
PathoProof™ mastitis PCR assay

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Finnzymes' PathoProof™ Mastitis PCR Assay is a revolutionary novel real-time PCR based method for bovine mastitis testing. The assay can identify and quantify 11 major mastitis-causing species or groups and the beta-lactamase penicillin resistance gene in just 3 hours. The assay has been optimized for use with even the most challenging milk samples from cows having clinical mastitis. The PathoProof Mastitis PCR Assay can also be used with bronopol-preserved samples, making it applicable for milk testing and dairy herd improvement schemes where mastitis testing has not been previously possible.

The PathoProof Mastitis PCR Assay identifies the following 12 bacterial targets:

1. *Staphylococcus aureus*
2. Coagulase negative *staphylococci* (all major mastitis species)
3. *Streptococcus agalactiae*
4. *Streptococcus dysgalactiae*
5. *Streptococcus uberis*
6. *Escherichia coli*
7. *Enterococcus faecalis* and *Enterococcus faecium*
8. *Klebsiella oxytoca* and *Klebsiella pneumoniae*
9. *Serratia marcescens*
10. *Corynebacterium bovis*
11. *Arcanobacter pyogenes* and *Peptoniphilus (Peptostreptococcus) indolicus*
12. Staphylococcal β -lactamase gene (penicillin resistance gene)

Prevalence of the species identified by the PathoProof Mastitis PCR Assay is known to vary between countries. Large-scale data sets indicate that the assay covers over 99% of pathogens responsible for clinical mastitis (e.g. Makovec and Ruegg, 2003; Pitkälä *et al.*, 2004).

The analytical specificity of the assay has been validated using an extensive culture collection of the target organisms, as well as non-target species having close phylogenetic relationships with the assay's targets. These data demonstrate excellent specificity (100%) for all mastitis pathogens identified by the test (Koskinen *et al.*, 2009).

**Bacterial targets
identified by the
assay**

Bacterial identification and quantification in three hours

The PathoProof Mastitis PCR Assay identifies all of its targets simultaneously in a total of only three hours (Figure 1). This throughput time is in many cases short enough for the veterinary practitioners to apply the test results for selecting the initial treatment for the animal. Importantly, this allows knowledge-based initial therapeutic decision, decreasing unnecessary use of antimicrobials. Furthermore, the rapid throughput time of the test may decrease the total duration of treatment, which has been suggested to improve therapeutic outcome (Barkema *et al.*, 2006), and to shorten the time required for returning to normal milk (Milner *et al.*, 1997).

The PathoProof Mastitis PCR Assay provides semi-quantitative (+, ++ or +++) results for all of its 12 bacterial targets. This is useful for example for detecting quantities of the different pathogens in multi-bacterial infections, for monitoring changes during an infection, or for accurately controlling the response of a pathogen to antimicrobial treatment.

High sensitivity even with difficult-to-grow samples

The PathoProof Mastitis PCR Assay identifies the DNA of the bacterial targets. Hence, it does not rely on the ability of the bacteria to grow in a laboratory environment and has high sensitivity for viable but growth-inhibited, as well as dead bacteria.

This is a major advancement in mastitis testing, because in approximately 25-30% of the bovine milk samples taken from animals with clinical mastitis, no bacterial growth can be detected in conventional culturing. It has been suggested that inflammation of the mammary gland, inhibitory metabolic products of bacteria and unfavorable conditions during sample transportation may decrease the viability of bacteria to grow in culture (Todhunter *et al.*, 1985).

High sensitivity with growth-inhibited bacteria is also beneficial when testing milk from animals treated with antimicrobials: conventional culturing cannot be reliably used with such samples, but the PathoProof Mastitis PCR Assay enables repeated sampling and reliable testing of mastitis pathogens in cases where the selected treatment fails to improve the symptoms of an animal. The procedure (shown in figure 1) is hereby described with the correspondent steps, as numbered in figure 1.

1. Bacterial DNA is extracted from milk samples. Bronopol-preserved milk is also suitable.
2. Extracted bacterial DNA and the PathoProof PCR solutions are used to set up real-time PCR reactions. Four real-time PCR reactions are performed for each milk sample. Each reaction identifies three bacterial targets and also contains an internal amplification control for verification of acceptable reaction conditions.
3. The PathoProof Mastitis PCR Assay identifies 11 major mastitis bacterial species/groups and the β -lactamase penicillin resistance gene in staphylococci. While one milk sample is typically positive for only one primary pathogen, the kit can identify all 12 targets simultaneously. The test result is a cycle threshold value (Ct), i.e. the number of PCR cycles required to reach a certain species-specific fluorescence level in the reaction tube. The fewer cycles it takes to obtain the fluorescence level, the greater the amount of bacterial DNA for the given target in the reaction. The blue curves present in the reactions represent the internal amplification controls. An automated software can be used for scoring the results for every sample and bacterial target and for preparing a results report according to the needs of the user.

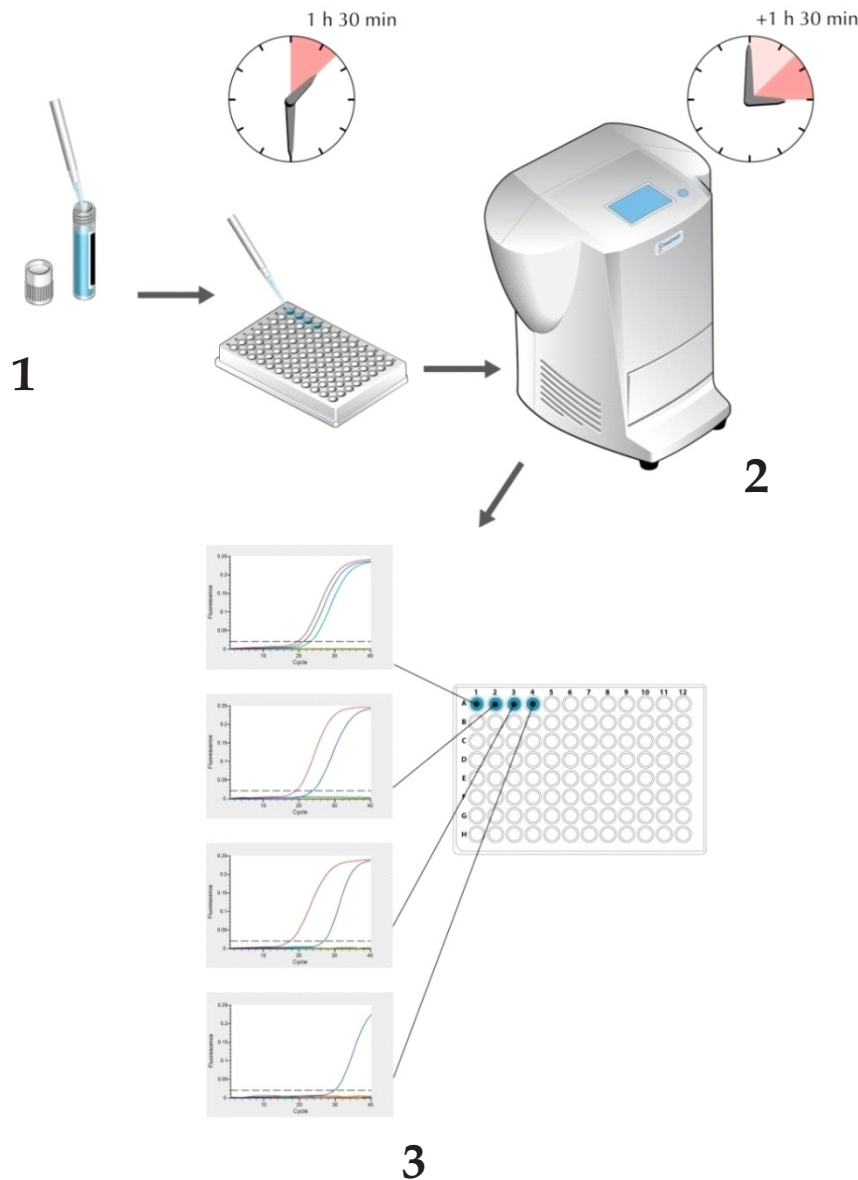


Figure 1. Laboratory workflow of the PathoProof Mastitis PCR Assay.

The PathoProof Mastitis PCR Assay has been validated for use with raw or bronopol-preserved milk samples. When preserving the milk samples when they are taken, the results obtained using the test reflect the true status of an animal at the time of sampling without any risk for bacterial growth during sample transportation.

Using the PathoProof Mastitis PCR Assay and bronopol-preserved milk is a convenient and full-proof solution to problems caused by uncontrolled microbial growth or death during transportation in ambient temperatures. In conventional testing schemes, this source of error is predicted to be common if milk samples arrive to the test laboratories without any cooling efforts (Dinsmore *et al.*, 1990).

**PathoProof
Mastitis PCR
Assay can be
used with
preserved milk
samples**

Importantly, the PathoProof Mastitis PCR Assay can also be integrated to routine milk monitoring and dairy herd improvement schemes utilizing preserved milk. Mastitis pathogen identification in connection to, for example, somatic cell count measurements from preserved samples has not been previously possible. As high somatic cell count is often due to mastitis, the PathoProof Mastitis PCR Assay can provide tremendous benefits for the dairy produce, if the assay is used for offering mastitis pathogen testing services in connection to somatic cell count measurements.

Further information

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