

Identification of subclinical mastitis in DHI samples using PCR testing

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Key Words

Subclinical Mastitis; Somatic Cell Counts (SCC); DHI screening; PathoProof PCR Assay testing; Mycoplasma

Summary

To investigate the identification of subclinical mastitis in Dairy Herd Improvement (DHI) samples, a study was performed using DHI samples from five farms in The Netherlands with cows showing no clinical symptoms. The results of this study identified a close correlation between the presence of mastitis-causing pathogens identified using Thermo Scientific™ PathoProof™ Assays (Thermo Fisher Scientific) and Somatic Cell Count (SSC) measurements. This suggests that PathoProof screening could be used as part of routine DHI testing, to identify which cows in the herd should be subsequently tested for identification of the contagious mastitis-causing pathogens. This could direct treatment and minimize the potential economic impact of leaving the cows in the herd.

Introduction

The economic impact of mastitis is estimated to be ~\$200 per cow per year¹, and the loss in productivity is a well-recognised issue for both farmers and dairy companies. It is vital for the industry sustainability that herds remain as healthy and productive as possible.

Less well known is the fact that subclinical mastitis is estimated to be present in anywhere from 30-70% of cows in a herd. While the cow appears to be unaffected by illness, and there are no visible changes to the milk produced, in reality there is a significant impact:

- Milk yield is significantly reduced
- Milk quality is affected
- The cow is a potential source of infection to the healthy herd mates.

Subclinical mastitis costs US dairy industry in excess of \$1 billion annually² with the overall production loss estimated at \$110/cow.³ Therefore, identifying cases of subclinical mastitis could have significant economic benefits for the farmer and result in a positive impact on milk supply and overall herd health.

The National Mastitis Council estimates there are 15-40 cases of subclinical mastitis for every 1 case of clinical mastitis, and is a major cause of milk loss.



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Identification of subclinical mastitis

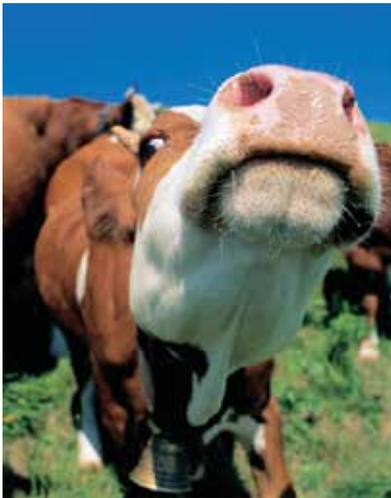
SCC level is often used as an indicator of sub-clinical mastitis.⁴ However, there is no consistent level for SCCs, and country-to-country, farm-to-farm, and animal-to-animal variations exist. Routine screening of standard DHI samples for the presence of mastitis – causing pathogens could rapidly and reliably identify sub-clinical cows, however classical culturing is not possible with samples that have been preserved for shipment. PathoProof Assays can be used with fresh and preserved samples, and results are available the same day. A study was thus performed on DHI samples using SCC levels together with PathoProof testing for pathogen detection in order to identify cows that should be subsequently tested for causative pathogen identification. This would deliver a cost effective method for farmers to identify, segregate and treat previously unidentified cases of subclinical mastitis.

Using SCC counts and PathoProof Assays to identify subclinical cases

A study was conducted using DHI samples from five Dutch herds over three time points and results were correlated between SCC counts and the results from two PathoProof products – Thermo Scientific PathoProof Complete 16 and Thermo Scientific PathoProof Major 4.2 Assay Kits. The PathoProof Complete Assay identifies bacteria responsible for >95% of all clinical and subclinical mastitis cases from both environmental and contagious sources, while the PathoProof Major 4.2 Assay identifies only major contagious pathogens.

PathoProof Major 4.2 Kit testing

Testing DHI samples with the PathoProof Complete 16 Assay detected a high degree of environmental pathogens in symptomless cows. This is due typically to the general environment, milking and sampling equipment and hygiene procedures. It is thus not recommended that PathoProof Complete 16 tests be used to routinely test DHI samples for subclinical mastitis. Further analysis was thus confined to PathoProof Major 4.2 Assay, which identifies the major contagious pathogens most significant to udder health: *Mycoplasma bovis*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Streptococcus Uberis*. Both SCC values and changes in SCC values were analysed alongside the results from the PathoProof Assay. A threshold value for the PathoProof assay result was selected (Cq < 32) that indicates the result was due to a real pathogen infection rather than as a result of any potential cross contamination.



This combination of testing can identify up to 79% of previously undetected subclinical mastitis cases

Study outline

Pathogen positive cows were identified using a PathoProof Major 4.2 Assay reading of Cq < 32
Pathogen positive cows were then analyzed for SCC values and % change in SCC values over time-points
Analysis showed pathogen positive cows displayed SCC higher values than those not selected by the Cq result
This enabled identification of threshold SCC values that correlate with positive pathogen presence and require identification of the causative pathogen to direct treatment.

Clear correlations between PathoProof Major 4.2 Assay results and SSC levels have been identified. Analysis of the data indicates that the following SCC thresholds will identify 71-79% of cows that had a positive PathoProof Major 4.2 Assay result.

Measurement	Threshold	% affected cows detected
SCC level	100,000 cells/ml	79% of sub-clinical cows detected
% change in SCC between two time points (even if SCC < 100,000 cells/ml)	>200%	71% of sub-clinical cows detected

If the objective is to reduce as many clinical and subclinical cases as possible, screening of all cows with the PathoProof Major 4.2 Assay would be the recommendation, with subsequent testing of positive samples with the PathoProof Complete 16 Assay to identify the infective agent and infected quarter. However, where screening of all animals is not economically feasible, this study identified a subset of cows based on SCC values that would identify up to 79% of cases. An alternative approach would be to use PathoProof on pooled DHI samples or bulk milk tanks samples, as shown to be effective in other studies.⁵

Using PCR testing with DHI samples is extremely convenient and can be integrated with routine somatic cell count (SCC) monitoring” CanWest DHI.⁶

Conclusions

Use of PathoProof Major Assays to screen preserved DHI samples offers a valuable tool in the early identification of subclinical mastitis. Additionally as PathoProof kits also test for highly contagious pathogens like *Mycoplasma bovis*, DHI screening also offers proactive early detection thus reducing the spread of mastitis throughout the herd, offering significant benefits to the overall health and productivity of the herd, as well as reducing economic losses.

About PathoProof PCR kits

- Identifies bacterial DNA so is 100% specific
- No "mixed growth" results, all bacteria detected can be reported
- Rapid results in three to four hours without the need for culturing
- Can be used with raw and preserved DHI samples
- Scalable products in both manual and automated formats
- Kits available that will detect the pathogens that cause >95% of all mastitis and subclinical mastitis cases
- Rapidly identifies *Mycoplasma bovis* in only four hours



For more information about PathoProof products or for details of DHI labs that offer PathoProof testing in your area please contact your local Thermo Scientific sales office.



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