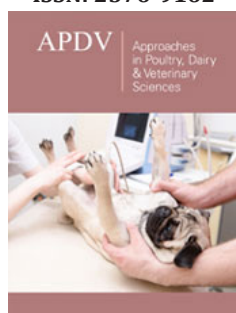


Comparison of Fluorescent Image Technique for Somatic Cell Counting and Traditional Microscopic Method

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ISSN: 2576-9162



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Submission:  August 05, 2019

Published:  August 12, 2019

Volume 6 - Issue 4

How to cite this article: Becheva Z, Ivanov Y, Godjevargova T. Comparison of Fluorescent Image Technique for Somatic Cell Counting and Traditional Microscopic Method. *Appro Poultry Dairy & Vet Sci* 6(4). APDV.000642.2019. DOI: [10.31031/APDV.2019.06.000642](https://doi.org/10.31031/APDV.2019.06.000642)

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Opinion

Somatic cell count (SCC) in milk is an important characteristic associated with udder health. Somatic cells are mainly leucocytes that protect the animal glands, and a smaller percentage of epithelial cells [1,2]. There are variety methods for somatic cell counting in milk. The standard technique, used all over the world, is the light microscopic method [3]. The probability of errors is high due to subjectivity of the counting. The operator must be well trained and properly educated. Therefore, automatic cell counters, in particular fluorescent image cytometers, are preferable. They save time and allow many samples to be processed in a short time. Moreover, there is lower risk for subjective counting and the operators do not need to have special and complex training or education [4]. Also, the fluorescence detection is more selective compared to methylene blue-based microscopic method [5]. There are several companies that offer fluorescent image cytometers: Lactoscan SCC (Milkotronic Ltd, Bulgaria); ADAM-SCC (NanoEnTech Inc, South Korea), NucleoCounter SCC-100 (Chemometec, Denmark) DeLaval Cell Counter (DeLaval Ltd, New Zealand).

In the present experiment cow milk samples ($n = 108$) with variety SCC were analyzed. Two different counting techniques were used and compared: a light microscope (reference method) and a fluorescent image cytometer (Lactoscan SCC). The standard microscopic method uses Methylene blue. It is cheap. But one of the disadvantages is the need of trained operator (for example veterinarian). Main problems of this method were artifacts from the dye or parts of cells, and in some cases cell aggregation. So the counting was time-consuming and labor-intensive. These analysis points were observed from other authors too [6]. The mentioned disadvantages were avoided when using the automatic cell counter Lactoscan SCC. The somatic cells in milk samples were stained with fluorescent dye (Sofia Green) which entered in cell nucleus and they turned intensive green. Main privileges of the fluorescent dye Sofia Green are the weak background and the high fluorescence intensity after DNA binding [7]. It was easier to count fluorescent green cells in a black field using the auto-counter than the blue cells in a bright field using the light microscope (Figure 1).

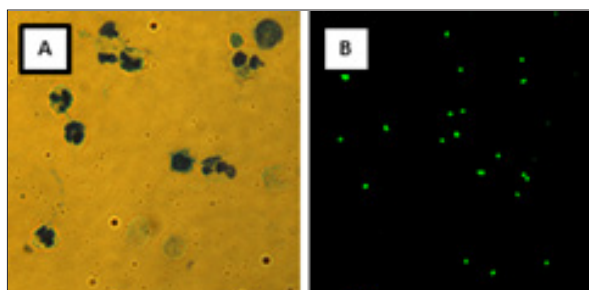


Figure 1: Images of milk somatic cells by a direct microscopic method – Olympus BX51 × 40 magnification (A) and by a fluorescent somatic cell counter – Lactoscan SCC (B).

Moreover, the Lactoscan SCC has an algorithm and counts the cells itself in a few seconds to 1 min, and displays the result. For example, ten samples were counted with Lactoscan SCC only for 10 min, but microscopic analysis was more than 60 min for the same quantity of samples. Therefore, it was a suitable method for the analysis of many samples at a time. That is a great advantage for research works or for farms with a very large number of animals. Furthermore, the fluorescent image technique is more accurate and reproducible. Convincing demonstration of the statements is a lot of sample analysis and statistical processing of the results.

Total number of the analyzed milk samples was 108. The range of SCC in the samples was wide. The lowest SCC was 0.9×10^5 cells/mL, and the highest SCC was 5.4×10^6 cells/mL. All of the samples were analyzed with both devices: the light microscope (Olympus BX51) and the fluorescent image cytometer (Lactoscan SCC). For the statistical processing, each sample was measured 6 times, and the mean value and standard deviation from the mean value were calculated. After that, the coefficient of variation (CV) was determined for each sample [8]. For better presentation of the results, milk samples were summarized into five classes due to their SCC (Table 1). The average values of CV in each class obtained from the light microscope were higher, compared with the values from Lactoscan SCC. The average CV of the counted cells with the microscopic method in the first class ($<2 \times 10^5$ cells/mL) was high (8.52%) due to fewer cells in the field of view. Also, the CVs of the samples that had more than 1.0×10^6 cells/mL increased significantly due to the big crowd of cells in the field of view (CV 18.30%). That was avoided while using the automatic cell counter. The CVs were much lower than those with the microscopic method. Furthermore, increasing of the SCC in the sample showed decreasing of the CV. It was found that there was a good correlation between the methods (correlation coefficient 0.96). Therefore, fluorescent image cytometers can be successfully used for SCC analysis. Consequently, automatic cell counting is preferable technique for SCC of a large number of samples with a wide range of SCC, and especially for SCC in mastitic milks, where the quantity of somatic cells is high.

Table 1: Comparison of SCC in fresh cow milk obtained by a light microscope and by a fluorescent somatic cell counter – Lactoscan SCC.

SCC Class, $\times 10^5$ Cells/mL	n	Average CV in the Class, % (Light Microscope)	Average CV in the Class, % (Lactoscan SCC)
<2	20	8.52	3.95
2 – 4	40	6.65	3.57
4 – 6	16	5.26	3.32
6 – 8	12	9.41	3.07
8 – 10	11	15.02	1.95
>10	9	18.30	3.50

SCC = somatic cell count; CV = coefficient of variation.

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