

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\$



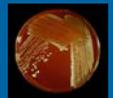
Fighting mastitis

- 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



Mastitis diagnosis

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

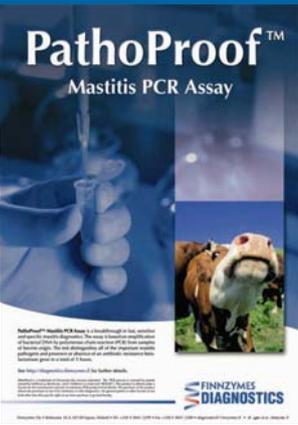
- exponential amplification of specific DNA sequences
- probes (primers) define sequence to be amplified
- million to billionfold amplification in about 1 hour



PathoProof™ Mastitis PCR Assay

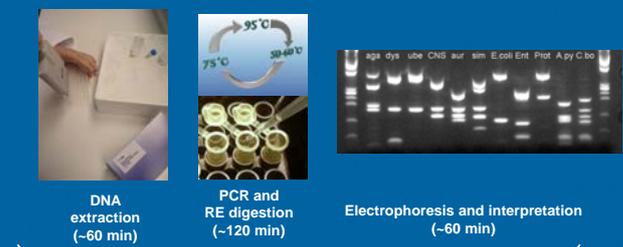
Pathogen identification in ~4 hours

- 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



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PathoProof™ Mastitis PCR assay



DNA extraction (~60 min) **PCR and RE digestion (~120 min)** **Electrophoresis and interpretation (~60 min)**

(~ 4 h)

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Method comparison milk samples (n=338)

	Positive	Negative
Bacterial culture (Finnish laboratory)	239 (71%)	99
Mastitis PCR assay	293 (87%)	45

Discordant positive result: 39

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DNA sequence analysis of discordant samples

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

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MasTest™

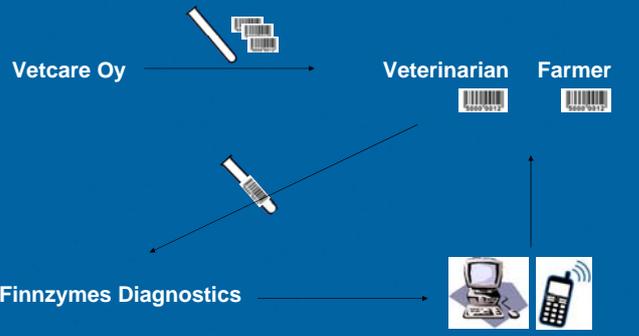
Service concept

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



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MasTest™ flowchart



Vetcare Oy → **Veterinarian** → **Farmer**

FINNZYMES DIAGNOSTICS → [Computer/Phone]

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MasTest™ preliminary results

- average reply time 2.30 pm
- bacterial finding in >90% of samples
- <5% of samples reanalyzed



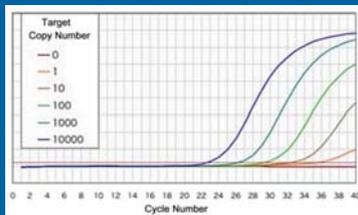
Implications

- 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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