Real-time PCR used in U.S. Slaughter Surveillance

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Objectives

- Review historical workflow
- Introduce current workflow
- Report performance of assay on slaughter surveillance samples
- Discuss control setup and performance of controls
- Discuss performance of assay in known affected herds
Historical workflow: partial parallel testing

Slaughter surveillance granuloma identified

Collect:
Borate sample for PCR/Culture
Formalin sample for Histopathology

Etiology determined by Histopathology

Results and Report ≤2 days

60%
Borate sample Not cultured

40%
Borate sample cultured

Results and Report ≤8 wks

1 day
8 weeks
## Culture/Histology performance

<table>
<thead>
<tr>
<th>Year</th>
<th>No. submitted</th>
<th>No.</th>
<th>% M. bovis confirmed</th>
<th>FFPE MTBC PCR results +/-total (%)</th>
<th>Histology false negative</th>
<th>% submissions cultured</th>
<th>M. bovis isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>7362</td>
<td>23</td>
<td>82.6%</td>
<td>17/22 (77.3%)</td>
<td>2</td>
<td>40.3%</td>
<td>21</td>
</tr>
<tr>
<td>2010</td>
<td>8584</td>
<td>17</td>
<td>58.8%</td>
<td>7/15 (46.7%)</td>
<td>0</td>
<td>42.3%</td>
<td>10</td>
</tr>
</tbody>
</table>
Disadvantage of culture

Culture
Slow (≤8 wks)

High cost

Low throughput (max 24 samples/day/person)

Results after histology released
## Advantages of PCR

<table>
<thead>
<tr>
<th>Culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow (≤8 wks)</td>
<td>Fast (≤24 hrs)</td>
</tr>
<tr>
<td>High cost</td>
<td>Low cost</td>
</tr>
<tr>
<td>Low throughput (max 24 samples/day/person)</td>
<td>High throughput (max 75 samples/day)</td>
</tr>
<tr>
<td>Results after histology released</td>
<td>Results prior to histology release</td>
</tr>
</tbody>
</table>
Current workflow: full parallel testing

Slaughter surveillance granuloma identified

Collect:
Borate sample for PCR/Culture
Formalin sample for Histopathology

PCR of borate sample

Etiology determined by Histopathology

Report PCR results

M. bovis specific PCR

Report M. bovis results

Compare

Compare

Results and Report ≤2 days

Borate sample cultured

Results and Report ≤8 wks

1 day

8 weeks
Development & Implementation

- Development May – Dec. 2013
- Validation started December of 2013
- Start parallel testing with culture January 2014
- Real-time PCR replaced culture in April, 2014 after 2000+ tests
Performance of real-time assay in slaughter surveillance

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity (95% CI)</td>
<td>ND</td>
<td>0.998 (0.994, 1.00)</td>
<td>0.992 (0.990, 0.994)</td>
</tr>
<tr>
<td>Sensitivity (95% CI)</td>
<td>0.96 (0.89, 0.99)</td>
<td>ND</td>
<td>1.00 (0.91, 1.00)</td>
</tr>
<tr>
<td>No. of samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hist +, PCR +</td>
<td>74</td>
<td>3</td>
<td>38* (+2)</td>
</tr>
<tr>
<td>Hist -, PCR -</td>
<td>3</td>
<td>1732</td>
<td>6077</td>
</tr>
<tr>
<td>Hist +, PCR -</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hist -, PCR +</td>
<td>0</td>
<td>3</td>
<td>47* (-2)</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>1738</td>
<td>6162</td>
</tr>
</tbody>
</table>
Control setup

Types of controls:
1) Negative tissue extraction control added every 5 samples thru 20 samples, then every 10
2) TB tissue extraction control added every 5 samples thru 20 samples, then every 10
3) One *M. bovis* tissue extraction control added to each plate
4) Every sample and control has an internal extraction control

Full 96-well plate would have 75 samples, 10 negative controls, 10 TB controls, and 1 *M. bovis* control.
Control Daily Average

- Extraction Control of the Negative Control Daily average
  - Graph showing daily averages with error bars and run numbers.

- TB Control Daily Average
  - Graph showing daily averages with error bars and run numbers.

- M. bovis Control Daily Value
  - Graph showing daily values with error bars and run numbers.
Post Validation Ct Results

![Graph showing Ct values for different groups including BCG Control, TB Control, 1081-3 False Pos, 1081-3 True Pos, L3 True Pos, 1081-3 BCG Cont. Subset, and L3 BCG Cont. Subset.](image-url)
Using the real-time PCR in affected herds

• 3 herds in Michigan (2) and Texas (1)

• 340 samples were parallel tested by either histology, culture or both.
## Performance in known affected herds

### MTBC

<table>
<thead>
<tr>
<th></th>
<th>Histology or culture +</th>
<th>Histology or culture -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +</td>
<td>297</td>
<td>1</td>
<td>298</td>
</tr>
<tr>
<td>PCR -</td>
<td>9</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>306</td>
<td>33</td>
<td>339</td>
</tr>
</tbody>
</table>

Sensitivity: 0.97 (95% CI 0.94, 0.99)

### M. bovis

<table>
<thead>
<tr>
<th></th>
<th>Histology or culture +</th>
<th>Histology or culture -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +</td>
<td>289</td>
<td>0</td>
<td>289</td>
</tr>
<tr>
<td>PCR -</td>
<td>14</td>
<td>31</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>303</td>
<td>31</td>
<td>334</td>
</tr>
</tbody>
</table>

Sensitivity: 0.95 (95% CI 0.92, 0.97)
Limits of the Assay

- Open system- High risk of laboratory contamination
  - Cross contaminations likely will occur, plan controls and their locations in plates well
  - Amplicons (PCR product) can build up in facility with daily use
- Requires highly trained molecular technicians and a workflow that protects against contamination
- Requires lesioned tissue
- Very small tissue amounts can be tested (0.3 g) with PCR vs 50 g for culture
Conclusion

USDA is now using real-time PCR in place of culture to screen submissions for routine slaughter surveillance

– Assay Sensitivity >97%, Specificity >99%

– Allows us to resolve discrepant results prior to the release of the histology report.

– Allows us to screen 100% of the samples with two independent tests, which greatly reduces error.
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Questions?