

# Providing Routine PCR Testing as Part of Official Milk Recording Options

Angela Dawn Coburn  
AgSource Cooperative Services  
Verona, Wisconsin, USA

## Introduction

Obtaining Somatic Cell Count (SCC) values from routine milk sample analysis is, historically, a primary reason U.S. dairy producers participate in official milk recording programs. With improved management of environmental conditions and general cow health, SCC levels have shown a steady decline over the past 4 years. Among AgSource herds, weighted average SCC for Holsteins have dropped from 263,000 in 2012 to 227,000 in 2016. The percent of cows with SCC over 200,000 SCC has dropped from 24.3% to 20.4%.

Although producers are improving udder health management and cases of mastitis are reduced, the use of antibiotic therapies in livestock is of growing concern and scrutiny. With the prospect of greater regulation of antibiotic use, new tools are required for producers to aid in more selective and specific treatment of cows for mastitis. Although SCC provides a good overall measure, it does not point at the specific pathogen causing the mastitis infection. With the introduction of Polymerase Chain Reaction (PCR) technology, in most cases strains of bacteria can be detected using a preserved milk sample.

## Research Trial

PCR testing services for herds on official milk recording was introduced in 2012. Compared to Europe, U.S. adoption of routine PCR testing using preserved milk samples has been slow. Cited reasons include costs, lack of options, and concern about milk sample carryover when using meters for sample collection. To address these concerns, a field study was conducted using individual cow and bulk tank milk samples. Basic elements of the research trial included the following:

- 1) Identify accuracy of bulk tank milk samples in identifying pathogens present in the herd
- 2) Utilize pre-screening criteria for SCC to limit the number of cows selected for routine PCR testing and compare pathogens of individual cow samples taken during routine milk meter sampling to pathogens found in bulk tank samples collected on the same day.
- 3) Identify pathogens present in bulk tank samples and customize the PCR test to identify those pathogens at the individual cow level.

## Materials and Methods

Somatic Cell Count data was collected using the Foss Fossomatic 5000 and Fossomatic FC cell counters while PCR analysis was conducted using the DNA Diagnostics Mastit-4 C and BDF kits using a Bio Rad DW40 deep well plate washer, and an Agilent Stratagene Mx3005P real time PCR Thermal Cycler. The Mastit-4 product comes in 6 different kits each allowing for the testing of 4 different combinations of pathogens. These kits can be combined to customize the diagnostics and minimize costs. The BDF kit examines samples for 11 pathogens and the  $\beta$ -Lactamase gene. Herds participating in the trial did not have to make any changes from the regular milk recording schedule. Milk samples from 11,241 cows were collected on 33 herds and

analyzed for milk components and SCC. Average herd size was 341 milking cows, with the largest herd milking 647 cows and the smallest 58 cows. In addition, each herd provided a bulk tank sample which was analyzed using the Mastit-4 BDF kit. Laboratory staff selected milk samples for PCR testing (PCRselect) for two groups of cows: individual cows where the milk sample had a SCC value of 200,000 or greater and samples collected from cows due to be dried within 30 days from test day, regardless of SCC., resulting in a total of 1,425 individual cow samples selected for PCR testing. 751 samples from 16 herds were run using the Mastit-4 C kit and the remaining from 17 herds using the Mastit-4 BDF kit.

### Results

The PCRselect group was comprised of 194 samples from fresh cows, 1,005 samples were from cows with a chronic infection, the remainder were pre-dry-off cows. There was significant variation between herds. The lowest herd had only 3% of samples pulled for PCR testing and the highest had 34% selected. Among the 33 bulk tank milk samples, there were 107 determinations of a specific pathogen presence, collectively. In 106 cases or 99%, one or more cows were identified matching the bulk tank pathogen. In one case where *Mycoplasma* species was identified in the bulk tank, there was no cow identified in the PCRselect group. To determine how close individual sample results mirror bulk tank results, bulk tank and individual cow PCR results were compared using data from 17 herds that had Mastit-4 BDF bulk tank and 5,078 individual cow results. Based on the screening process, 674 cows were included in the PCRselect group. of these, 134 cows had no pathogen detected. From the remaining 540 cows there were a cumulative total of 888 pathogens identified. From those 888 pathogens, 485 or 55% had a corresponding bulk tank determination. Individual pathogen results showed a lot of variation, for example *Staphylococcus aureus* was found in 85 PCRselect cows in 13 herds. The same pathogen was found in 8 of the 13 herds' (62%) bulk tank sample. In the case of *Mycoplasma* species 13 herds with 30 PCRselect cows were found, however only 3 herds representing 8 (27%) PCRselect cows had a matching bulk tank determination.

### Conclusions

Based on the results from the field trial, a bulk tank sample can point towards pathogens that have a significant presence in the herd. Furthermore, it is very likely that cows with high SCC counts have the greatest risk of a current mastitis infection and will be found carrying these same pathogens. Although the bulk tank sample can provide indication of pathogens with a high presence, during the field trial, pathogens were also found in the individual cow sample that were not found in the bulk tank sample. Missing certain pathogens can be a concern and can be addressed by testing a random sample of cows with SCC greater than 200,000 using the Mastit-4 BDF kit. The individual cow samples will identify additional pathogens not found in the bulk tank and also provide an indication of the level at which they are present in the herd. The field study conclusions support customization of the individual cow PCR test by selection of the Mastit-4 kit that provides the greatest coverage while reducing the overall cost of routine PCR testing.