

● **PathoProof™ Mastitis PCR Assays**



**2011 NALMA Meeting**

**Kevin Foth,  
Market Manager PathoProof products**

# The World Leader in Serving Science



## Who We Are

**Leading provider of analytical instruments, equipment, reagents and consumables, software and services for research, analysis, discovery and diagnostics**

## Strengths

- |                     |   |
|---------------------|---|
| <b>Scale</b>        | <ul style="list-style-type: none"><li>• \$11 billion revenues</li><li>• 34,000 employees</li><li>• 350,000 customers</li><li>• 150 countries served</li></ul> |
| <b>Capabilities</b> | <ul style="list-style-type: none"><li>• Complete portfolio</li><li>• World-class technologies</li><li>• Commercial reach</li></ul>                            |
| <b>Experience</b>   | <ul style="list-style-type: none"><li>• 150 years of combined experience</li></ul>  |
| <b>Brand Equity</b> | <ul style="list-style-type: none"><li>• Pre-eminent brands</li></ul>  |

*Enabling customers to make the world healthier, cleaner and safer*

\* Figures from Thermo Fisher Scientific Corporate Profile

# Thermo Fisher Scientific – what we do



## In research and analysis

- Advanced analytical instrument technologies
- Solutions to improve laboratory workflows



## In healthcare and clinical laboratories

- Diagnostic kits, reagents and consumables
- Clinical trial support and total supply



## In manufacturing and the field

- Safety-related products and personal protection
- Environmental and process instruments

***World-class analytical technologies and the most complete portfolio of laboratory products and services***

● Microbiology Division



# About the Microbiology Division:

- Specialist business focused on Microbiology for diagnosis of infectious disease and detection of bacterial contamination in industrial applications.
- Legacy brands of Oxoid and Remel, world renowned in culture media and diagnostic assays, combining with Thermo Scientific brand which represents technology and innovation.
- Direct sales and service in 24 countries
- Focused R&D with an expanded Finnzymes team to continue to grow the product range



*Combining expertise in the science of microbiology with leading edge technology to continue to develop better ways to detect and identify micro-organisms`*

# Bovine Mastitis

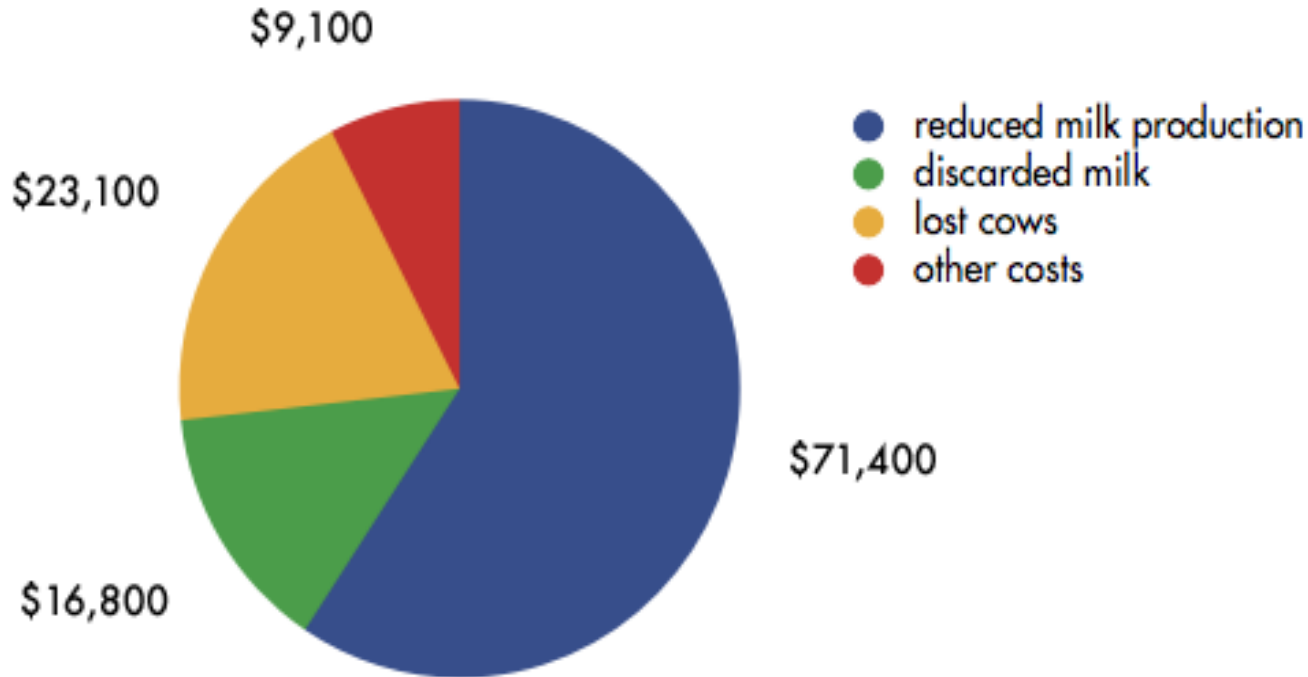
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- Economical losses ~200 USD/cow/year (dependent on cause)
- Total losses in the USA alone ~ 2.0 billion dollars per year
- Bacterial culturing is still regarded as the golden standard in mastitis diagnostics – it is considered a "flawed" standard
  - Slow
  - Unspecific
  - Sensitive to errors
  - Can not be used with preserved milk samples

# Bovine Mastitis

- Responsible for huge losses each year to the dairy industry due to many reasons, for example reduced milk production and discarded milk unfit for human consumption

**MASTITIS LOSSES FOR TYPICAL 500-COW FARM EXCEED \$120,000\***



# Ford Truck & Manure Spreader



# You have the tools – how do you use them?

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**The truck is great and the spreader is great , but they do not normally work together**

What can you do instead of treating the right mastitis with the wrong drug?

Find out first what you are fighting.

(A huge thank you to Jere High for letting me steal the previous couple of slides)

## PathoProof Mastitis PCR Assay



[Link to Brainshark presentation](#)

**Thermo**  
SCIENTIFIC

# Why use PCR-based Mastitis testing?



## 'No-growth' study

(Taponen et al., *Journal of Dairy Science*, Vol.92, No.6, June 2009)

- Approximately **25-40% of all mastitis samples yield no growth** in conventional bacterial culturing, even after 48 hours of incubation
- The reasons for the no-growth samples are unclear
- A total of 79 mastitic no-growth milk samples (from clinical mastitis) were studied using the PathoProof Mastitis PCR Assay
- Of the 79 samples, 43% were positive in PCR
- The bacterial copy number in the positive samples was ~10<sup>3</sup>-10<sup>7</sup> genome copies per milliliter of milk
- It was concluded that all common mastitis bacteria can occur in large quantities in clinical mastitis samples that exhibit no growth in conventional culture, and that the PathoProof Mastitis PCR Assay is a useful tool for bacteriological diagnosis of such samples

# Why use PCR-based Mastitis testing?

## Analytical specificity and sensitivity

(Koskinen et al., *Journal of Dairy Science*, Vol.92, No.3, March 2009)

- Analytical specificity and sensitivity validated using 643 culture isolates (from 80 species or families) representing the assay's targets and their phylogenetically closely related non-target species
- Specificity and sensitivity of beta-lactamase detection validated using 150 staphylococcal culture isolates
- Study serves as the basis for clinical field trial validations of the assay (especially for culture negative, but PCR positive cases, to show that those are not false positives)



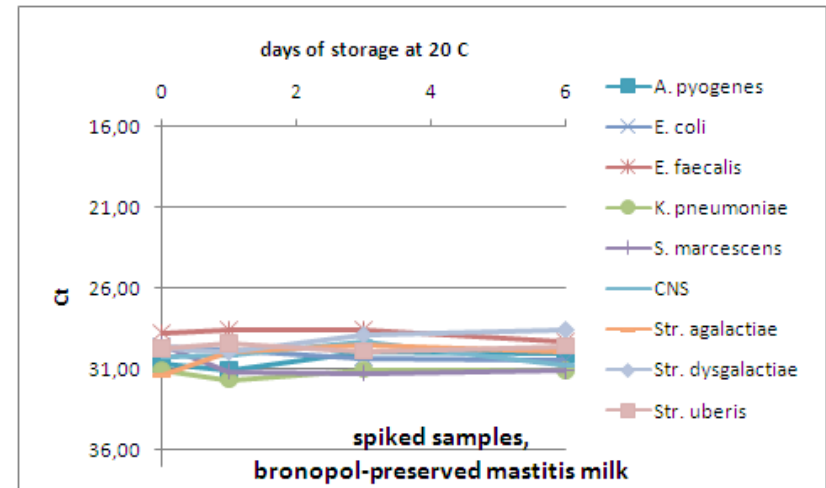
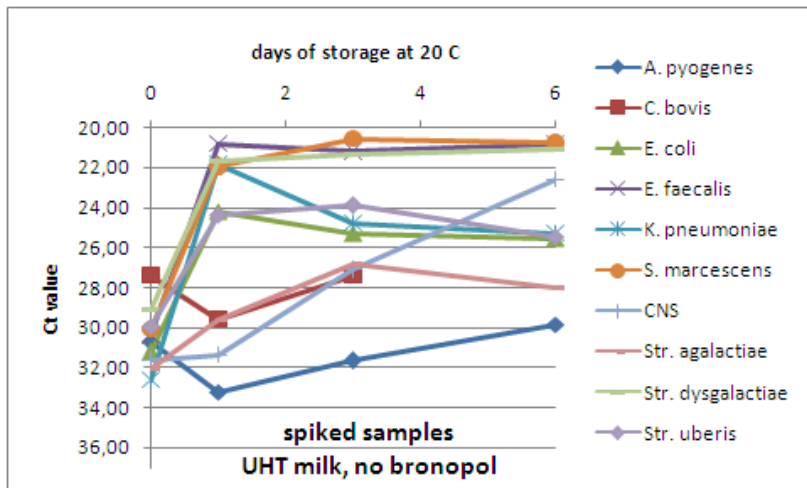
# Analytical specificity and sensitivity

| Bacterial target              | Analytical specificity | Analytical sensitivity |
|-------------------------------|------------------------|------------------------|
| <i>Staph. aureus</i>          | 100%                   | 100%                   |
| <i>Staph. sp.</i>             | 100%                   | 100%                   |
| <i>Str. agalactiae</i>        | 100%                   | 100%                   |
| <i>Str. dysgalactiae</i>      | 100%                   | 100%                   |
| <i>Str. uberis</i>            | 100% (99,0%)           | 100%                   |
| <i>E. coli</i>                | 100% (99,5%)           | 100%                   |
| <i>C. bovis</i>               | 100%                   | 100%                   |
| <i>Enterococcus sp.</i>       | 100%                   | 100%                   |
| <i>Klebsiella sp.</i>         | 100%                   | 100%                   |
| <i>Serratia marcescens</i>    | 100%                   | 100%                   |
| <i>A. pyogenes</i>            | 100%                   | 100%                   |
| Staphylococcal beta-lactamase | 100%                   | 100%                   |

# Advantages of PCR Testing

- Bronopol study

- In conventional mastitis testing schemes, samples are often transported to the laboratory without any form of refrigeration
  - sample storage using bronopol is not possible
  - this may result in bacterial growth during sample transportation
- Conventional mastitis testing methods cannot be integrated to milk monitoring programs utilizing bronopol-preserved samples
- Can the PathoProof Mastitis PCR Assay be used with bronopol-preserved milk?



# Validation with DHI metered milk samples

- The aim of the study was to answer the following primary questions:
  - What is the level of concordance in bacterial detection from milk recording samples using the PathoProof Mastitis PCR Assay, compared to bacterial culture-based testing from udder samples?
  - Is there a risk that bacteria present in the previous sample can contaminate the next sample coming through the same milking meter?
- Five herds with *Staph. aureus* history were selected
- A total of 248 animals were sampled. Three sample types were taken and analyzed from every animal:
  - Udder sample- bacterial culture
  - Udder sample- PathoProof Mastitis PCR Assay
  - Milk recording sample (DHI sample)- PathoProof Mastitis PCR Assay
    - 34 udder samples were positive for *Staph. aureus* in culture. Of these, 34 were positive with the PCR assay
    - Of the 34 culture positive animals, 32 milk recording samples were positive for *Staph. aureus*

# Validation with DHI metered milk samples

- 11 PathoProof DHI sample positive, but culture sample negative cases:
  - re-sample the animals
  - Re-culture
- Seven animals were available for re-sampling. Of these, five provided *Staph. aureus* positive results in culture
- PathoProof PCR Assay –based testing of DHI samples is more sensitive for *Staph. aureus* detection than bacterial culture-based testing from dedicated udder samples
- For dairy producers, the pilot indicates that at least as accurate *Staph. aureus* results are available by fast PCR testing of DHI samples, compared to dedicated sampling and time consuming bacterial culture testing

**Carry-over contamination: no signs of carry-over contamination were detected!**

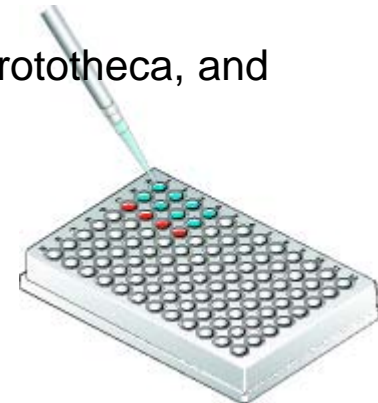
Currently waiting on publication

# Additional validation studies

- Accuracy of beta-lactamase detection in staphylococci. Pitkälä et al. (2007) *J. Clin. Microbiol.* 45: 2031-2033.
- Analytical specificity and sensitivity validation using 650 bacterial strains from 6 countries. Koskinen et al. (2009) *J. Dairy Sci.* 92: 952-959.
- Sensitivity with 'no growth' milk samples from clinical mastitis. Taponen et al. (2009) *J. Dairy Sci.* 92: 2610-2617.
- Field comparison against conventional bacterial culturing using quarter milk samples from clinical, subclinical and healthy cows from Finland and The Netherlands. Coming soon.
- Detection limit for identification of *Mycoplasma bovis* from preserved milk. Published at NMC 2010 conference in February.
- Accuracy of *Str. agalactiae* detection from preserved DHI samples against conventional bacterial culturing from composite milk samples. Coming soon.

# PathoProof kit formats

- PathoProof reagent kits
  - Two kit configurations: PathoProof Mastitis Complete-12 and PathoProof Mastitis Major-3 (contagious panel)
  - Two kit formats: Small (50 samples) and Large (4 x 96 samples) kits
  - Major-3 kit mainly used for bulk tank milk testing and DHI samples
  - 6 months shelf life
  - Delivery time 3 - 5 weeks (soon to be stored in Lenexa, KS warehouse)
  - Stored in room temperature and -20 °C (two separate boxes)
  - Option to add 1 target to Major-3 kit and 4 bacterial targets to Complete-12 kit
    - Major-4 already used by CanWest DHI, includes BlaZ detection
    - Complete-16 beginning beta-site testing (M. bovis, M. species, Prototheca, and yeast targets will be added to Complete-12 panel)



# PathoProof Software

- Automatically analyzes the PCR results
- Creates reports, which show the amount of each bacteria separately
- If one bacteria is present in over 90 % of the total bacterial load, then that is also reported

| Category | Explanation                                    |
|----------|--|
| -        | Bacterial DNA not detected                     |
| +        | Bacterial DNA detected in small amounts        |
| ++       | Bacterial DNA detected in intermediate amounts |
| +++      | Bacterial DNA detected in large amounts        |

# Additional tool used by Lancaster DHIA

| Classification   | Bacteria   | Contagious or Environmental  | Source                                       | Spread                                       | Control  | Treatment                        |
|--|--|------------------------------|--|--|--|----------------------------------|
| <i>Staphylococcus</i> spp.                             | <i>Staph. aureus</i>                             | Contagious                   | Infected udders, hands of milkers            | Milking time                                 | Post-dip, DCT <sup>1</sup> , segregation and cull if necessary       | Please consult your veterinarian |
|  | Coagulase (-) staph. & <i>S. hyicus</i>          | Neither                      | Skin flora & occasionally environment        | Infect teat canal from skin sources          | Post-dip, DCT  |                                  |
| <i>Streptococcus</i> spp. and <i>Enterococcus</i> spp. | <i>Strep. agalactiae</i>                         | Contagious                   | Infected udders                              | Milking time                                 | Milking time hygiene, post-dip, DCT                                  |                                  |
|  | <i>Strep. dysgalactiae</i>                       | Contagious and environmental | Infected udders and environment              | Milking time & environmental contact         | Milking time hygiene, pre- & post-dip, DCT, teat seal                |                                  |
|  | <i>Strep. uberis</i>                             | Environmental                | Environment – early dry period               | New IMI <sup>2</sup> during early dry period | Milking time hygiene, pre- & post-dip, DCT, teat seal                |                                  |
|  | Environmental strep & <i>Enterococcus</i> spp.   | Environmental                | Environment                                  | Environmental contact                        | Milking time hygiene, pre- & post-dip, DCT, teat seal                |                                  |
| Coliform   | <i>Escherichia coli</i>                          | Environmental                | Bedding, manure, soil                        | Environmental contact                        | Cows clean & dry, use of sand bedding, pre-dip, a J5 vaccine         |                                  |
|  | <i>Klebsiella</i> spp.                           | Environmental                | Organic bedding                              | Environmental contact                        | Avoid sawdust & recycled manure, pre-dip, J5 vaccine                 |                                  |
|  | <i>Enterobacter</i> spp.                         | Environmental                | Bedding, manure, soil                        | Environmental contact                        | Cows clean & dry, use of sand bedding, pre-dip, a J5 vaccine         |                                  |
|  | <i>Serratia</i> spp.                             | Environmental                | Soil and plants                              | Environmental contact                        | Cows clean & dry, pre-dip (no chlorhexidine products)                |                                  |
|  | <i>Pseudomonas</i> spp.                          | Environmental                | Water & wet bedding                          | Environmental contact                        | No water use in parlor, no cooling ponds, sand bedding, a J5 vaccine |                                  |
|  | <i>Proteus</i> spp.                              | Environmental                | Bedding, feed & water                        | Environmental contact                        | Not much known, use of sand bedding, a J5 vaccine                    |                                  |
|  | <i>Pasteurella</i> spp.                          | Probably contagious          | Upper respiratory tract of mammals and birds | Unknown – likely cow to cow                  | Prevent teat injuries, remove affected cows from herd                |                                  |
| Other  | Yeast & mold                                     | Environmental                | Soil, plants, water                          | Dirty infusions                              | Aseptic infusions  |                                  |
|  | <i>Corynebacterium bovis</i> & other coryneforms | Contagious                   | Infected udders                              | Cow to cow                                   | Post-dip   |                                  |
|  | Prototheca                                       | Environmental                | Soil, plants, water                          | Dirty infusions, infected udders             | Aseptic infusions, eliminate infected cow                            |                                  |
|  | <i>Bacillus</i> spp.                             | Environmental                | Soil, water, air                             | Dirty infusions                              | Aseptic infusions  |                                  |
|  | <i>Arcanobacterium pyogenes</i>                  | Contagious/Environmental     | Teat injuries                                | Flies  | Fly control  |                                  |

Information obtained from NMC Laboratory Handbook on Bovine Mastitis and veterinary consultation for treatment recommendations).

\*These are general treatment recommendations; actual recommendations may vary from herd to herd. Please consult your veterinarian.

\*\*Extra label usage, please consult your veterinarian before starting this protocol.

\*\*\*Nonsteroidal anti-inflammatory drugs.

<sup>1</sup> – DCT, dry cow therapy; <sup>2</sup> – IMI, intramammary infection; <sup>3</sup> – IMM, intramammary.

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# Work with the herd Veterinarian



