Early Detection of MAP infection

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**The Challenge / Issue**

Animals shed MAP and spread infection – but remain largely undetectable by serological assays during early infection.

- Sensitivity ranges from ~10–~90% depending on stage of infection.

- Culture based assays take weeks for results.
- PCR based assays have low sensitivity, particularly during early infection.
- Current ELISAs are whole cell Ag based; low sensitivity during early infection.
Dynamics of Serological Response

A. Total normalized signal intensities from the clinical, sub-clinical and uninfected groups: Red Clinical; Yellow, Sub-clinical; Blue, negative control
B. Heat map of intensities from individual cow to each recombinant protein
Some antigens are recognized earlier than fecal shedding and ELISA.

Detection of MAP infection:
Fecal (culture /PCR) ~ 7 months post infection;
Commercial serological test (ELISA) ~ 8 months post infection.
Patterns of Serological Reactivity

A & B. Antigens recognized at later stage of infection
C. Antigens recognized from early through the late stage
D. Antigen recognized at early and declining at later stage
Orthogonal approach to MAP Antigen Discovery
Whole Proteome Arrays – Data Analysis Toolkit

Sample 1

Sample 2

Normalized intensities

(Methods based on Kunnath-Velayudhan (2010) PNAS 107:14703)
# JD Diagnostics Standards Study

## Johne’s Disease Diagnostics Standards Study

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cows</td>
<td>917</td>
</tr>
<tr>
<td>No. of farms (Herd Size)</td>
<td>13 (66-1,400)</td>
</tr>
<tr>
<td>US. States</td>
<td>CA, GA, MN, PA</td>
</tr>
<tr>
<td>Within herd prevalence of MAP</td>
<td>0-53.3%</td>
</tr>
<tr>
<td>Fecal Culture Assays</td>
<td>HEYM, MGIT, TREK</td>
</tr>
<tr>
<td>PCR Assays</td>
<td>LifeTechnologies; Tetracore</td>
</tr>
<tr>
<td>Serum ELISA Assays</td>
<td>IDEXX; ParaChek</td>
</tr>
<tr>
<td>Milk ELISA Assays</td>
<td>IDEXX</td>
</tr>
</tbody>
</table>

## Stratification of Samples

<table>
<thead>
<tr>
<th>Group</th>
<th>NL</th>
<th>NH</th>
<th>F+E-</th>
<th>F+E+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence in Herd</td>
<td>−</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Fecal Positive</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ELISA</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Group</td>
<td>NL</td>
<td>NH</td>
<td>F+E-</td>
<td>F+E+</td>
</tr>
<tr>
<td>-------</td>
<td>----</td>
<td>----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td><em>n</em></td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

**In NH only**

- Rv1536
- Rv1969

**In F+E- only**

- Rv1028c
- Rv1568

**In All Groups**

- Rv0341
- Rv0954

**Only In F+E+**

- Rv1860
- Rv2878c
Patterns of Serum reactivity to the 47 MTB proteins
Increased Sensitivities with Antigen Combinations

![Graphs showing increased sensitivities with antigen combinations.](image-url)
# MAP ELISAs

<table>
<thead>
<tr>
<th>Group</th>
<th>NL</th>
<th>NH</th>
<th>F+E-</th>
<th>F+E+</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MAP1272c</th>
<th>MAP1569</th>
<th>MAP2121c</th>
<th>MAP2942c</th>
<th>MAP2609</th>
<th>MAP1201c+2942c</th>
<th>LacZ_MBP</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean_NL</td>
<td>0.09</td>
<td>0.07</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean_F+E+</td>
<td>0.29</td>
<td>0.32</td>
<td>0.10</td>
<td>0.73</td>
<td>0.37</td>
<td>0.36</td>
<td>0.10</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Ratio of Mean F+E+/Mean NL**

![Graph showing the ratio of Mean F+E+ to Mean NL for different MAP ELISA samples.](image)
MAP ELISAs

MAP2942c (Rv2878c)

ELISA OD: MAP2942c

p < 0.001

MAP2942c ortholog Rv2878c (identity 77.6%)
Evolution to Multiplex Platforms

- Tubes
- Microwell plates
- Microarrays
- Bead-based Liquid Arrays
Bead-Based Sandwich Assays

Coat beads with Antigen (s)

Incubate with sample (serum / milk / etc.)

Bind labeled reporter antibody

Bead ID and Reporter quantity Determined by Laser Detection

Red laser reads the bead, i.e. the target

Green laser detects the amount of the target
Map Bead-Arrays (Serum, $n = 180$)
Map Bead-Arrays (Milk, $n = 90$)
ROC Curves (Serum; NL and F+E+)

MAP1272c ELISA BeadArray
Sensitivity 0.800 0.617
Specificity 0.867 0.800
AUC 0.897 0.780

MAP1569 ELISA BeadArray
Sensitivity 0.667 0.800
Specificity 0.933 0.833
AUC 0.817 0.908

MAP2121c ELISA BeadArray
Sensitivity 0.517 0.633
Specificity 0.500 0.600
AUC 0.486 0.736

MAP2942c ELISA BeadArray
Sensitivity 0.817 0.833
Specificity 0.900 0.833
AUC 0.904 0.898

MAP2609 ELISA BeadArray
Sensitivity 0.700 0.800
Specificity 0.733 0.933
AUC 0.741 0.885
ROC Curves (Milk; NL and F+E-)

<table>
<thead>
<tr>
<th>Test/Antigen</th>
<th>AUC</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDEXX</td>
<td>0.625</td>
<td>0.064</td>
<td>0.567</td>
<td>0.767</td>
</tr>
<tr>
<td>MAP1272c</td>
<td>0.801</td>
<td>1641</td>
<td>0.667</td>
<td>0.800</td>
</tr>
<tr>
<td>MAP1569</td>
<td>0.746</td>
<td>1409</td>
<td>0.767</td>
<td>0.633</td>
</tr>
<tr>
<td>MAP2942c</td>
<td>0.771</td>
<td>2044</td>
<td>0.600</td>
<td>0.833</td>
</tr>
<tr>
<td>MAP2609</td>
<td>0.752</td>
<td>628</td>
<td>0.733</td>
<td>0.633</td>
</tr>
</tbody>
</table>
Good potential for further improvement – TB arrays
Good potential for further improvement – MAP arrays
Concluding Comments

• MAP Dx with serology, culture or PCR remains a challenge, particularly early during infection
• Several MAP antigens reveal potential to identify animals early during infection (much sooner than fecal shedding or ELISA)
• Bead based liquid arrays show promise for early detection of MAP infected animals based on serum and milk samples
• Continued validation of candidate antigens and development of robust assays for detection of early MAP infection are in process
The Challenge / Issue

Animals shed MAP and spread infection – but remain largely undetectable by serological assays during early infection.
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RESEARCH ARTICLE

Identification of sero-reactive antigens for the early diagnosis of Johne’s disease in cattle

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