Development and Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Bovine Tuberculosis

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Introduction

Detection of bovine tuberculosis

• Skin Tests
  • Measurement of delayed-type hypersensitivity response to the intradermal injection of purified mycobacterial protein (PPD or tuberculins)
  • Caudal Fold Test (CFT) or Single Cervical Test (SCT)
  • Comparative Cervical Test (CCT)
  • Standard or primary test: EU (64/432/EEC), OIE, USDA

• Gamma-Interferon Test (BOVIGAM™)
  • Measurement of cell mediated immune response
  • Ancillary test: EU (64/432/EEC), OIE, USDA
  • Parallel/serial testing strategies
  • Primary test in Mexico
Introduction

Detection of bovine tuberculosis

• Serology Tests
  • Measurement of serologic antibody responses to M. Bovis antigens
  • MPB70, MPB83, etc.
  • Not approved according to EU and USDA
    but listed at OIE
Introduction

Detection methods from Thermo Fisher Scientific for bTb:
Bovigam™, bovine Gamma Interferon Test kit

- in vitro laboratory test for the diagnosis of bovine tuberculosis in cattle, sheep, goats, buffalo, bison and other bovidae
- monoclonal antibody-based sandwich enzyme immunoassay (EIA) for the detection of interferon-γ (IFN-γ)
- widely used as an ancillary test to the tuberculin skin test
Tuberculin PPD for skin test

- High quality product – GMP facility (formerly Production Lelystad)
- Standardized product - Eu. Ph./OIE
- Matched Avian/Bovine Product (SICCT)
- Trusted by leading TB eradication programs (UK, Ireland, France)
- Potency tested in guinea pig assay and 2 - 3 assays on naturally TB infected bovines per year in Ireland
## Introduction

### Detection following experimental infection with M. bovis (Waters et al., 2011)

<table>
<thead>
<tr>
<th>Test</th>
<th>dpi</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOVIGAM®</td>
<td>14 – 17</td>
</tr>
<tr>
<td>Serology</td>
<td>90 – 100</td>
</tr>
</tbody>
</table>

→ CMI is the earliest immune response after infection with *M. bovis*

→ Antibody responses develop later than CMI responses but are a useful tool to complete the diagnostic portfolio for bTB testing

→ Serological tests can be used in anamnestic animals and in very low prevalence areas as a confirmatory test

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Vordermeier *et al.*, The Veterinary Record, July 10, 2004
Regulatory

Skin Test:
- The only test approved for official herd testing as a primary test (OIE, EU, USDA)

BOVIGAM Test:
- Recognized by many authorities (USDA, FLI, EFSA, SENASA, APHA, DFAM etc.) as ancillary test to Skin Test
- OIE approval to use BOVIGAM™ as primary test and to confirm a herd free of TB after outbreak, since may 2015

Serology Test:
- Not official tuberculosis diagnostic tool (but listed at OIE)
ELISA

- Mycobacterium bovis antigen
- Blocking
- Serum Samples
- Conjugate
- Stop
- Measurement at $\lambda = 450/620 \text{ nm}$
- Analysis: Cut-Off 15% PP

$$PP = 100 \times \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{PC} - OD_{NC}}$$
Sample Preparation

Pos. Con
Neg. Con
Test Work Flow

Sample incubation

- Transfer of controls and samples from the dummy plate to the Test Plate.
- Final dilution 1:100
- Sample incubation for 60 minutes
- Washing of plate
Test Work Flow

Conjugate incubation

- Adding of POD labelled detection antibody
- Conjugate incubation for 60 minutes
- Washing of plate
Detection

- Adding of substrate (TMB) for 15 minutes
- Stopping of color development by adding of Stop Solution
- Reading of the optical density at $\lambda = 450$ nm / 620 nm
Results

Determination of Sensitivity and Specificity

Sensitivity / Specificity – results

• Data from 835 samples
• Cut-off defined with ROC

<table>
<thead>
<tr>
<th>Cut-off (PP)</th>
<th>Sensitivity %</th>
<th>95% CI</th>
<th>Specificity %</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 15 %</td>
<td>65.99</td>
<td>60.3 - 71.4</td>
<td>98.34</td>
<td>96.9 - 99.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin</th>
<th>total samples</th>
<th>Status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>APHA 105</td>
<td>105</td>
<td>105</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>APHA 99</td>
<td>99</td>
<td>99</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IRL</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>USDA 300</td>
<td>300</td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>APHA positive</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>UCD 150502</td>
<td>38</td>
<td>0</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Chamau</td>
<td>56</td>
<td>56</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>APHA 138</td>
<td>138</td>
<td>0</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>USDA spec.</td>
<td>38</td>
<td>31</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>∑</td>
<td>835</td>
<td>541</td>
<td>294</td>
<td></td>
</tr>
</tbody>
</table>
Determination of the Analytical Specificity

<table>
<thead>
<tr>
<th>Status</th>
<th>Number of samples</th>
<th>Result Neg / Pos</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. Paratuberculosis</em> positive</td>
<td>48</td>
<td>48 / 0</td>
<td>USDA (7), NL (10), IRL (31)</td>
</tr>
<tr>
<td><em>M. kansasii</em> positive</td>
<td>8</td>
<td>8 / 0</td>
<td>USDA</td>
</tr>
<tr>
<td><em>M. avium</em> positive</td>
<td>8</td>
<td>8 / 0</td>
<td>USDA</td>
</tr>
</tbody>
</table>

No Cross Reactivity was detected
## Results

### Determination of Variances

<table>
<thead>
<tr>
<th>Plate</th>
<th>Mean OD</th>
<th>Stdev</th>
<th>% CV</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.850</td>
<td>0.026</td>
<td>3.12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.944</td>
<td>0.028</td>
<td>2.95</td>
<td>Intraplate variance</td>
</tr>
<tr>
<td>3</td>
<td>0.948</td>
<td>0.023</td>
<td>2.44</td>
<td></td>
</tr>
<tr>
<td>1 - 3</td>
<td>0.914</td>
<td>0.052</td>
<td>5.70</td>
<td>Interplate variance</td>
</tr>
<tr>
<td><strong>Negative sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.021</td>
<td>0.001</td>
<td>4.72</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.021</td>
<td>0.001</td>
<td>5.02</td>
<td>Intraplate variance</td>
</tr>
<tr>
<td>3</td>
<td>0.022</td>
<td>0.001</td>
<td>6.20</td>
<td></td>
</tr>
<tr>
<td>1 - 3</td>
<td>0.022</td>
<td>0.001</td>
<td>6.19</td>
<td>Interplate variance</td>
</tr>
</tbody>
</table>
Conclusion

The PrioCHECK™ Tuberculosis Complex Ab is a suitable and reliable tool for confirmatory testing of bovine tuberculosis.

The determined sensitivity of 66 % and specificity of 98 % makes it an ideal tool to complement the diagnostic portfolio for the detection of bovine tuberculosis.
Acknowledgements

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