Validation of a rBP26-based Commercial Brucella ovis Antibody ELISA

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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

www.aphis.usda.gov
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

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

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

<table>
<thead>
<tr>
<th>MI-ELISA</th>
<th>NVSL ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>220</td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>248</td>
</tr>
</tbody>
</table>

- 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

<table>
<thead>
<tr>
<th></th>
<th>NVSL ELISA</th>
<th>MI-ELISA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>220</td>
<td>248</td>
<td>488</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>4</td>
<td>30</td>
<td>34</td>
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<tr>
<td>Negative</td>
<td>9</td>
<td>230</td>
<td>239</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>508</td>
<td>737</td>
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</tbody>
</table>

- 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