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Differential somatic cell count—A novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs

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ABSTRACT

Somatic cell count (SCC) is used as key indicator in mastitis screening programs typically applied in the frame of Dairy Herd Improvement (DHI) testing programs. However, mastitis is still causing tremendous economic losses to the dairy industry. Hence, new biomarkers are needed that can be used for enhanced management of mastitis on dairy farms. Besides the determination of SCC, differentiation of cells has been described to be beneficial for a more definite description of the actual udder health status of dairy cows. The aim of this study was to develop a new method for rapid and simultaneous determination of SCC and a new parameter, differential somatic cell count (DSCC), in individual cow milk samples using flow cytometry. The method is sought to be applied in central milk testing laboratories, so that existing DHI infrastructures can be used. The DSCC represents the combined proportion of polymorphonuclear leukocytes (PMN) and lymphocytes expressed in percentage. The proportion of macrophages can be calculated by subtracting DSCC from 100%. Our research revealed increasing proportions of PMN, but decreasing proportions of macrophages as SCC increased. However, lymphocytes occurred fairly constantly with low proportions across the entire SCC range. Hence, the DSCC parameter reflects the antidromic trend of PMN and macrophages. Fluorescence microscopy was used to evaluate the specificity of the new Foss DSCC method in terms of DSCC and a high correlation was found. Apart from that, the accuracy of cell differentiation using the Foss DSCC method was confirmed in a cell sorting trial. Total SCC could be determined equally well using the new method as compared with existing methods. The new method was further proven to be robust toward a range of method and milk-sample-related factors. In an initial field trial, regular DHI samples of a local dairy herd were analyzed. The DSCC values occurred in a broad range from 34 to 79% in samples with <400,000 cells/mL. Higher DSCC values (53–89%) were found in samples with >400,000 cells/mL. In conclusion, the new Foss DSCC method allows reliable, repeatable, fast, robust, and accurate determination of both DSCC and SCC at low cost. This, in turn, provides more accurate information on the actual udder health status of dairy cows. The practical application of DSCC in the frame of DHI testing programs, however, needs further investigation.

Key words: mastitis, udder health, differential somatic cell count, somatic cell count

INTRODUCTION

Mastitis is one of the most prevalent and costly diseases in the dairy cattle industry worldwide, and adversely affects dairy cow welfare as well (Menzies et al., 1995). A major problem of the spread and persistence of mastitis within dairy herds is subclinical mastitis, a condition where the udder and the milk appear normal although the mammary gland is inflamed, infected, or both. Subclinically infected cows act as a reservoir for bacteria, resulting in an unnoticeable spread of mastitis to healthy herd mates (Halasa et al., 2007). The effective control of subclinical mastitis can clearly result in large economic profit for dairy farmers (van den Borne et al., 2010). However, the effectiveness of control is highly dependent on how fast these cows are detected, and hence the efficacy of the udder health monitoring program (van den Borne et al., 2010).

Somatic cell counts in milk provide an indication of the inflammatory response in the mammary gland and hence a proxy for measuring IMI and milk quality at quarter, cow, herd, and population levels (Schukken et al., 2003). The optimal SCC cutoff point to distinguish between infected and uninfected at the individual cow level has been established at 200,000 cells/mL (IDF, 2013). Regular SCC testing and associated udder health monitoring programs have a substantial positive influence on single animals as well as the entire herd

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(Barkema et al., 1998). Nevertheless, a major need for new biomarkers that are specific for mastitis and easy to detect at an early stage of the disease has been described (Viguier et al., 2009).

Somatic cell count is a robust quantitative measurement, but does not divide the cells present in milk, which are mainly lymphocytes, macrophages, and PMN, into different cell types (Kehrli and Shuster, 1994; Rivas et al., 2001). These immune cells play an important role in inflammatory responses within the mammary gland (Paape et al., 1979; Sordillo and Nickerson, 1988). Lymphocytes regulate the induction and suppression of immune responses (Nickerson, 1989; Sordillo et al., 1997). Macrophages are active phagocytic cells capable of ingesting bacteria, cellular debris, and accumulated milk components (Sordillo et al., 1997). Moreover, they recognize invading pathogens and initiate an immune response (i.e., rapid recruitment of PMN into the mammary gland; Paape et al., 2002; Oviedo-Boyso et al., 2007). The main task of PMN is to defend against invading bacteria at the beginning of an acute inflammatory process (Paape et al., 1979; Oviedo-Boyso et al., 2007).

Due to the specific functions of the individual cell populations, the distribution of leukocytes differs between normal milk and mastitic milk (Nickerson, 1989). Specifically, proportions of PMN can reach up to 95% in milk from cows with mastitis (Paape et al., 1979; Kehrli and Shuster, 1994). In the uninfected mammary gland, however, SCC is low and consists predominantly of macrophages and lymphocytes (Schwarz et al., 2011a,b; Pilla et al., 2012). Nevertheless, recent studies investigating the composition of milk cells in the range <100,000 cells/mL interestingly revealed evidence for inflammatory reactions based on elevated proportions of PMN (Schwarz et al., 2011a,b; Pilla et al., 2012, 2013). This indicated a correlation between elevated proportions of PMN and IMI or at least an active immune response (i.e., triggered by pathogens). Hence, in addition to SCC, determination of proportions of individual immune cell populations in milk is beneficial for describing the actual udder health status of dairy cows in more (Pillai et al., 2001; Rivas et al., 2001; Pilla et al., 2013). So far, various research groups around the world have developed methods for cell differentiation. However, no routine method is available yet that could be applied in high-throughput milk analyzers that would typically be used in central milk testing (**CMT**) laboratories.

The objective of this study was to describe and present the development of a new method for simultaneous measurement of a new parameter called differential somatic cell count (**DSCC**) and SCC.

MATERIALS AND METHODS

Dairy Farms

Milk samples used in this study originated mainly from 3 dairy farms. Farms A and B were located in North Zealand, Denmark, whereas farm C was situated in the central part of Jutland, Denmark. In farms A to C, between 120 and 270 dairy cows of the breed Holstein-Frisian were housed in pen barns and milked twice per day in milking parlors, except farm C which used milking robots (DeLaval, Tumba, Sweden). A TMR mainly consisting of grass and maize silage, rape grist, and cereals was fed on all of the dairy farms. The average herd milk yields ranged between 10,500 and 12,500 kg/yr (3.79–3.91% fat, 3.33–3.41% protein). The average number of parity and DIM varied from 1.8 to 2.1 and 165 to 198, respectively. Bulk tank milk SCC ranged between 130,000 and 220,000 cells/mL.

Types of Milk Samples Used

Routinely Available Cow-Composite Samples. Samples used for method development originated from dairy farm A (120 dairy cows) and were received on a weekly basis. Briefly, freshly collected individual cow milk samples, obtained with milk meters (Tru-Test, Auckland, New Zealand), were available from 20 cows (30 to 200 DIM and parity 1 to 4) every Monday morning (d 0). Subsamples of each individual cow milk sample were preserved with Broad Spectrum Micro tabs (Advanced Instruments Inc., Norwood, MA) on d 0 to a final concentration of bronopol and natamycin of 0.27 and 0.01 mg/mL of milk, respectively. These samples were used for experiments within a maximum period of 4 d.

DHI Samples. Routinely collected DHI samples (bronopol-preserved) from farms A to C were available for various investigations upon request. Samples were available from all lactating animals. These samples were used for different experiments within a maximum period of 4 d after collection.

Routine DHI Samples. In total, 655 routinely collected bronopol-preserved samples from CMT laboratories in Canada, Denmark, France, and New Zealand were received. Due to the lengthy transportation in some cases, the age of these samples was between 3 and 5 d on arrival. Samples were processed within 1 d upon arrival.

Reference Milk Samples. Commercial reference materials were available from Cecalait (Poligny, France), Hüfner (Hergatz, Germany), Max Rubner-Institut (Kiel, Germany), and Eastern Laboratory