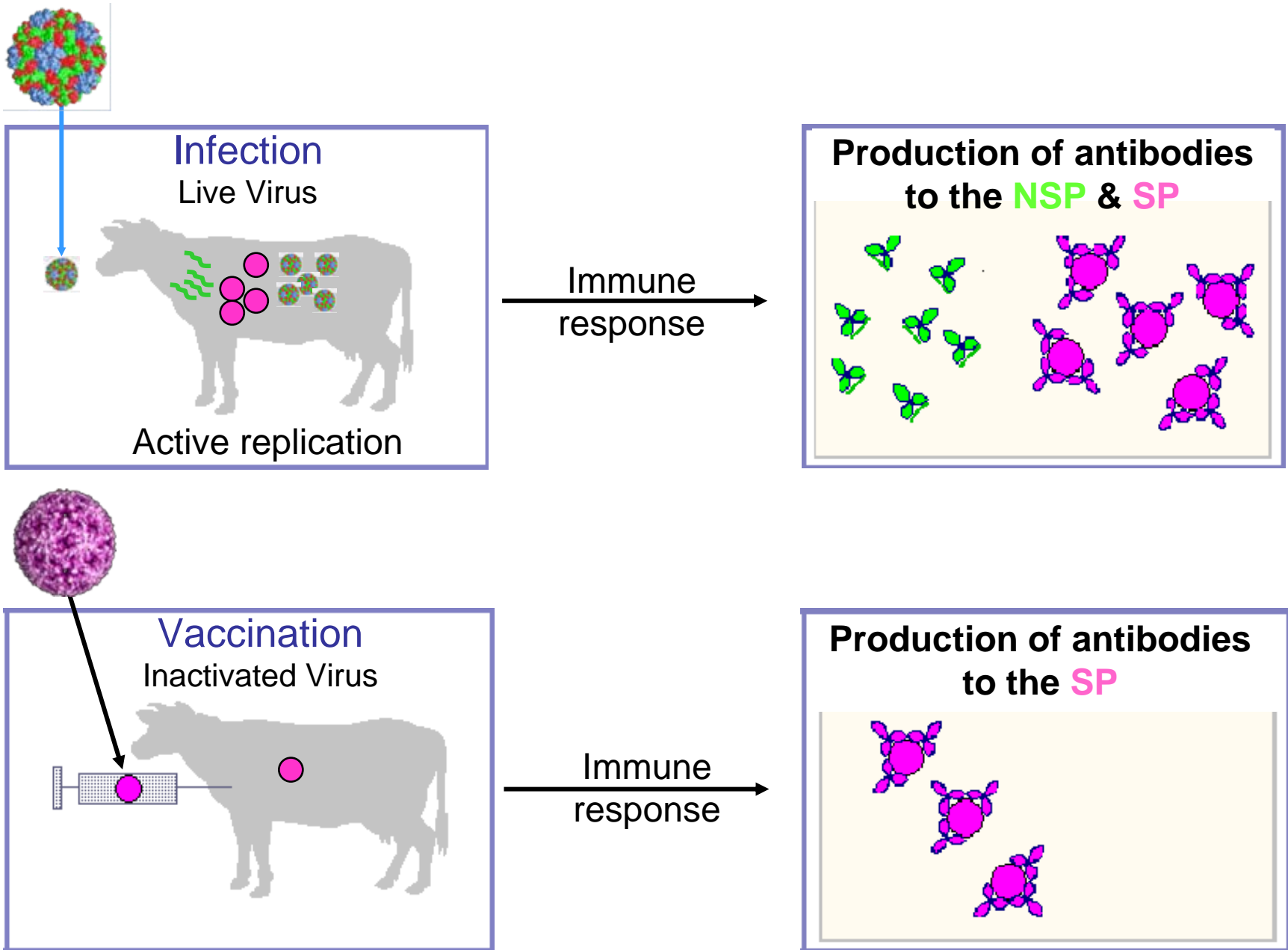
# DEVELOPMENT OF A ONE-STEP ELISA FOR FMDV ANTIBODY DETECTION BASED SOLELY ON RECOMBINANT REAGENTS

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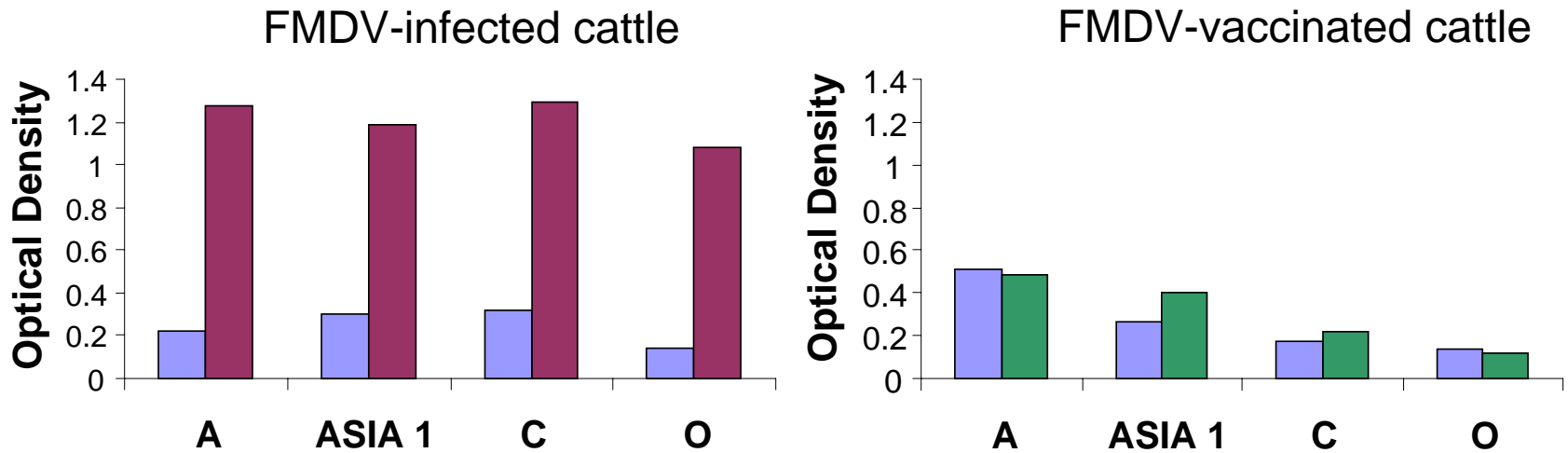
**J. Muller<sup>1</sup>, A.Foord<sup>2</sup>, H.Heine<sup>2</sup>, M.Yu<sup>2</sup> & L-F.Wang<sup>2</sup>**

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<sup>2</sup>CSIRO Livestock Industries, AAHL Australia



# Antibody response to the non-structural protein 3B in FMDV-infected and FMDV vaccinated cattle

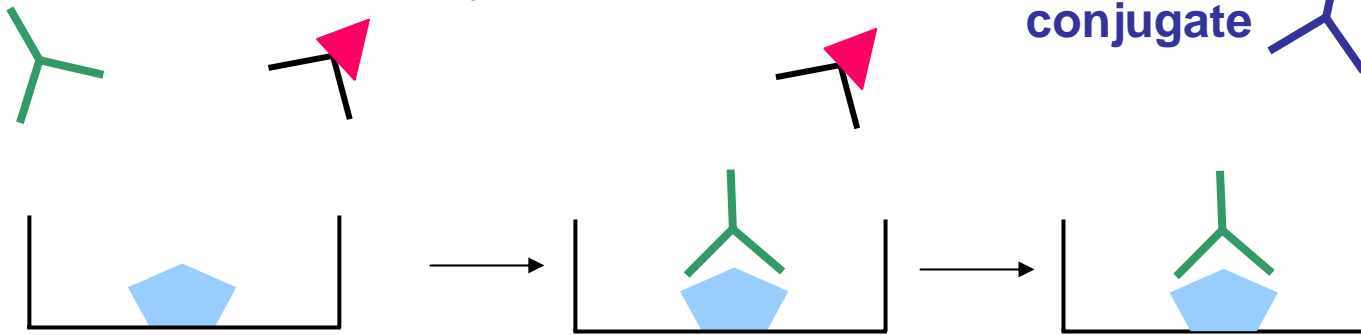


# Competition ELISA (C-ELISA)

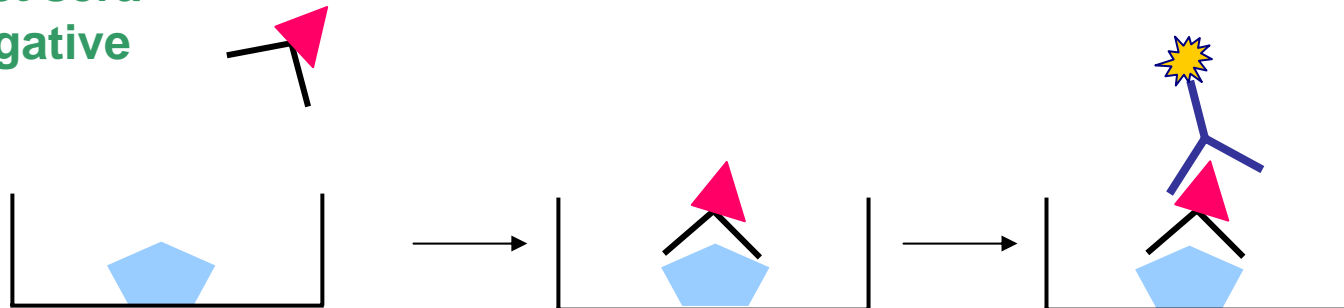
Test sera  
Positive

Detecting  
Antibody

Tag-specific  
conjugate

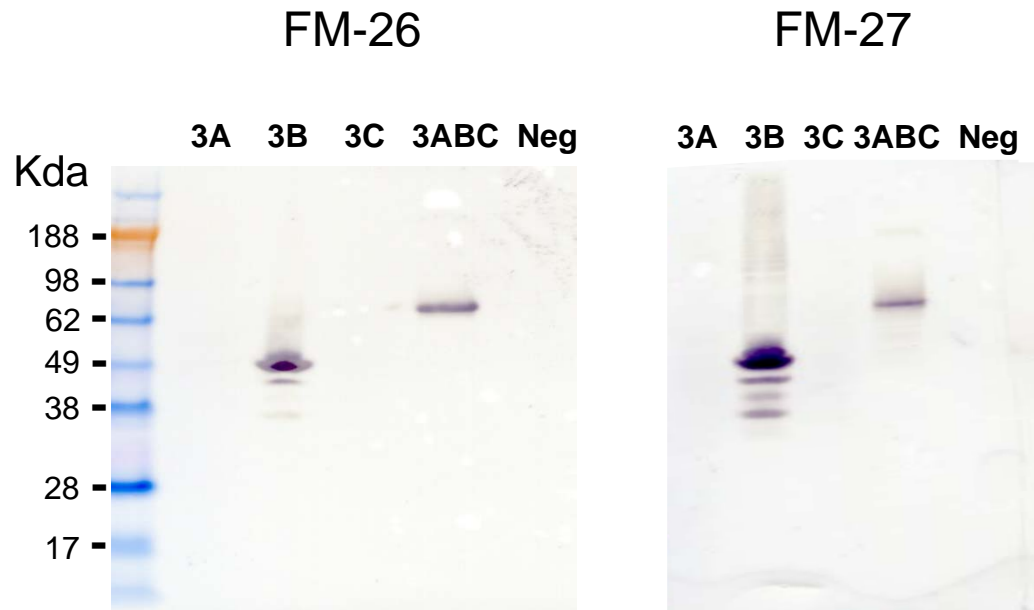


Test sera  
Negative

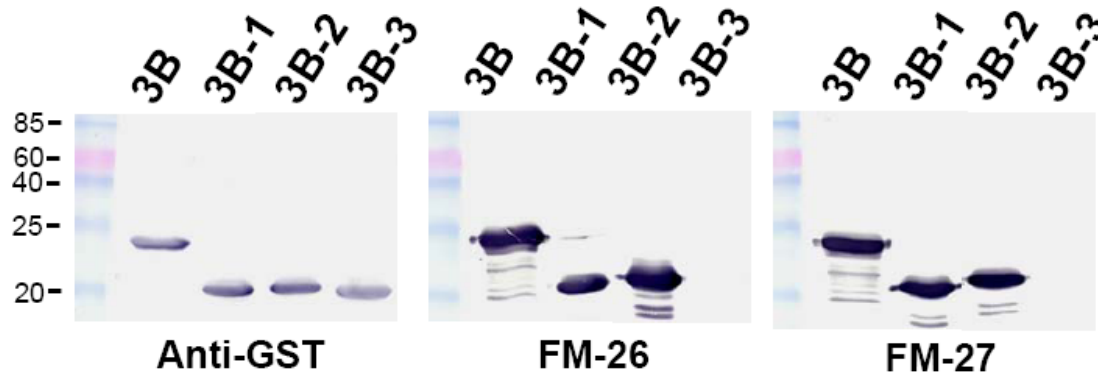
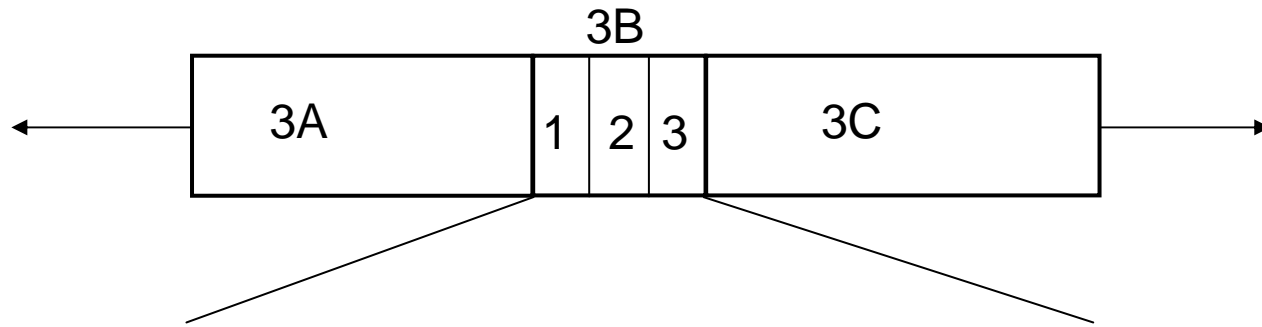


**CHICKEN RECOMBINANT  
ANTIBODIES SPECIFIC TO  
FMDV-3ABC**

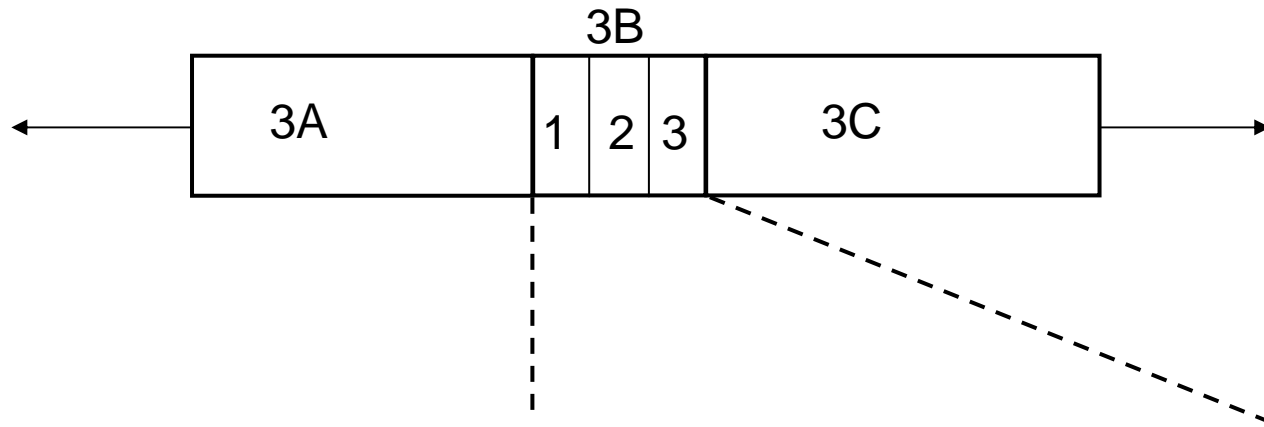
# FMDV 3ABC-specific chicken recombinant antibodies



# Reactivity of FM26 & FM27 to 3B

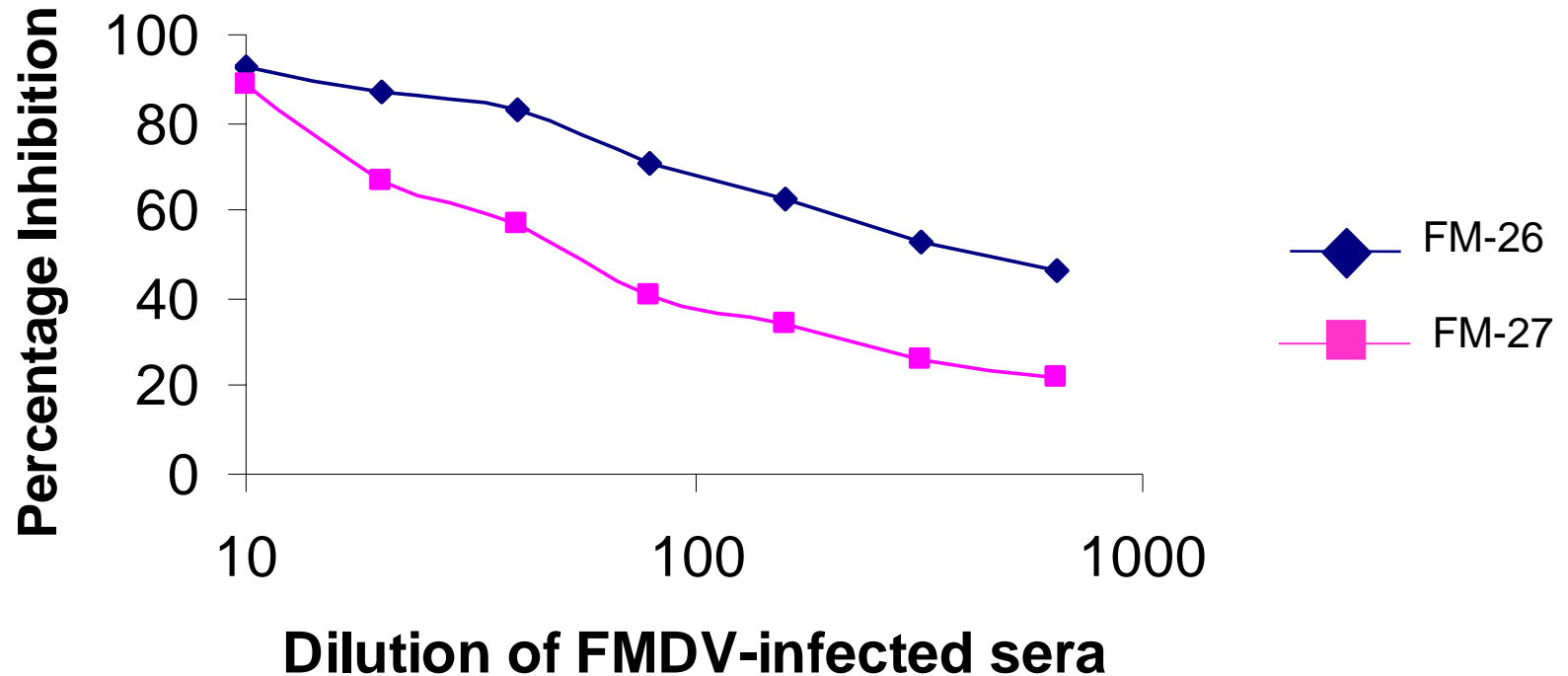


# 3B - Amino acid sequence alignment

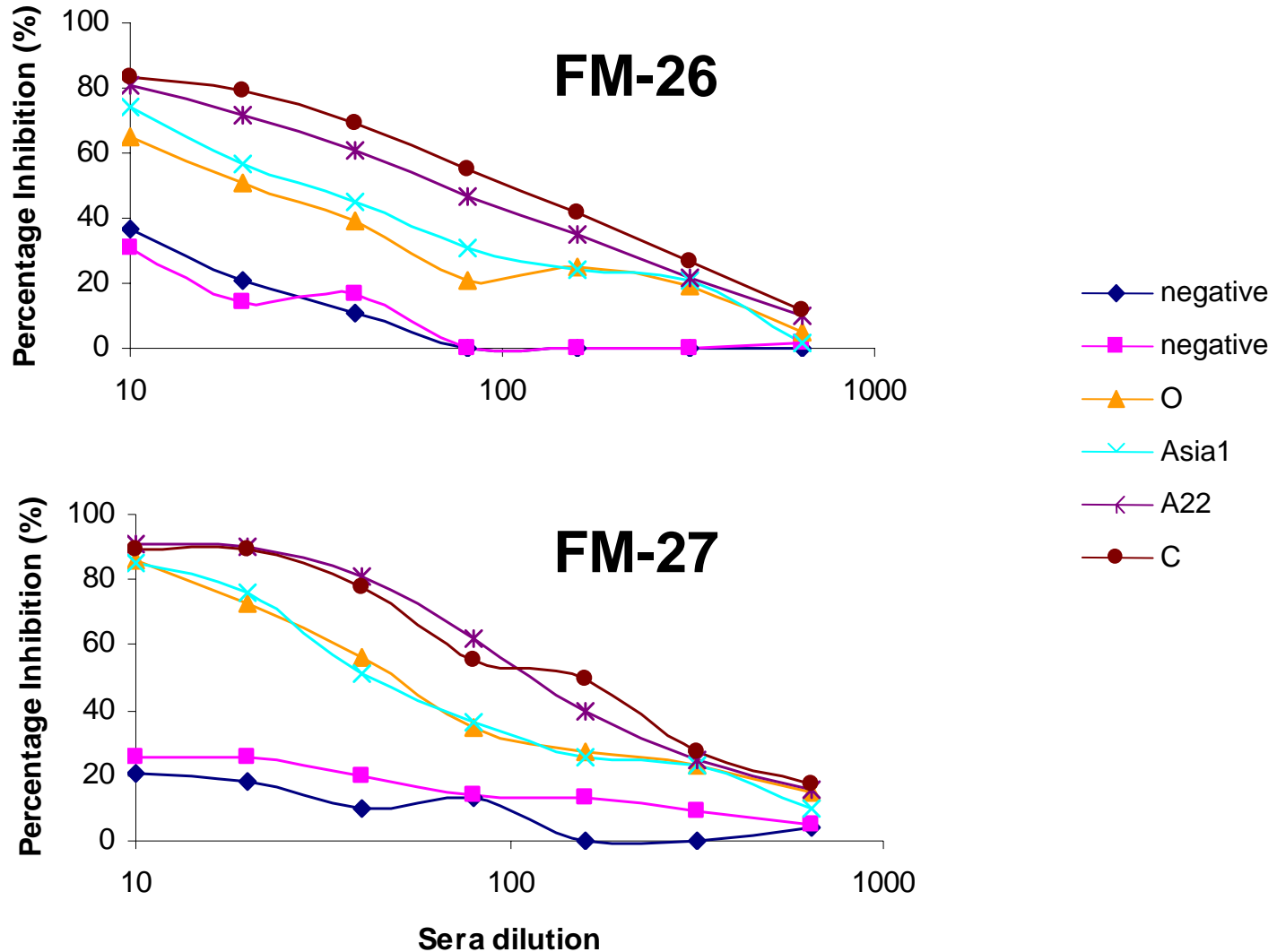


3B-1 GPYAGPLERQKPLKVRAKLPQQE-  
3B-2 GPYAGPMERQKPLKVKAKAPVVKE  
3B-3 GPYEGPVKKPVALKVKAKNLIVTE

# Binding specificities of FM-26 & 27 in a C-ELISA

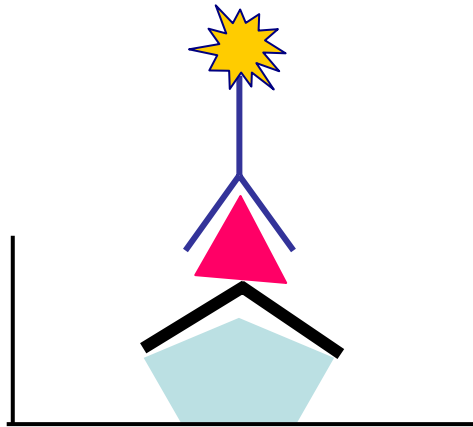


# Further comparison of FM-26 & 27



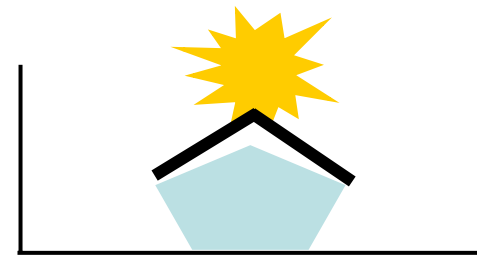
**ONE-STEP C-ELISA BASED  
SOLELY ON RECOMBINANT  
REAGENTS**

# Towards one-step detection in the C-ELISA



Current detection

**TWO-STEP**

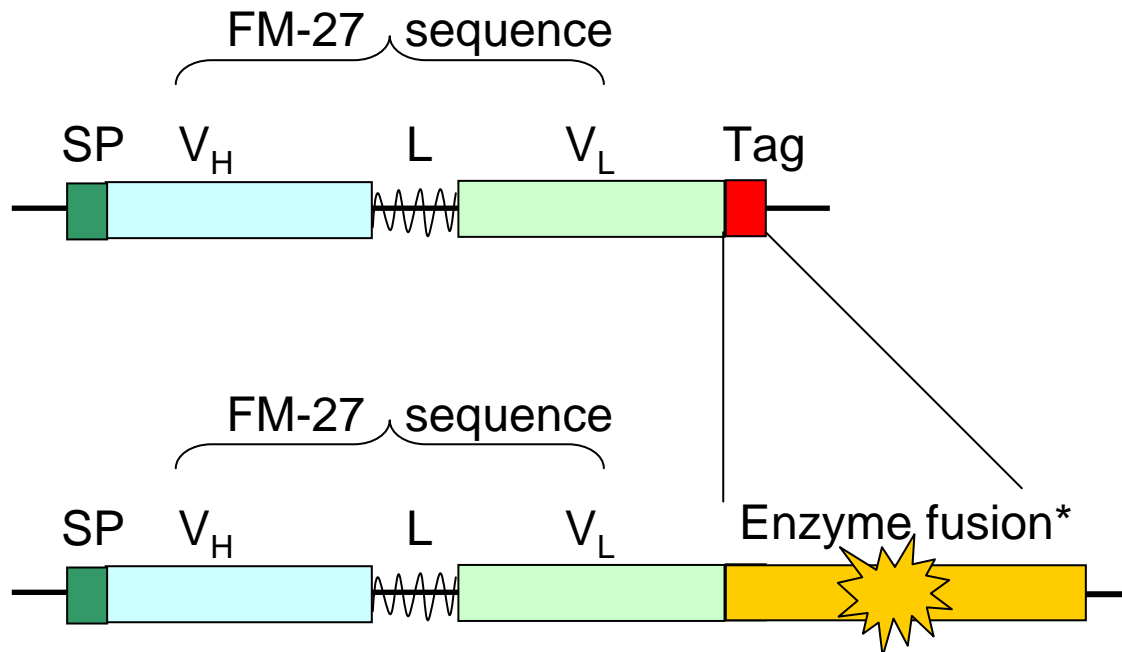


Enzyme fusion

Direct detection

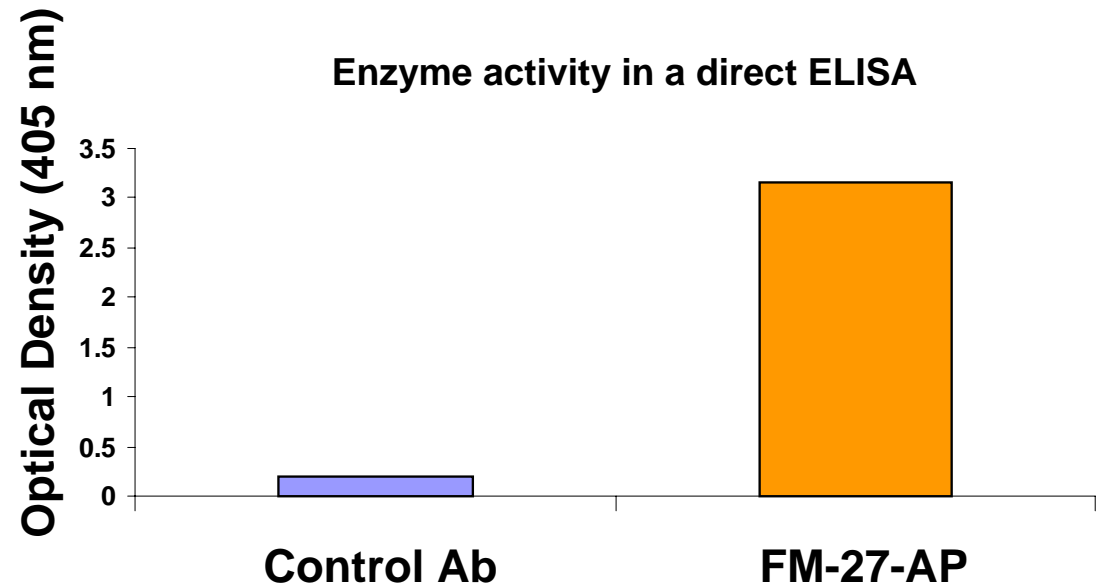
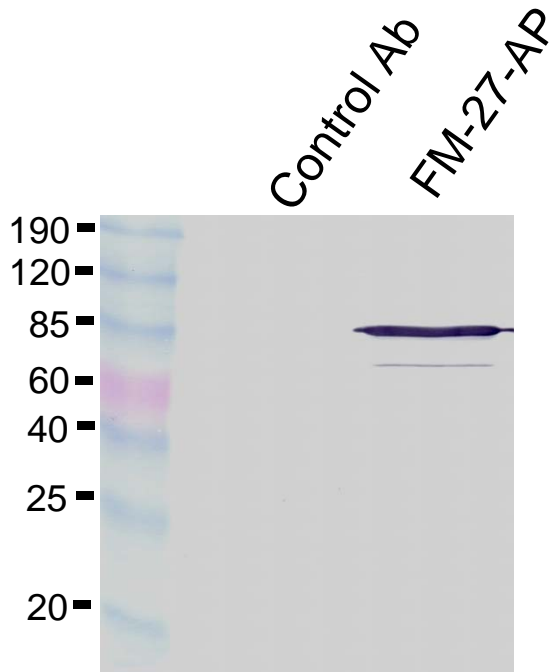
**ONE-STEP**

# From an epitope tag to an enzyme fusion

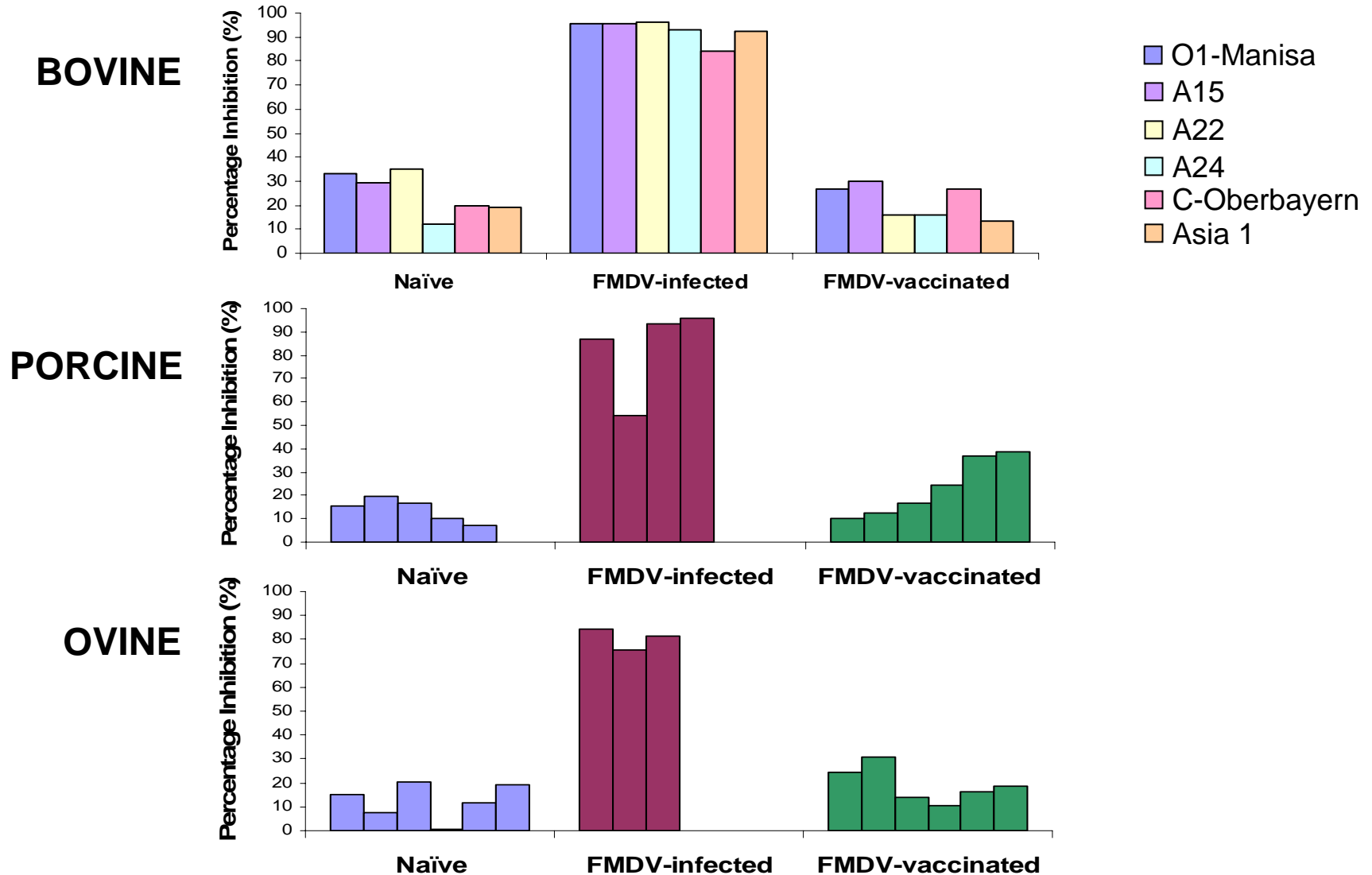


\* Kerschbaumer, R.J. et al. 1997. *Analytical Biochemistry* 249:219-27

# Specific enzyme activity



# One-step detection C-ELISA



# In Summary

- The FM-27 bound a well defined epitope region of 3B.
- FM-27 is an ideal replacement for the monoclonal antibodies and FMDV-specific serum currently used in the C-ELISA.
- We have developed a DIVA C-ELISA based solely on recombinant reagents that is simple, reliable and rapid.

# Acknowledgements

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## Supervisors

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## Collaborators

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Dr Olan Dolezal

Ms Jennifer McEachern

Mr Chris Morrissy

Dr. Jef Hammond

Dr. David Boyle

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Dr. Alfonso Clavijo