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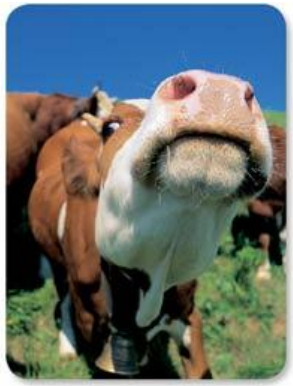
PathoProof® Mastitis PCR Assay



Martina Kahila, Product Manager PathoProof products

The World Leader in Serving Science

ThermoFisher SCIENTIFIC

Who We Are

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

Strengths

Scale	• $11 billion revenues • 34,000 employees • 350,000 customers • 150 countries served
Capabilities	• Complete portfolio • World-class technologies • Commercial reach
Experience	• 150 years of combined experience
Brand Equity	• Pre-eminent brands

Enabling customers to make the world healthier, cleaner and safer

* Figures from Thermo Fisher Scientific Corporate Profile

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

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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.
- Long-standing collaboration with DuPont Qualicon to commercialise the BAX range of PCR products for food safety in Europe, Australia and NZ.
- Direct sales and service in 24 countries
- Focused R&D with an expanded Finnzymes team to continue to grow the product range

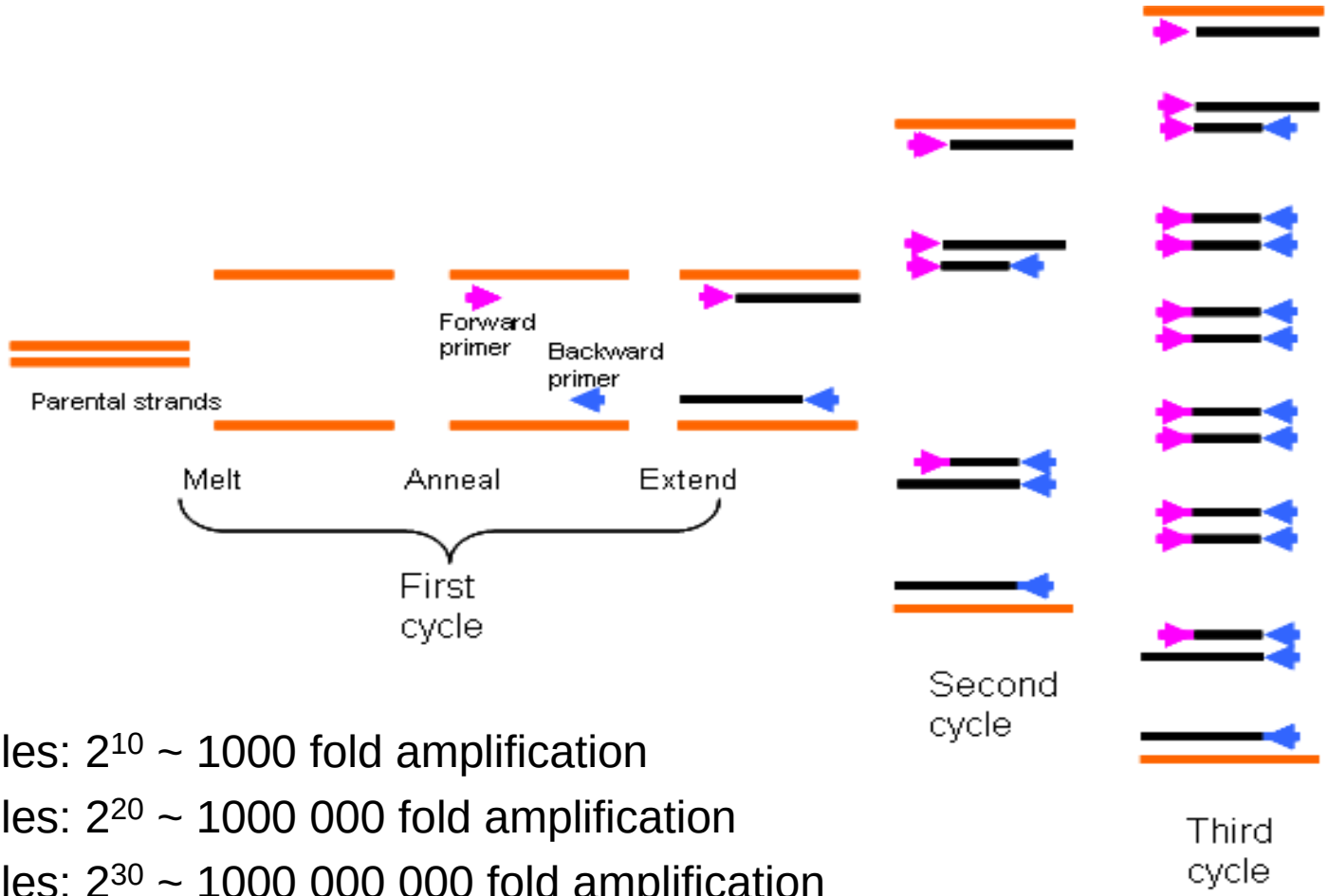
OXOID remel Thermo SCIENTIFIC

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

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
- Economical losses ~200 USD/cow/year. Total losses in the USA alone over 1,7 billion dollars per year
- Bacterial culturing is still regarded as the golden standard in mastitis diagnostics
- However, there are many problems related to culturing, for example:
  - Slow
  - Unspecific
  - Sensitive to errors
  - Can not be used with preserved milk samples

PCR=Polymerase Chain Reaction



10 cycles: 2^{10} ~ 1000 fold amplification

20 cycles: 2^{20} ~ 1000 000 fold amplification

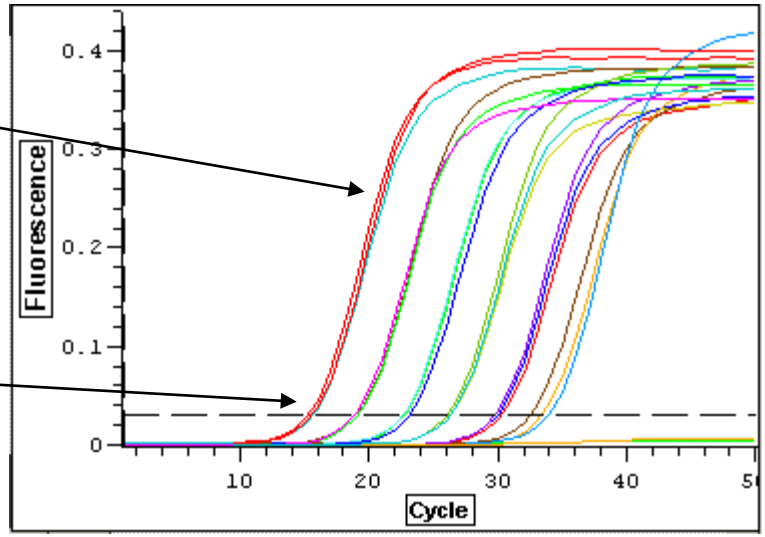
30 cycles: 2^{30} ~ 1000 000 000 fold amplification

Real-time PCR

- PathoProof is based on real-time PCR, which is the new golden standard in for example human clinical diagnostics and food diagnostics
- When using real-time PCR, the amplification and the detection of DNA is done simultaneously
- Real-time PCR is one-step, single tube detection, and the sample tube stays closed during amplification, minimizing contamination risks
- Real-time PCR is a quantitative method, which means that the initial amount of each bacteria can be detected

Fluorescence curves represent the bacterial amounts in the sample

C(t) value = the PCR-cycle where the fluorescence grows over a certain threshold level



PathoProof Mastitis PCR Assay

- Fast
- Sensitive
- Accurate
- Easy to use
- Can be used with:
  - Bulk tank milk samples
  - Individual quarter milk samples
  - DHI samples

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Speed, accuracy and sensitivity

- 3-4 hours from sample to result
- 100 % specificity – the test detects the bacterial DNA and interpretation of results is done by a software designed for PathoProof
- No ’mixed growth’ results. All bacteria are detected simultaneously. The most prevalent pathogen is reported if it accounts for >90% of the total bacterial quantity
- 40-50 % of samples yielding no growth in conventional culturing provide positive results with the PathoProof Mastitis PCR Assay
- Equally sensitive for viable and growth inhibited bacteria

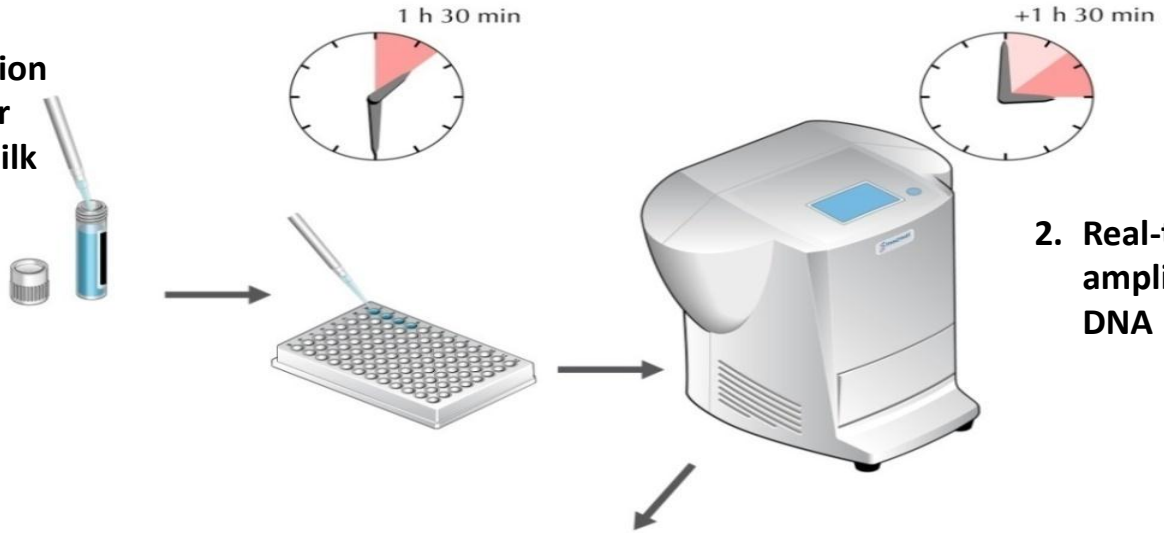
PathoProof vs. culturing

Benefits of PathoProof over bacterial culturing in mastitis diagnostics are tremendous	
Bacterial culturing	PathoProof Mastitis PCR Assay
48-72 hours	4-5 hours for all pathogens, incl. *Mycoplasma*
25-40% of no-growth cases	Detects growth-inhibited and dead bacteria -significantly less (>40% less) 'no growth' results
User experience affects reliability	Objective results for all bacteria -after product training high accuracy can be reached in all laboratories
Mainly qualitative	Qualitative and quantitative -calculates bacterial proportions in 'mixed growth' cases
Unreliable during antibiotic treatment	Not affected by antimicrobials -can be used for following and studying treatment efficacy -reliable for repeated testing even if antibiotics have been/are being used
Prone to error due to bacterial growth during sample transportation	Can be used with bronopol-preserved milk (elimination of error)
Cannot be used for testing of DHI samples	Can be used for testing of DHI samples

Easy to use

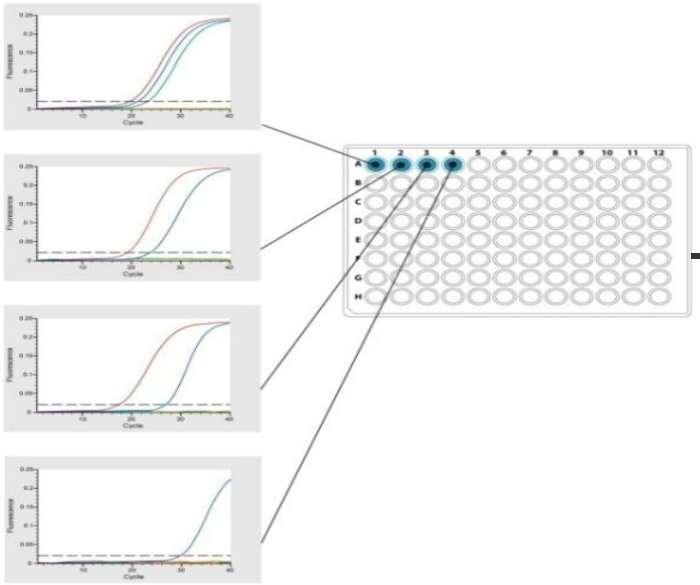
- Laboratories with no previous microbiology or molecular biology experience use the assay successfully and get equally accurate results
- Kits include all DNA extraction and PCR reagents
- The software automatically analyze the results and create results reports
- We provide a 3-4 days training to use the kits and the instruments. The training is free of charge in combination to the real-time PCR instrument purchase

1. **DNA-extraction from fresh or preserved milk samples**

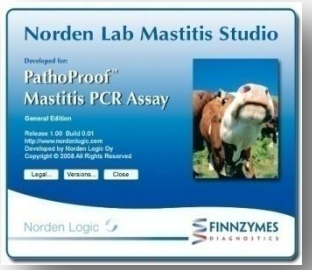


2. **Real-time PCR amplification of bacterial DNA**

3. **Simultaneous detection and quantification of the 11 most important mastitis species/groups and the most relevant antibiotic resistance gene (blaZ)**



4. **Analysis of results**



PathoProof kit configurations

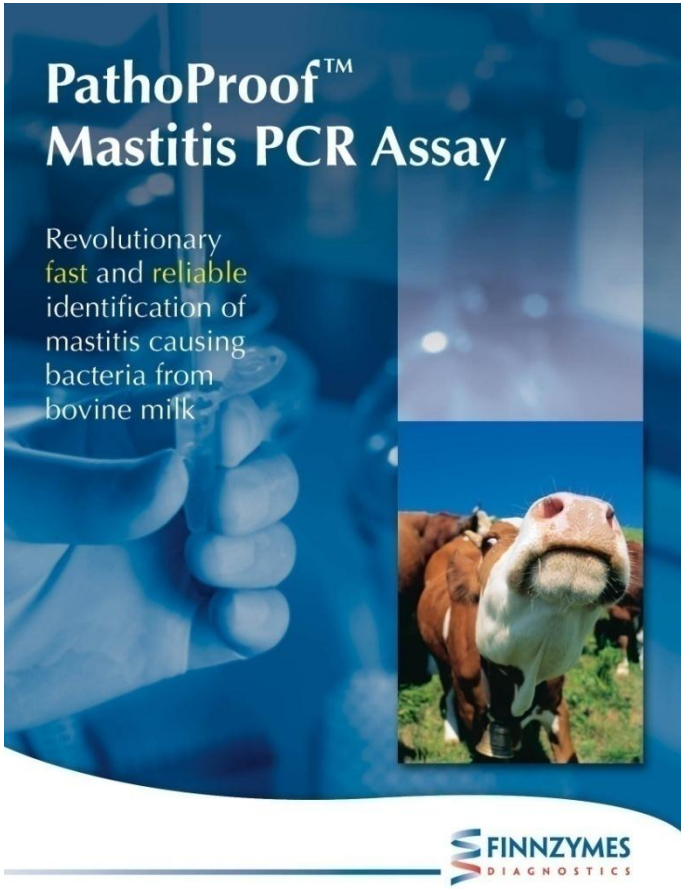
PathoProof Mastitis Complete-12

Staphylococcus aureus
Coagulase negative staphylococci
Streptococcus agalactiae
Streptococcus dysgalactiae
Streptococcus uberis
Escherichia coli
Corynebacterium bovis
Enterococcus faecalis and faecium
Klebsiella pneumoniae and oxytoca
Serratia marcescens
Arcanobacterium pyogenes and Peptostreptococcus indolicus
Staphylococcal beta-lactamase gene

⇒ **These bacteria are responsible for >95 % of all clinical and subclinical mastitis cases**

PathoProof Mastitis Major-3

Mycoplasma bovis
Staphylococcus aureus
Streptococcus agalactiae



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

Field study: PCR vs. bacterial culturing

Koskinen et. al., *J.Dairy Sci,.* In press

- First comprehensive field comparison about PCR and culturing from mastitic milk samples
- Included 1000 milk samples from cows with clinical or subclinical mastitis or from clinically healty quarters with low SCC
- 77 % of the clinical samples were positive in culturing and 89 % in PCR
- ”Healthy samples” provided a negative result in most cases (4,3 % of samples positive in culturing and 7,5 % in PCR), indicating that PCR is not ”too sensitive”
- Benefits of PCR were clear: speed, automated interpretation of results as well as increased sensitivity

PathoProof customers

- The PathoProof system has been installed into about 30 laboratories in 16 countries
- Currently about 60 % of the laboratories are DHI laboratories and 40 % animal health laboratories
- During the first 9 months in 2010, about 90 % of all installations have been to DHI laboratories
- The highest throughput is currently in Finland at Valio laboratories, where about 100 000 samples are analyzed per year
- Our service laboratory in Finland analyzes about 15 000 samples per year
- We organized the first PathoProof User's Day on September 1st, 2010 in Finland. This will be a yearly event in the future.

PathoProof feedback from laboratories and their customers

- Convenience is very important, many laboratories use the DHI samples to analyze mastitis
- Different sample types are being used: individual quarter milk samples, string samples, combined quarter samples, DHI samples, bulk tank milk samples..
- More expensive, but:
  - Lower cost of sample transportation
  - Less resampling (no growth results)
  - Faster results (the same day)
  - Integration with the DHI results
  - SCC results can be utilized efficiently

- In Finland, Valio made a survey about the milk producers interest in PathoProof testing, 98 % answered that they want their samples tested with PCR

PathoProof kit formats

- Large kits (F-870L and F-914L)
  - Reagents for 4 x 96 samples
  - DNA extraction is done on 96-well plates

- Small kits (F-870S and F-914S)
  - Reagents for 50 samples
  - DNA extraction is done in single tubes

Thank you for your attention!

Questions?