Next-day results in bovine mastitis diagnostics using DNA technology and electronic reporting

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Mastitis

-inflammation of the udder in dairy cattle
-annual economic loss: billions of US$

Fighting mastitis

-treatment based on diagnostics
-breeding for mastitis resistance

Mastitis diagnosis

Traditional approach

- culture and biochemical assays
- inexpensive
- require up to several days
- some pathogens difficult to culture
- difficult to automatize

DNA approach

- DNA amplification by PCR
- more expensive than culture method
- results in 3-5 hours
- independence of culture -> efficiency
- nucleotide level analysis -> accuracy
- possible to automatize

PCR

polymerase chain reaction

- exponential amplification of specific DNA sequences
- probes (primers) define sequence to be amplified
- million to billionfold amplification in about 1 hour
Pathogen identification in ~4 hours

- most common mastitis pathogens (Str. agalactiae, Str. dysgalactiae, Str. uberis, E. coli, Enterococcus sp., Klebsiella sp., Coagulase Negative Staphylococci, S. aureus, L. lactis, C. bovis, A. pyogenes etc.)
- staphylococcal beta-lactamase gene

PathoProof™ Mastitis PCR assay

DNA extraction (~60 min)

PCR and RE digestion (~120 min)

Electrophoresis and interpretation (~4 h)

Method comparison milk samples (n=338)

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial culture (Finnish laboratory)</td>
<td>239 (71%)</td>
</tr>
<tr>
<td>Mastitis PCR assay</td>
<td>293 (87%)</td>
</tr>
<tr>
<td>Discordant positive result:</td>
<td>39</td>
</tr>
</tbody>
</table>

DNA sequence analysis of discordant samples

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Bacterial culture (# of mismatches)</th>
<th>PathoProof™ (# of mismatches)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8040414932</td>
<td>S. aureus (25)</td>
<td>CNS (0)</td>
</tr>
<tr>
<td>8040414933</td>
<td>CNS / Str. uberis (23-78)</td>
<td>L. lactis (0)</td>
</tr>
<tr>
<td>8040414950</td>
<td>CNS (11)</td>
<td>S. aureus (0)</td>
</tr>
<tr>
<td>8040414964</td>
<td>CNS (11)</td>
<td>S. aureus (0)</td>
</tr>
<tr>
<td>8040415021</td>
<td>S. aureus (21)</td>
<td>Str. agalactiae (0)</td>
</tr>
</tbody>
</table>

MasTest™

Service concept

Mail milk sample, get result next day (internet, SMS)

MasTest™ flowchart

Vetcare Oy → Veterinarian → Farmer

Finnzymes Diagnostics
MasTest™ preliminary results
- average reply time 2.30 pm
- bacterial finding in >90% of samples
- <5% of samples reanalyzed

Implications
- health monitoring and breeding for mastitis resistance
- species-related variability in heritability (several genes)
  - pathogen information facilitates the finding of marker genes

Prospective improvements
Real time PCR (qPCR)

Real Time PCR in Mastitis Diagnostics
- improved speed (no electrophoresis needed)
- quantitative detection of species
- facilitates automatization

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