Somatic cell count and differentiation in raw milk using flow cytometry

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Introduction

For the milk producers, mastitis is probably one of the main problems. Mastitis is a common inflammation of the cows udder that makes the milk unfit for human consumption. Apart from the veterinary issues that it generates, the economic loss is significant. Actually, mastitis is detected by the total number of cells in the milk (above 350'000 cells per milliliter). But this does not give any indication about the evolution stage of the disease. Indeed, an acute mastitis is defined by a large proportion of polymorphonuclear cells (PMN) in the milk, while a chronic or recovering mastitis shows more antigen presenting cells (APC). This is the reason why Agroscope established a new method that allows to count and differentiate those two cell group without centrifugation steps that may enrich some cell type due to the complexity of the milk matrix.

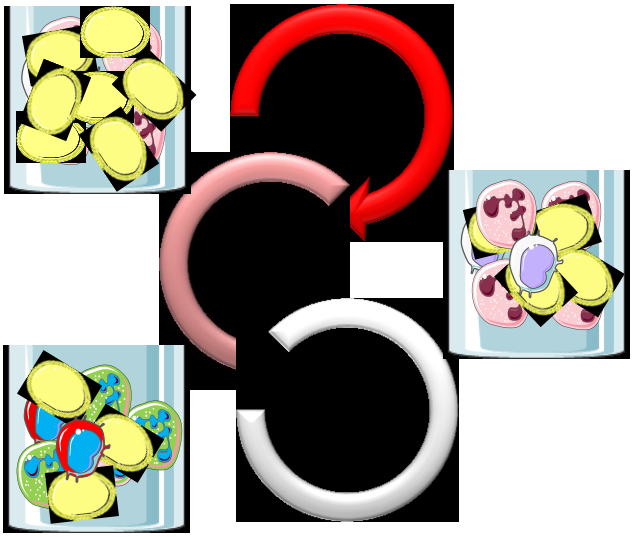

Figure 1 :
Scheme of the clearing-staining process:
In raw milk, the amount of MFG is so high that analysis is not possible (a). By adding our Clearing solution, part of the MFG's are removed (b). The cells are stained using labelled antibody and DNA staining directly in milk (c).

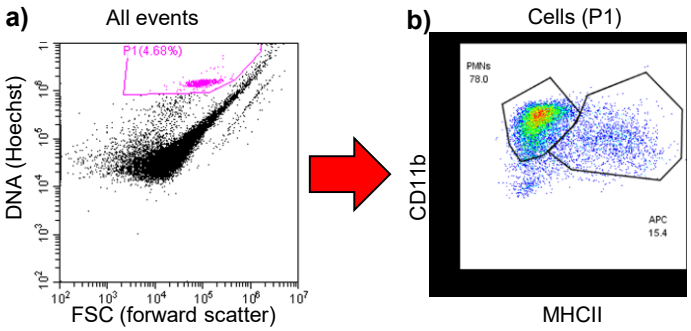

Figure 3 :
a) Cell count (P1) based on DNA staining (Hoechst channel). Negative events are remaining MFG after clearing.
b) In gate 1 (P1) only, CD11b and MHCII are compared, APC are expressing MHCII, their population is shifted to the right.

Method

Milk is mainly composed of milk fat globules (MFG) with a similar size and membrane structure as somatic cells. To allow the analysis of cells, a clearing step is performed, capable of inducing changes in the critical micellar concentration (CMC) (Figure 1). Total cells counts are performed based on DNA stain (Hoechst , blue). Its ability to enter both live and dead cells was determinant, especially for a method that should be used on fresh milk as well as with milk containing preservatives. To differentiate the PMN cells from APC, the surface markers CD11b and MHCII were selected. Both cell types express CD11b (green) but only APC are expressing MHCII (red) (Fig. 2 and 3).

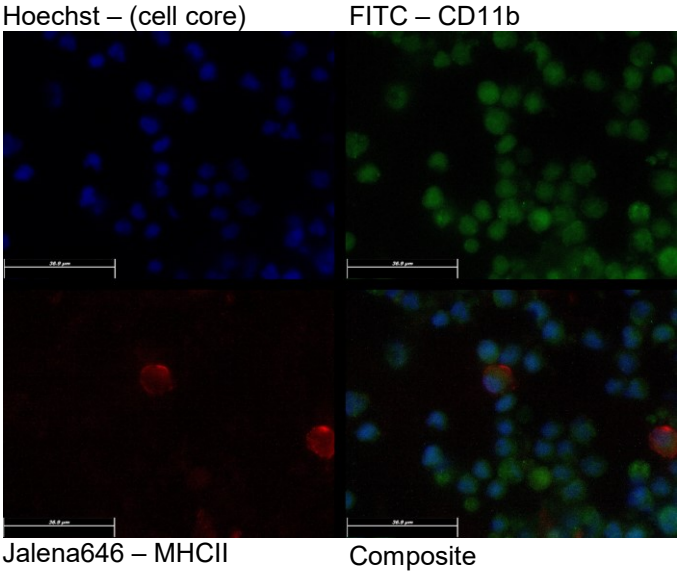

Figure 2 :
The Flow Cytometry method was confirmed by using Immunofluorescence microscopy (IFM). This method is semi-quantitative and allowed the qualitative confirmation that the antibodies are indeed specific for the two cell types.

Summary
Until now precise somatic cell counts in raw milk are performed on dedicated automates and the differentiation of the different cell populations was only performed at a research scale using several washing steps to avoid MFG to interfere with the flow cytometry measurements. Those washing steps led to the enrichment of some cell populations due to the complex matrix milk. Indeed, as PMNs are engulfing MFGs, their relative density is changing and they can be floating in the cream or sinking in the pellet.
The newly developed method is a powerful tool especially for the research on mastitis. With only one in situ run, it is now possible to count precisely the total number of somatic cells in the milk but also give a precise value for two cells populations without any loss or selection of cells.