Somatic cell counting using the LUNA-FX7™ Automated Cell Counter

INTRODUCTION

Somatic cell count (SCC) is a well-established parameter used in the dairy industry to manage udder health. An increase in SCC indicates intramammary gland infection (IMI) leading to mastitis. This condition can result in poor pregnancy rates and impact milk production. Hence, dairy farms use flow cytometry-based cell counters such as the FossoMatic. Although this approach is efficient for high-throughput analysis, there is a demand for inexpensive methods of enumerating smaller sized samples in the research field.

However, SCC determination in milk is challenging due to fat and protein debris. To overcome this issue, the *Somatic Cell Staining Solution* has been developed for uncomplicated and straightforward SCC determination with the LUNA-FX7™. Here, we show the ability of the LUNA-FX7™ Automated Cell Counter to efficiently count somatic cells in milk using *Somatic Cell Staining Solution*.

MATERIALS AND METHODS

Raw milk samples were obtained from POSTBIO, Inc. in high, medium, and low concentrations. Only low concentration had SCC below 2 x 10^5 cells/mL. Each milk sample was mixed with the *Somatic Cell Staining Solution* (Cat# F23101) at a 1:4 ratio. Cell counting was performed with the LUNA-FX7™ using 1-channel slides (Cat# L72011). A modified default protocol was used in the Fluorescence Cell Counting mode (Table 1).

STAINING PRINCIPLE

SCC determination in brightfield (BF) channel is not effective due to the fat and protein debris in milk (Figure 1A). Since these debris in milk have little to none fluorescence signal at the wavelengths that can be detected by the LUNA-FX7™, SCC determination was conducted using *Somatic Cell Staining Solution* contains propidium iodide (PI). It also contains a detergent to induce cell lysis as PI is not able to permeate intact cells. Once cells are permeabilized, PI binds to DNA by intercalating between base pairs and its fluorescence is increased 20- to 30-fold upon binding. These allow for the accurate distinction of somatic cells from debris in milk. Accordingly, SCC can be determined without being interrupted by debris in milk.

RESULTS

The results from the LUNA-FX7™ were closely analyzed and inspected to confirm whether the PI labeled and identified somatic cells are accurately. The LUNA-FX7™ successfully distinguished somatic cells from debris in milk which can be seen in tagged images (Figure 1A). Moreover, SCC determination results from the LUNA-FX7™ were compared with the known concentrations from the flow-cytometry based cell counter. The results showed strong correlation between two different platforms (with the low concentration being below 2 x 10^5 cells/mL) (Figure 1B).

Table 1. *The optimize parameter settings for SCC in Fluorescence Cell Counting mode of the LUNA-FX7™*

Counting Mode	Fluorescence Cell Counting
GF exposure level	5
RF exposure level	5
Min. cell size	5
Max. cell size	30
Declumping sensitivity	5
Min. FL intensity	0
Min. roundness	3
Dilution factor	5

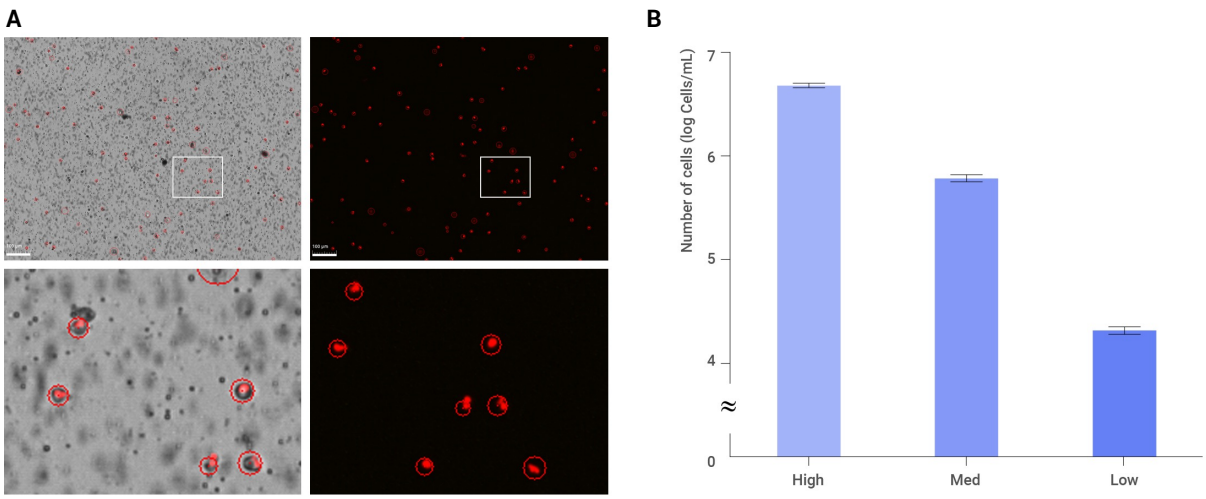

Figure 1. *(A) The image shows successfully stained and tagged somatic cells in milk samples using the LUNA-FX7™. (B) The bar graph shows strong correlation of SCC data obtained from the LUNA-FX7™ and the flow-cytometry based cell counter.*

CONCLUSION

We showed that LUNA-FX7™ automated cell counter could effectively count somatic cells using Somatic Cell Staining Solution. Moreover, the SCC results comparison between the LUNA-FX7™ and the flow cytometry-based cell counter showed a strong correlation to each other. Therefore, the LUNA-FX7™ automated cell counter is versatile enough to be used for many applications including SCC determination.

REFERENCES

Sharma, Neelesh, N. K. Singh, and M. S. Bhadwal. "Relationship of somatic cell count and mastitis: An overview." Asian-Australasian Journal of Animal Sciences 24.3 (2011): 429-438.