Validation of a rBP26-based Commercial *Brucella ovis* Antibody ELISA

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vmrd Veterinary Medical Research & Development

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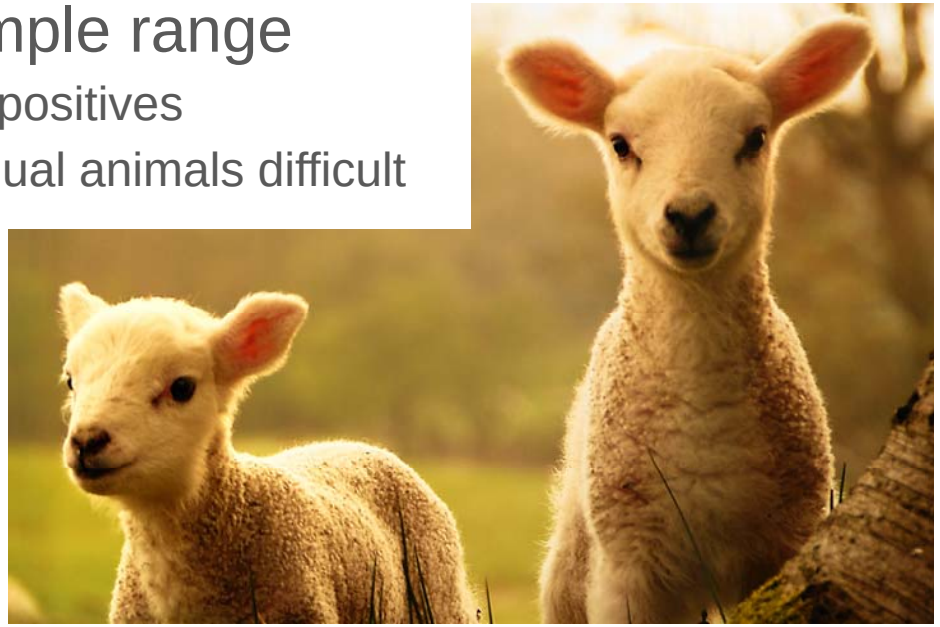
The challenges of *Brucella ovis* serology

- Currently performed by indirect ELISA
  - Antigen and components provided by NVSL
  - Plates coated at individual labs
- Variation among laboratories
  - SOP and proficiency testing provided by NVSL
  - Lack of consistency still occurs, discrepant results between labs
- Problematic indeterminate sample range
  - Established to avoid missing any positives
  - Can make management of individual animals difficult



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NVSL Reagent Manual

NVSL SOP-SERO-0035 (4)

Impact

- Inconvenience and expense for producers
- Lack of confidence in labs
- Difficulty determining proper management

[1] USAHA Comm on Sheep and Goats, 2014, Res 31
[2] USAHA Comm on Sheep and Goats, 2005, Res 33

USAHA resolutions

- Develop better diagnostic tests
  - Consistent and reliable
  - Suitable for individual animal diagnosis
- Address current challenges
  - Cross-reactivity
  - Colostral antibody interference
  - Indeterminate sample range
  - Variation between laboratories

Unmet needs

- Standardization
- Improved specificity
- Better sample resolution

[1]USAHA Comm on Sheep and Goats, 2005, Res 33
[2]USAHA Comm on Sheep and Goats, 2007, Res 68
[3]USAHA Comm on Sheep and Goats, 2009, Res 41
[4]USAHA Comm on Sheep and Goats, 2014, Res 31

Brucella ovis rBP26

BP26 protein

- Immunodominant Ag located in periplasm
- Well-conserved in the *Brucella* genus
- Absent in *Y. enterocolitica* O:9

ELISA antigen

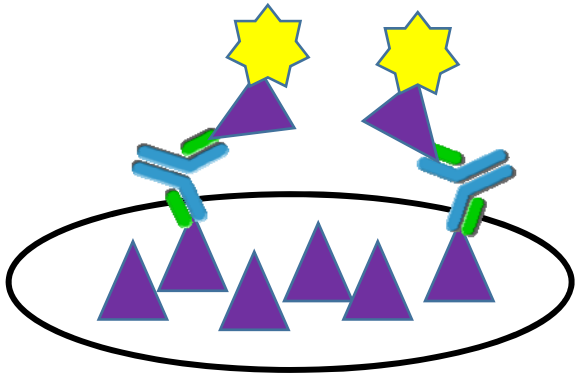
- Partial BP26 sequence, recombinant protein
  - Optimize specificity
  - Consistent production
- Coated on plate and HRP-conjugated
  - MI-ELISA format

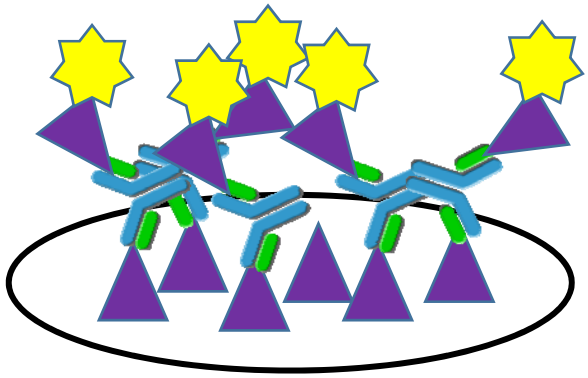
Seco-Mediavilla P et al. 2003. Clin Diag Lab Immunol 10(4):647-651.

MI-ELISA format

Detection of antibody in serum

- Ag coated on plate
- Addition of sample
  - If present, Ab in sample bind to Ag on plate
- Conjugated Ag binds to Ab from sample bound on plate
  - Increased Ab = increased bound conjugate
  - Conjugate reaction with substrate produces color
  - Increased color = increased Ab present





MI-ELISA comparison to NVSL ELISA

NVSL clear positives/negatives

NVSL ELISA

MI-ELISA	Positive	Negative	Total
Positive	**220**	9	229
Negative	28	**221**	249
Total	248	230	478

- 478 samples, obtained from Colorado State
- 92.3% concordance, kappa = 0.845*
- NVSL positive/MI-ELISA negative
  - Possible cross-reactivity in NVSL assay?

*https://graphpad.com/quickcalcs/kappa1.cfm

Refereed against western blot

MI-ELISA

- Cutoff of 3.4 S/N
  - Sensitivity 95.3%
  - Specificity 95%
- Cutoff of 2.0 S/N
  - Sensitivity 97.7%
  - Specificity 86.6%
- No indeterminate region

NVSL

- Only clear positives and negatives (no indeterminate samples included)
  - Sensitivity 96.7%
  - Specificity 86.6%

MI-ELISA comparison to NVSL ELISA

Including 30 NVSL indeterminate samples

NVSL ELISA

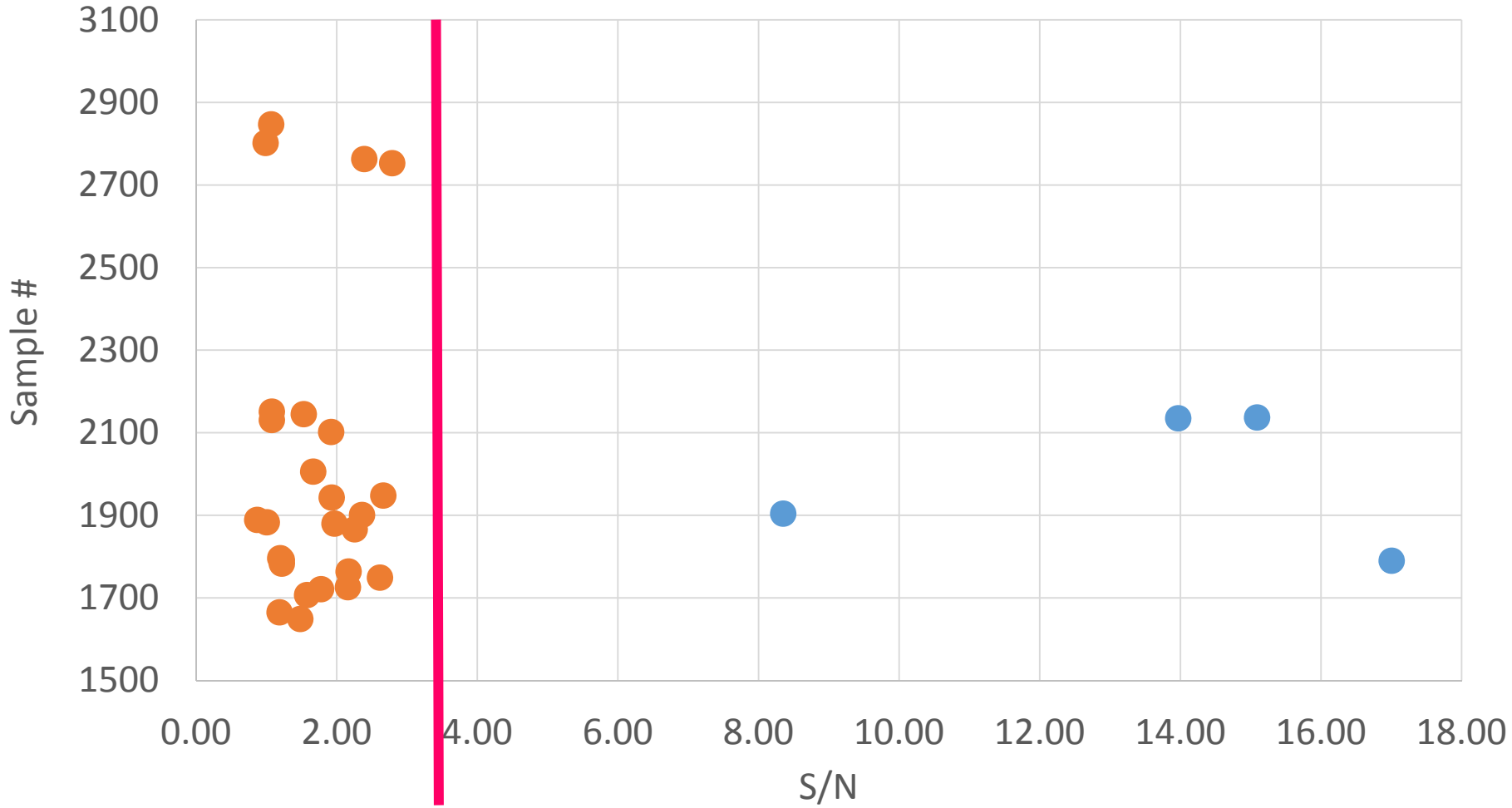
MI-ELISA	Positive	Indeterminate	Negative	Total
Positive	220	**4**	9	233
Negative	28	**26**	221	275
Total	248	**30**	230	508

- 508 samples
- No indeterminate range for MI-ELISA
  - 86.8% concordance
  - Kappa score of 0.796* (weighted)

*https://graphpad.com/quickcalcs/kappa1.cfm

Indeterminate samples

Good resolution of test positive/test negative samples



Ongoing work

Further validation

- Address indeterminate sample issue
  - Testing of additional NVSL indeterminate samples
  - Establish cutoff with optimal sample resolution
- Evaluate with larger sample set
  - More extensive validation against NVSL assay
  - Include positives characterized from experimental infection
  - Expand negative sample set
- Continued refinement of referee assay

Benefits of the VMRD MI-ELISA

- Standardized commercial product
  - Less subject to individual lab variation and protocol drift
- Very good concordance with NVSL clear positives & negatives
  - Possible improved specificity
- Improved resolution of NVSL “indeterminate” samples
  - Characterization of individual animals for show/sale/export
- Not dependent on *B. ovis* culture

- Balancing sensitivity and specificity
  - Recombinant antigen increases specificity vs. bacterial extract
  - MI-ELISA format improves sensitivity vs. cELISA format

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