Overview

- Remind everyone again
- Reasons for national testing protocol
- Benefits of standardization
- Protocol for cattle, bison and cervids
- 2013 National SOP published
- New VS Guidance
- What if you don’t use it?
- Questions?
Historical Serologic Protocols

• Not consistent across labs
• Different numbers and types of tests used in each state
• Can they all be equivalent quality?

“Where’s the rivanol? I love the rivanol!”

“What the Hell do you know about Bangs testing?”

“The Bangs is coming back!!”

“What? No card test!”

“You idiot!!”
Loss of Control

• “I don’t mind if everyone uses the same protocol as long as ...the protocol we use is mine!”

Historical Serologic Protocols

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of states using test (out of 32 unique state responses)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAPA</td>
<td>23</td>
<td>71.9%</td>
</tr>
<tr>
<td>RST</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>RAP</td>
<td>19</td>
<td>59.4%</td>
</tr>
<tr>
<td>CARD</td>
<td>8</td>
<td>25.0%</td>
</tr>
<tr>
<td>CITE</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>FPA</td>
<td>2</td>
<td>6.3%</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>3.1%</td>
</tr>
</tbody>
</table>
Historical Serologic Protocols

Situation Prior to Standardization

- Prior to 2007, not consistent across labs
- Different numbers and types of tests used in each state
- Interpretation of tests varies?
  - Parallel testing increases sensitivity
  - Series testing increases specificity
- Can they all be equivalent quality?
National Standardized Testing Protocol

<table>
<thead>
<tr>
<th>Test</th>
<th>Studies</th>
<th>N</th>
<th>Sensitivity (Mean)</th>
<th>Specificity (Mean)</th>
<th>LR(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAPA</td>
<td>15</td>
<td>60,634</td>
<td>95.4%</td>
<td>97.7%</td>
<td>37</td>
</tr>
<tr>
<td>Card</td>
<td>11</td>
<td>6434</td>
<td>90%</td>
<td>55%</td>
<td>2</td>
</tr>
<tr>
<td>Rivanol</td>
<td>12</td>
<td>4,845</td>
<td>89%</td>
<td>63%</td>
<td>2.4</td>
</tr>
<tr>
<td>FPA</td>
<td>7</td>
<td>39,934</td>
<td>97.5%</td>
<td>99%</td>
<td>97.5</td>
</tr>
<tr>
<td>CF</td>
<td>38</td>
<td>28,537</td>
<td>89%</td>
<td>83.5%</td>
<td>5.4</td>
</tr>
</tbody>
</table>


National Standardized Testing Protocol

<table>
<thead>
<tr>
<th>Test</th>
<th>Parallel interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP</td>
<td>Sensitivity 99.99%</td>
</tr>
<tr>
<td>FPA</td>
<td>Specificity 80.68%</td>
</tr>
<tr>
<td>CF</td>
<td>Number false negative 0</td>
</tr>
<tr>
<td></td>
<td>Number false positive 193,177</td>
</tr>
</tbody>
</table>

Source: Eric Ebel, VS Epidemiologist, 2002
### National Standardized Testing Protocol

**Total number tested**: 1,000,000  
**Prevalence**: 0.0010%

<table>
<thead>
<tr>
<th>Test name</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Number false negative</th>
<th>Number false positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP</td>
<td>82.78%</td>
<td>99.996%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td></td>
<td></td>
<td>2</td>
<td>42</td>
</tr>
</tbody>
</table>

Source: Eric Ebel, VS Epidemiologist, 2002

---

### National Standardized Testing Protocol

**Total number tested**: 1,000,000  
**Prevalence**: 0.0010%

<table>
<thead>
<tr>
<th>Test name</th>
<th>'Current' interpretation (2007 confirmatory parallel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP</td>
<td>Sensitivity 95.14%</td>
</tr>
<tr>
<td>FPA</td>
<td>Specificity 99.60%</td>
</tr>
<tr>
<td>CF</td>
<td>Number false negative 0</td>
</tr>
<tr>
<td></td>
<td>Number false positive 4,006</td>
</tr>
</tbody>
</table>

Source: Eric Ebel, VS Epidemiologist, 2002
National Standardized Testing Protocol

- Total number tested: 1,000,000
- Prevalence: 0.0010%
- Test name: RAP > FPA in Series alone
  - RAP Sensitivity: 93.02%
  - FPA Specificity: 99.97%
  - CF: Number false negative: 1, Number false positive: 253

Note: CF is supplemental information for classification

Source: Eric Ebel, VS Epidemiologist, 2002

- Due to extremely low prevalence the US program requires very high specificity (minimize costs of false positives)
- Standardized Testing protocol (2014) = BAPA/RAP >> FPA in series to maximize specificity, minimize cost
- Screening test, if positive, followed by confirmatory test
National Standardized Testing Protocol

Classification of Brucellosis Tests in US testing protocol:

- Screening Test
  - BAPA
  - RAP
- Primary Confirmatory Test
  - FPA
- Secondary Confirmation Test
  - Complement Fixation
- Supplemental Test
  - 8% Card
  - ELISA/BRT/HIRT
  - Plate/Standard Plate Test
  - Rivanol
  - Tube/Standard Tube Test
  - Western Blot

We calculated an expected responder rate of about 25 per 100,000 samples tested should be seen with our current protocol...

...but we actually only detect about one half or less than that at slaughter = ~10 responders per 100,000.
National Standardized Testing Protocol

2013 Review of Brucellosis Slaughter Lab Responder rates

<table>
<thead>
<tr>
<th>Lab</th>
<th>Testable Samples</th>
<th>Total FPA tests</th>
<th>FPA(+) Rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KY Lab</td>
<td>1,181,166</td>
<td>24</td>
<td>2.0</td>
</tr>
<tr>
<td>TX Lab</td>
<td>1,515,444</td>
<td>122</td>
<td>8.1</td>
</tr>
<tr>
<td>2016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KY Lab</td>
<td>855,522</td>
<td>21</td>
<td>2.4</td>
</tr>
<tr>
<td>TX Lab</td>
<td>628,305</td>
<td>71</td>
<td>11</td>
</tr>
</tbody>
</table>

* FPA response rates are reported in FPA positives per 100,000 samples

The average responder rate of the national testing protocol is roughly .01% or 10 per 100,000 or a specificity of around 99.99%.

Significant variations away from 10 responders per 100,000 will trigger further investigation

2016 responder rate = 6.2 per 100,000
Published in 2013
Official protocol for cattle, bison and cervids

7.8.2 Non-Negative FPA Specimens

• If a specimen yields a non-negative FPA test interpretation, the specimen shall be forwarded to NVSL for confirmation....
• Disease classification will be made based on official protocol using all available epi info
NVSL guidance for performing confirmatory testing on a sample from a brucellosis lab that did NOT follow the national testing protocol.

- If a specimen yields a non-negative test result from a non-approved brucellosis testing protocol, then the specimen shall be forwarded to NVSL where the official brucellosis protocol will be used.
- Classification based on standard protocol + epi

NVSL guidance for performing confirmatory testing on a sample from a brucellosis lab that followed the national testing protocol.

- If a specimen yields a non-negative test result to the screening test only, the NVSL will perform the primary confirmatory test (FPA).
- NVSL will perform secondary confirmatory test (CF) if FPA is non-negative.
NVSL guidance for performing confirmatory testing on a sample from a brucellosis lab that followed the national testing protocol.

- If a specimen yields non-negative test results to the screening and confirmatory tests, then NVSL will rerun both confirmatory tests if initial test results are provided on 10-4 form.
- If initial test results not provided to NVSL, then confirmatory tests in series.

NVSL guidance for performing supplemental brucellosis testing

- Supplemental testing can be completed at NVSL according to the Standard Operating Procedure upon request... use VS form 10-4 “Examinations requested.”
Summary

• There is a national brucellosis testing protocol:
  BAPA/RAP >> FPA

• There is a published brucellosis testing SOP and a new guidance document

• All non-negatives must be confirmed at NVSL

• Follow the protocol!

Questions?