Differential Somatic Cell Count with the Fossomatic 7 DC - a novel parameter

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Dedicated Analytical Solutions

Somatic cell count (SCC) represents the total number of cells occurring in milk and is the key indicator used for detection and management of mastitis in dairy herds around the globe. Although the udder health situation of dairy cows has been improved significantly over the last 40 years (e.g. Sampimon et al., 2005), mastitis still causes tremendous economic losses to the dairy industry (e.g. Seegers et al., 2003). The challenge is that mastitis is a very complex disease influenced by multiple factors such as the environment and the keeping and feeding of cows. In addition, the pathogens that cause mastitis are constantly evolving and this requires altered mas¬titis management programmes. Apart from that, dairy cows have changed and are producing evidently more milk today, which requires a different type of dairy cow management.

Given that cells in milk mainly consist of lymphocytes, macrophages, and polymorphonuclear neutrophils (PMN), researchers found out that besides the determination of the total SCC, differentiation of cells is beneficial for a more precise description of the actual udder health status of dairy cows (Pillai et al., 2001; Rivas et al., 2001; Pilla et al., 2013). The three main cell populations occurring in milk play a vital role in inflammatory responses within the mammary gland (Sordillo et al., 1997; Oviedo-Boyso et al., 2007). Briefly, lymphocytes regulate the induction and suppression of immune responses. Macrophages recognize invading mastitis pathogens and initiate the immune response by starting a massive influx of PMN. Beyond that, macrophages ingest bacteria, cellular debris, and accumulated milk components and carry out tissue repair. PMN cells defend against invading bacteria at the beginning of mastitis.

During mastitis both the total SCC and composition of the cells change evidently. Milk from healthy mammary glands is low in SCC that consist mainly of macrophages and lymphocytes (Lee et al., 1980; Schwarz et al., 2011a, b; Pilla et al., 2012). However, SCC increases significantly and PMN are the predominant milk cell population in the presence of infection (Paape et al., 2002).

The key elements of FOSS's new technology

FOSS has developed a new patented method for simultaneous determination of both SCC and a new parameter – Differential Somatic Cell Count (DSCC). The newly developed Fossomatic 7 DC determines the two parameters at measurement throughputs of up to 600 samples per hour. For this, the basic measuring principle of the current Fossomatic model has been rethought and extended. As a result, the key elements of the new Fossomatic for measuring SCC and DSCC are: *new chemistry, new incubation unit and new measuring module.*

The *new chemistry* is based on the fluorescence dye acridine orange (Fossomatic DC dye). Characteristics of milk cells are well-described in the literature (e.g. Lee et al., 1980) and utilised when applying acridine orange because cell nuclei as well as compartments are stained.

The *new incubation* unit is added to the instrument to ensure proper and standardised incubation of the cells with Fossomatic DC reagents. Each samples remains for exactly one minute at 40 C in the incubation unit.

The *new measuring module* is the heart of the Fossomatic 7 DC. While a long-lasting LED laser is used as light source, three detectors receive fluorescence and morphology information of each cell passing through the laser. Fluorescence signals are measured

in two fluorescence channels, FL1 and FL2. Morphology characteristics, specifically the granularity, of each cell are captured on the side scatter (SSC) detector.

The combination of the described key elements enables the Fossomatic 7 DC to measure SCC and DSCC simultaneously as follows: A defined volume of a milk sample is analysed to be able to calculated the number of cells per millilitre. Somatic cells can be identified, separated from the milk background (e.g. bacteria, fat globules, etc.) in the FL1 vs. FL2 dot plot (Figure 1 A). In addition, SSC information is used in the background for a more precise determination of SCC. The somatic cells identified in figure 1 A will be further investigated in a second FL1 vs. FL2 dot plot with different scales to differentiate between macrophages and a group containing lymphocytes and PMN (Figure 1 B). The DSCC parameter represents the group of lymphocytes and PMN in percent. The percentage of macrophages is 100 – DSCC.

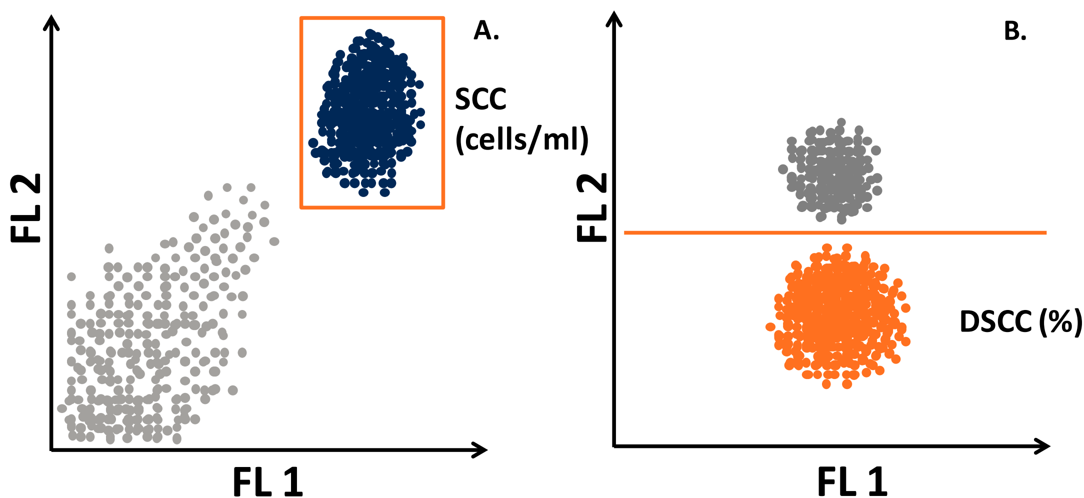

Figure 1. Schematic diagram of the measuring principle of the new Fossomatic 7 DC. A: Separation between background (grey dots) and milk cell (dark blue dots) fluorescence information and determination of SCC (orange box). B: Only somatic cells from figure 1 A are used for determination of DSCC by differentiating macrophages (grey dots) from lymphocytes and PMN (orange dots). FL = fluorescence emission

Properties of the new method

The objective of developing a new method applied for the current DHI samples was to meet high requirements on specificity, accuracy, reliability, repeatability, robustness, as well as allowing high sample throughput.

In the absence of a reference method for DSCC, FOSS developed an internal reference method using fluorescence microscopy. Comparing DSCC results of 113 routinely available DHI samples analysed with the DSCC method on a flow cytometer and a fluorescence microscope revealed a high correlation coefficient of r = 0.8391 (Figure 2).

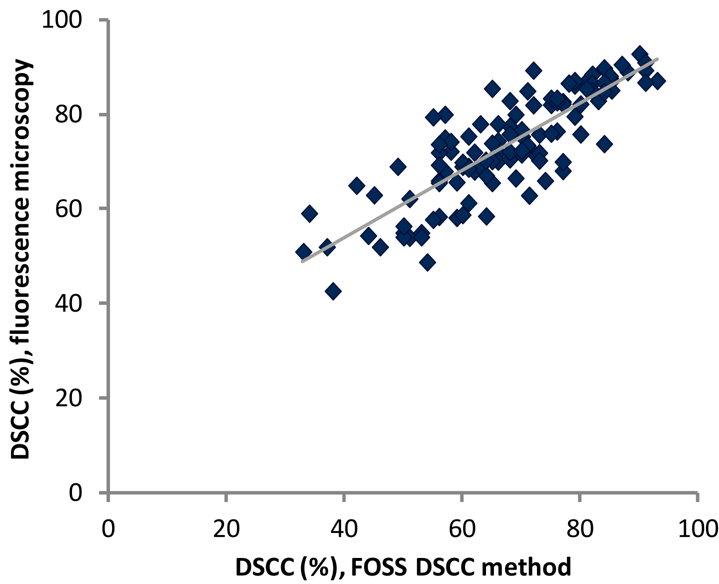

Figure 2. DSCC results of 113 routinely available DHI samples analysed with the FOSS DSCC method on both a flow cytometer and fluorescence microscope.

In a next step, the accuracy of the new method was tested in a cell sorting trial on routinely available DHI samples. Somatic cells were sorted from the two different groups (Figure 1 B), respectively, and differentiated using the fluorescence microscope method subsequently. A high proportion of 83% of cells in the DSCC gate (orange dots, Figure 1 B) could be confirmed to actually be lymphocytes or PMN under the microscope.

While a correct differentiation of the cells was confirmed in above experiments, the correct determination of SCC was proven comparing SCC results from 640 routinely available DHI samples measured with the new FOSS DSCC method and the Fossomatic FC. This experiment revealed that all cells occurring in milk were stained and considered correctly (Figure 3, r = 0.9937).

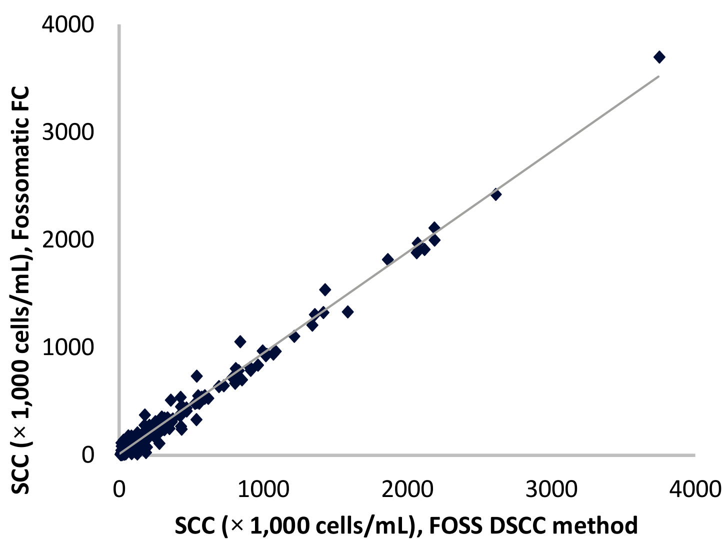

Figure 3. SCC results for 640 routinely available DHI samples analysed with the new FOSS DSCC method and on the Fossomatic FC.

The method repeatability was determined by analysis of routinely available cow-composite samples, run in five replicas, and found to be good (standard deviation of 2%).

The robustness of the method was tested towards a range of sample-specific factors. To obtain reliable DSCC values, the age of the milk samples should not exceed four days. Bronopol has been used as preservative during development of the methods. However, reduced stability of DSCC was observed in samples preserved with azidiol.

Given that only a defined volume of 50 microliter of milk gets tested per sample, the number of cells available to determine DSCC clearly depends on the SCC. To obtain sufficient statistics for the output (SCC and DSCC), the performance range for the method was defined to be 50,000 to 1,500,000 cells/ml.

In conclusion, the new Fossomatic 7 DC is the first instrument that enables standardised and routine analysis of two parameters, SCC and DSCC, simultaneously using untreated individual cow milk samples (i.e. DHI samples) at low cost. The new instrument allows specific, reliable, and repeatable determination of SCC and DSCC at a speed of up to 600 samples per hour. Besides the well-established SCC, DSCC indicates the combined proportion of PMN and lymphocytes occurring in milk. Hence, a more detailed picture of the actual inflammatory status of the mammary gland can be provided. In turn, this opens up the possibility to develop new tools for enhanced mastitis management.

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