

Multiplex ELISA kit for measuring antibodies to VMv/CAEv, CLA and MAP in sheep and goat flocks



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Introduction

1. The challenges faced in commercial flocks
pyramid diseases
2. An escalating problem?
3. Multiplex plate
4. Strength and limitations
5. Potential applications in the field



Establishing disease status in commercial sheep flocks- the challenge

- Lack of perceived impact of diseases
- Cost of disease?
- Cost of testing?
- Lack of availability of high health replacements
- Lack of demand for high health replacement
- If positive- what next?



Prevalence of disease (VMv, CLA, Map)

- **VMv:** Increasing individual and flock prevalence of (Ritchie et al, 2014)
- **CLA:** 18% terminal sire flocks >1 positive animal (Baird et al., 2004)
- **OJD:** Unknown in UK (6% Lovatt and Strugnell, 2013)



Cost of disease

- Limited studies looking at reduced performance in UK situation
- VMv seroprevalence increase pre-weaning lamb mortality (Arsenault et al., 2003)
- CLA (Australia) reduced fleece yields, trimming (Paton et al., 1994)
- Johnes prevalence and ewe mortality variable (Bush et al., 2006)

How did we get here?

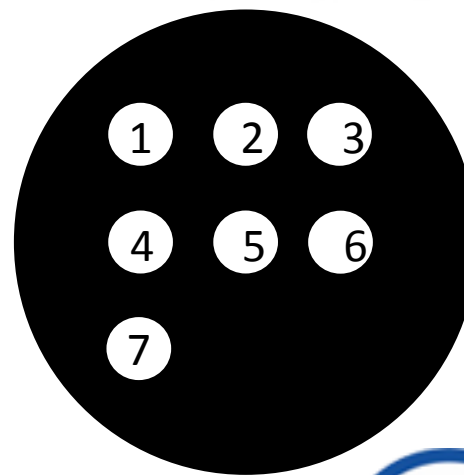
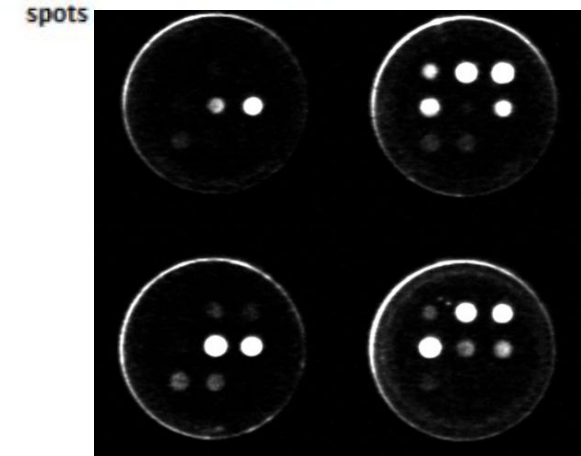
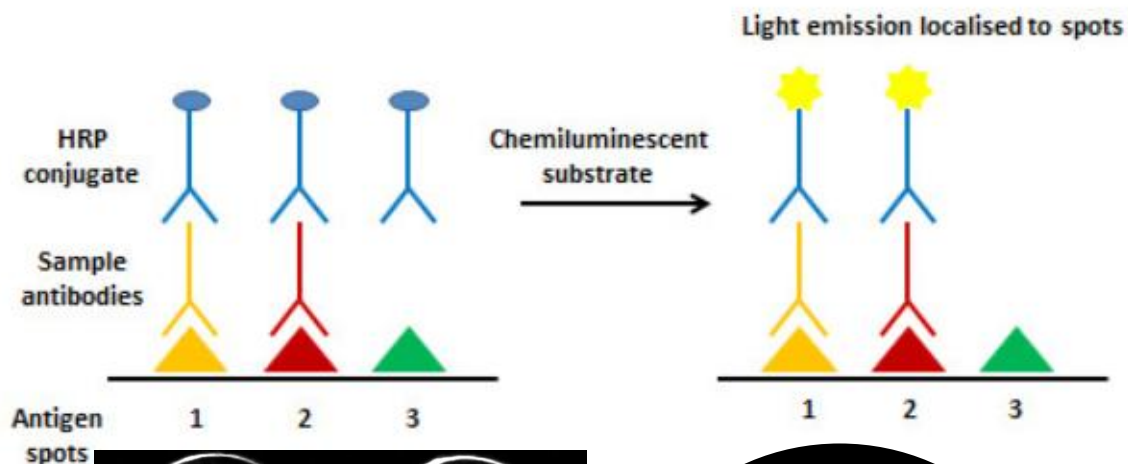
- Alpaca TB false positives using serological testing
- Test specificity improved by looking at multiple antigens simultaneously on same plate
- Same technology used in Norway

(Nagel-Alne et al., 2014)



The Multiplex ELISA kit

MVD-Enferplex array for detecting antibodies



Enferplex Bovine TB antigen array



SURE
FARM LTD

The antigens

- **MVV/CAEV:** Recombinant p25 core protein, TM1 gp46 synthetic peptide
- ***Corynebacterium pseudotuberculosis:***
Recombinant phospholipase D and CP40
- ***M. avium* subsp *paratuberculosis:*** PPA3
protoplasmic antigens

Strengths and limitations of the Multiplex

Strengths

1. Simultaneous pathogen testing
2. Reduced cost
3. Repeatability
4. Increased Sp VMv component relative to 5 other commercial test
5. Opportunities for development

Limitations

1. Challenges of Sn of Johnes diagnostics
2. Limitations of serological testing in iceberg diseases esp. Johnes and MV e.g age profile of disease



The properties and performance of the test

	<u>Relative Sn</u>	<u>Relative Sp</u>
VMv	99.4	97.4
CLA	97.5	98.8

Above relative to the Elitest (MVV/CAEV and CLA ELISAs, EAVLD Piza Proceedings 2014)

Johnes

Challenging to define, lack of gold standard. Must be interpreted bearing in mind biology.
Comparable



Applications:

- Easy, lower cost serological test, comparable Sn and Sp
 - Screening tool to establish likelihood of infection in flock
 - Cull ewe screen
 - Inform biosecurity/buying habits?
 - Pre-accreditation screen?
- Sn and Sp
 - Not an individual animal test (esp. Johnes), screening tool for flock
 - Not an accreditation scheme on offer
 - Not a one off screen- Johnes



Conclusion

- Comparable performance to other tests
- £15 to vets plus carriage fee, minimum 12 samples per flocks
- Screening test establish likelihood of infection

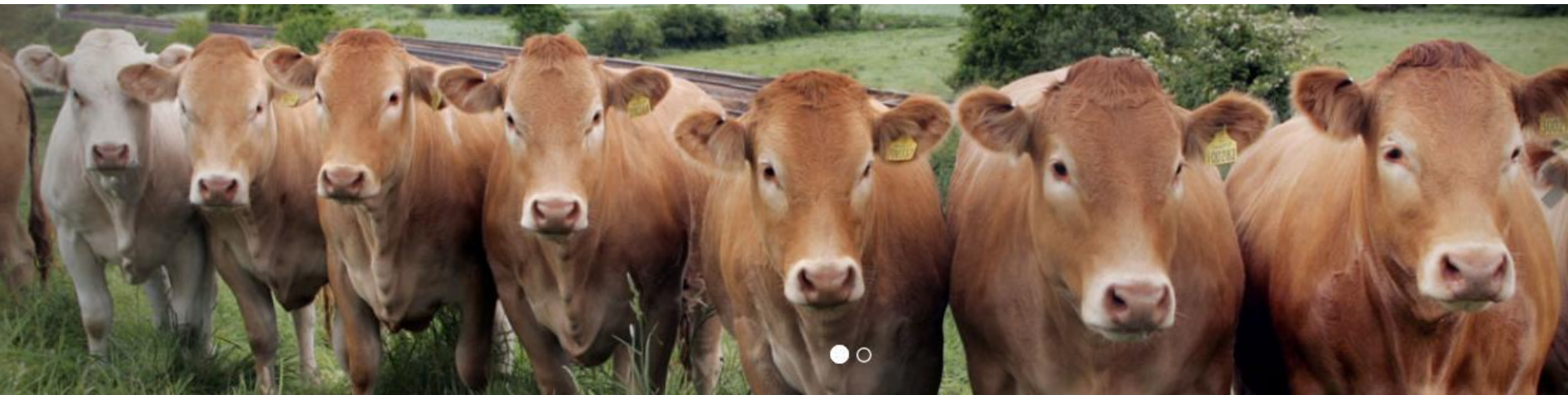


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