DNA profiling – a revolutionary method for mastitis testing

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Mastitis is responsible for greater economic losses for the dairy industry than any other disease due to reduced milk yield and the cost of discarding milk unfit for human consumption.

Bacterial culturing has been the standard method for mastitis testing for many years. However, this method has several drawbacks which cause it to be an inefficient method in mastitis analysis.

Bacterial culturing is slow, inaccurate, prone to errors and most importantly, it cannot be used with preserved milk samples. Dairy herd improvement samples that are used for example for somatic cell counts cannot be utilised in mastitis testing when bacterial culturing is the method of choice.

The PathoProof Mastitis PCR Assay (Finnzymes, Finland) is based on real-time PCR technology, a technology widely used in molecular biology and various diagnostic applications, for example. It requires no bacterial culturing steps, thus overcoming the problems related to culturing.

The assay detects the bacterial DNA in the milk sample and takes only 3-4 hours to perform in the laboratory. The milk sample can be either fresh or preserved. For the first time a normal dairy herd improvement (DHI) sample can also be used for mastitis testing, making it very convenient for the milk producer.

Straightforward protocol

The PathoProof method includes three steps: DNA extraction, DNA amplification, and analysis of the results.

In the first step, the DNA of the bacteria present in bovine milk is isolated. Bacterial DNA is released by breaking the cells and purified from all other components in milk such as lipids, proteins and bacterial cell debris. The DNA extraction protocol of the PathoProof mastitis PCR assay has been developed for use with even the most difficult milk samples.

The next step is real-time PCR, in which species-specific fragments of bacterial DNA are amplified and quantified.

The PathoProof Mastitis PCR Assay kits include reagents sufficient for 50 or 4 x 96 tests.

Real-time PCR is the most specific and sensitive diagnostic method available, having an accuracy of 100%.

In the final step, the real-time PCR results are imported into a tailored software, which automatically analyses and reports the results. The assay detects the 11 most common mastitis causing pathogens, like Staphylococcus aureus and Streptococcus agalactiae.

The bacteria detected by the assay are responsible for over 95% of all mastitis cases worldwide.

In addition to mastitis causing pathogens, presence of the beta-lactamase gene responsible for penicillin resistance in Staphylococcus sp. is also reported in the test results. Penicillin resistance is the only relevant antimicrobial resistance in mastitis.

All results obtained using the PathoProof mastitis PCR assay are quantitative, which means that the exact amount of each bacterial species or species group in the sample is measured and reported. Also, the most prevalent bacterial species is separately reported, if it covers for more than 90% of the total bacterial load in the sample.

A very common situation with milk samples is that they contain more than just one bacterial species. These cannot be identified on a bacterial culturing plate and the results are therefore reported as 'mixed growth'.

In contrast to bacterial culturing, the PathoProof Mastitis PCR Assay can distinguish the different bacteria, even if all 12 bacterial targets are present in the milk sample providing additional information for mastitis management.

Another common problem with bacterial culturing is the 'no growth' results. Based on the clinical symptoms the cow can be suffering from mastitis but the bacteria in the milk sample is either growth inhibited or dead and thus does not grow on a culture plate.

Since the PCR based method is completely independent on the viability of the bacteria, the PathoProof assay gives significantly less 'no growth' results than conventional bacterial culturing methods.

Convenience to customers

The PathoProof Mastitis PCR Assay is an easy and convenient method for mastitis testing. It is already in use in several laboratories worldwide.

For example CanWest DHI, a milk recording organisation based in Canada, is one of the companies now using it to offer mastitis testing service from DHI samples.

According to Richard Cantin, director of customer service for DHI, convenience is the key selling point of this new service. "Sample collection for mastitis testing has always been time consuming and inconvenient, which means it often did not get done. The fact that the DHI sample can now be used makes it incredibly convenient".

Taking the assay into use in a laboratory does not require previous molecular biology or microbiology experience. Finnzymes provides help in setting up the process as well as a thorough technical training for all new PathoProof users. ■