



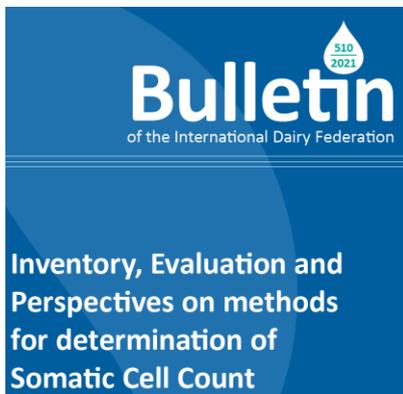
# IDF/ICAR Project on Reference System for Somatic Cell Counting in Milk

## Newsletter 8 – May 2021



### Global status on implementation of the new primary SCC reference material

Read all about the new primary SCC reference material ("EC JRC CRM") including a status report on its implementation around the world.

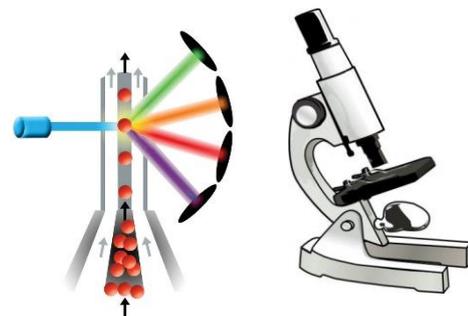


### New: Bulletin of the IDF n°510 on Technologies for somatic cell counting and their performance

A [new IDF Bulletin](#) describing different methods and technologies available for somatic cell counting and their performance including recommendations for criteria of a new reference method for somatic cell counting was just published and is summarised here.

### On-going activities to investigate candidates for a new reference method for somatic cell counting

Summaries on first results and the current status of two projects investigating possible candidates for a new reference method for somatic cell counting are provided by the respective project groups.



### Outlook on future work within this joint IDF/ICAR project

The recent change in project leadership is briefly described and the new project leaders introduce themselves and their ambitions regarding the project.





Substantial achievements have been made in the world of somatic cell counting and will be described in more detail in this 8<sup>th</sup> Newsletter on the joint IDF/ICAR Project on a Reference System for Somatic Cell Counting in Milk (i.e. IDF Action Team S09). The launch of the new primary reference material for somatic cell counting (SCC) is clearly a milestone in the project's aim to create better equivalence with somatic cell counting in milk worldwide. A global overview on the status of the implementation of the material including two best practise cases will be described here. Several different methods and technologies for somatic cell counting have been investigated and tested lately and the results were just published in an IDF Bulletin, which will be briefly summarised here. This work actually led to exciting follow-up activities bringing fundamental insights for the development of an improved reference method – read all about it below. Last, Harrie van den Bijgaart, who was heading this project since its beginning in 2010, decided to hand over. All project members thanked Harrie for his outstanding work and great leadership. Vesela Tzeneva and Daniel Schwarz were elected to lead the project moving forward as a team. We will share our short- and long-term ambitions for the project further below.

### *Vesela Tzeneva and Daniel Schwarz*

#### About Vesela Tzeneva

Since 2013 I operate within IDF and am actively involved in the work of several Action Team within the Standing Committee on Statistics and Automation. In 2006, I obtained a PhD degree in Microbiology at Wageningen University and during my career I gained knowledge on food safety and quality and developed my skills in project management to a senior level. Last 10 years I was part of the Innovation and Business Development team at Qlip where I became experienced with milk and dairy. Currently, I am working as an expert and senior project manager at NIZO in the Netherlands. There I am responsible for maintaining up-to-date knowledge about microbiological hygiene as well as residues and chemical contaminants in milk and milk products on for the interest of the Dutch dairy sector.



#### About Daniel Schwarz

I grew up on a dairy farm in Germany and have had an interest in dairy cows and milk production since my early childhood. After graduation as an Animal Scientist and completion of a PhD in the field of bovine mastitis at the University of Goettingen, Germany, I started working at FOSS in 2013. In my role as Dairy Farming Senior Specialist, I am focused on developing tools that allow to improve dairy herd management and milk quality through milk analysis and data generated therefrom. My involvement with IDF started in 2014 and I have been part of several Action Teams since.



## Global status on implementation of the new primary SCC reference material

By Vesela Tzeneva (NIZO, NL) and Daniel Schwarz (FOSS, DK)

The new primary SCC reference material<sup>1</sup> is available since spring 2020 and can be ordered [here](#). IDF and ICAR conducted a webinar entitled “Development and application of a certified reference material for somatic cell counting in milk” in December 2020 and more than 150 participants confirmed high interest in the SCC reference material. A recorded version of the full webinar and pdf-copies of the presentations are available [here](#). Furthermore, the IDF Action Team S09 in collaboration with the ICAR Milk Analysis Subcommittee developed a guideline on the application of the new SCC certified reference material. This guideline was recently published as “Bulletin of the IDF N° 508/ 2021 and is available for free [here](#).”



We conducted a small survey to learn more about the actual status on the implementation of the new SCC certified reference material and observed that different countries are mainly in four different phases of the implementation:

- 1) Material tested and adopted - in Lithuania and Switzerland.
- 2) Material not tested so far – In countries such as China and Chile the new material has not yet been tested so far. Among other reasons, the shipment of the SCC reference material was not possible due to COVID 19 restrictions.
- 3) Material tested, no need for adjustment of SCC level – The new primary SCC reference material has been tested and it was observed that the current SCC level and the SCC level of the primary reference material are well in alignment. Thus, it was concluded that no adjustment of SCC counting levels is needed. Nevertheless, the new primary SCC material is considered a valuable product because it opens up the possibility to monitor SCC counting levels on a regular basis (e.g. once every quarter) and verify correctness. This situation applies to many countries around the world, e.g. Denmark, Germany, Italy, Japan, New Zealand, UK, USA. A dialogue on using the new primary SCC reference material as an official and mandatory material has been initiated with the respective authorities in Japan and USA. Below a case report for this implementation phase coming from Germany.
- 4) Material tested, need for adjustment of SCC level – The test of the new primary SCC reference material revealed that the current SCC level requires some adjustment. Following this finding, the respective laboratories started to initiate the dialogue with their stakeholders and agreed upon a strategy for transition of SCC levels. Regular application of the new SCC reference material is seen as highly valuable in the transition period and thereafter. This situation applies to, e.g. Canada, France, Israel, and the Netherlands and is further described in below case report from the Netherlands.

<sup>1</sup>Official name: EC JRC CRM® ERM-BD001



It is common practise in most milk testing laboratories around the globe to work with secondary SCC reference material. In this context, we would like to recommend that secondary SCC reference materials are to be checked for alignment with the new primary reference material and that users of secondary reference materials ask their providers for such alignment checks.

**Case report from Germany**

By Christian Baumgartner (mpr Bayern, GER) and Silvia Orlandini (mpr Bayern, GER, and ICAR)



In September 2020 Milchprüfing Bayern e.V. (DE) collaborated with ICAR to characterize the ICAR proficiency test materials. With regard to somatic cell count (SCC) the goal was to assign target values traceable to the first internationally available certified reference material for somatic cell counting produced by the Joint Research Centre of the European Commission (EC JRC CRM SCC) (1). Two fluoro-optoelectronic instruments, routinely calibrated with secondary reference material (produced by QSE GmbH), were used for this task. The samples were analyzed before and after calibration adjustment, using five different levels of concentration of the EC JRC CRM SCC samples. Finally, “mpr anchor” values were calculated for the QSE samples, for the pilot samples (KM) and ICAR materials following the approach reported in the publication of Kuselman et al. (2).

The graph below (Fig. 1) shows the mean results of mpr instruments for the samples tested and reference values traceable with EC JRC CRM SCC. The samples are ordered by concentration from the left to the right.

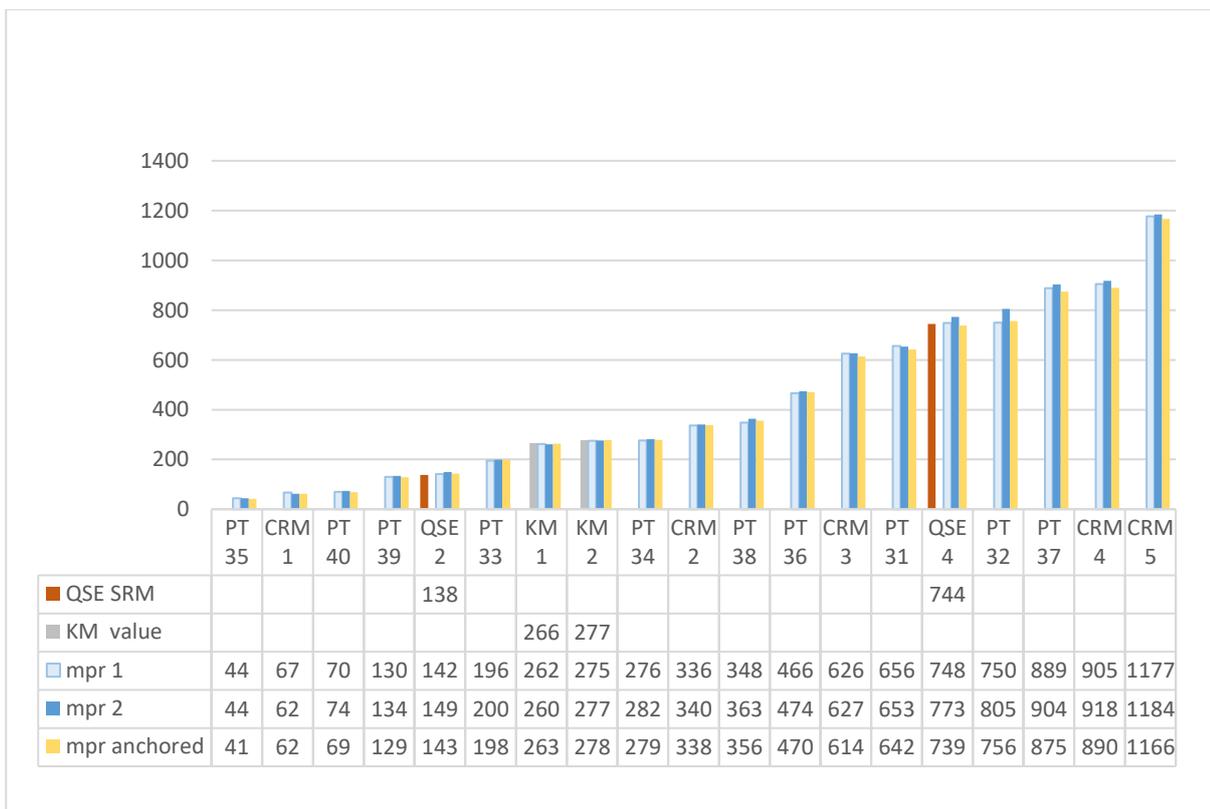


Fig. 1 – Somatic Cell Count (SCC) for different materials obtained by two instruments before calibration adjustment with EC JRC CRM SCC; mean values after calibration adjustment are reported as “mpr anchored” (yellow bar).

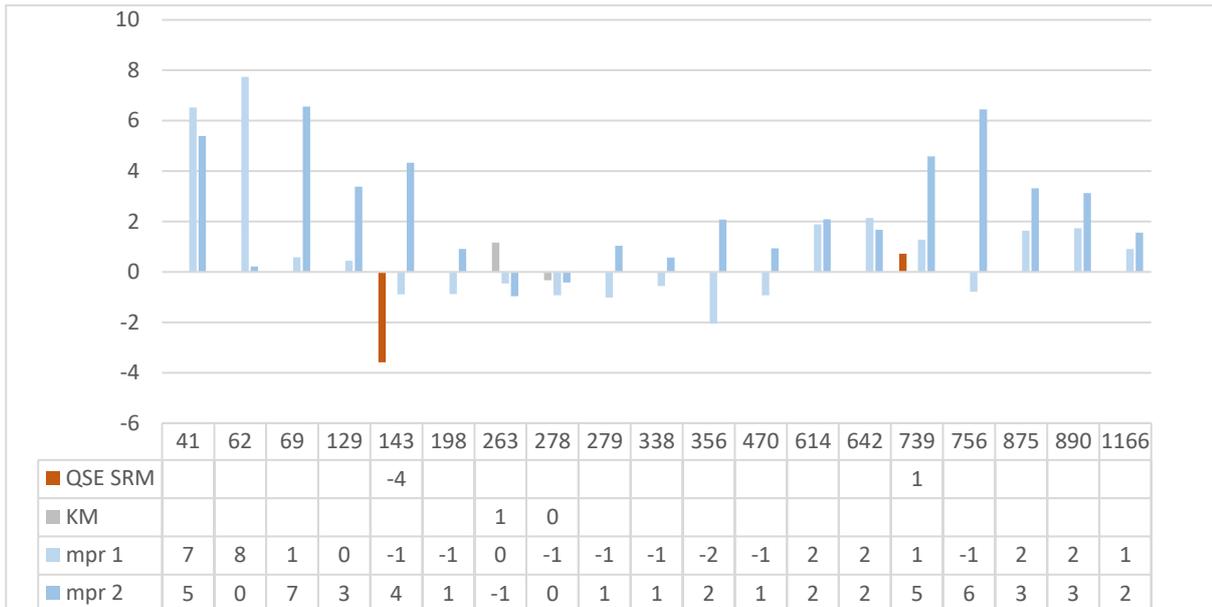


Fig. 2 – Relative bias (%) for different materials caused by calibration adjustment with EC JRC CRM SCC (ordered by SCC values “mpr anchored”)

Figure 2 shows the bias caused by calibration adjustment for the different materials tested. Overall, the mean bias of mpr instruments caused by the calibration adjustment with EC JRC CRM SCC is 1% (mpr 1) and 2% (mpr 2). This small bias indirectly indicates that the secondary reference material used for routine calibration (QSE SRM) and pilot samples (KM) are properly aligned with the EC JRC CRM SCC as well.

The collaboration with ICAR in this characterisation exercise gave us the chance to test the draft IDF guidance how to apply the EC JRC CRM SCC (3) and to check our instruments’ SCC level compared to the certified reference material of JRC, which is regarded as worldwide anchor for SCC now.

From the data obtained and illustrated above we conclude that our eleven fluoro-opto electronic instruments at Milchprüfing Bayern and the QSE secondary reference material are well aligned with the certified reference material of JRC as all instruments are routinely calibrated with QSE secondary reference material. As we are participating in a German national proficiency testing (PT) scheme on a regular base, the good performance in terms of z-score obtained in those PTs could provide the information that also most instruments in Germany are aligned as well to the EC JRC CRM SCC. QSE is already characterizing the new batches traceable with the EC JRC CRM SCC. In order to provide robust evidence of metrological traceability, it is our intention to further apply the procedures of IDF Bulletin 508/2021 and collect and report the relevant data in the future.

Milchprüfing Bayern e.V. and German DHI Laboratories are linked to the EC JRC Certified Reference Material for Somatic Cell Counting

## Bibliography

- 1) Orlandini S. 2020. ICAR PT SCC- Traceability with SCC Certified Reference Material <https://www.icar.org/index.php/technical-bodies/sub-committees/milk-analysis-sub-committee-landing-page/>
- 2) Kuselman, I., Weisman, A. & Wegscheider, W. 2002. Traceable property values of in-house reference materials. *Accred Qual Assur* 7 p122-124.
- 3) van den Bijgaart, H., Orlandini S., Luginbühl W. 2021. IDF Bulletin 508/2021. Guidance and application of EC JRC Certified Reference Material for somatic cell counting in milk.

## Case report from the Netherlands



Harrie van den Bijgaart, Qlip: “In the Netherlands somatic cell counting in milk is conducted in the frame of milk payment testing and checking compliance with EU 853/2004 (1 laboratory), milk recording analysis (3 laboratories), animal health service (1 laboratory) and by an unknown number of veterinary practices and individual farmers. Throughout the years Qlip has used her in-house microscopic method conform ISO 13366-1|IDF 148-1 as reference for routine somatic cell counting with fluoro-opto-electronic instruments. In the autumn of 2020 Qlip made a comparison with the counting level resulting from the application of the EC JRC Certified Reference Materials. It appeared that some shift in counting level would result. Subsequently, Qlip has therefore sought contact with the competent Dutch authorities and the involved stakeholders in the field (dairy organisations, milk recording organisations, animal health service) to inform them about the intended reanchoring and the consequences for the counting level. In mutual consultation a plan for implementation and communication in the field was initiated. Thereby it was considered relevant to keep in alignment with reanchoring processes in neighbouring geographies and to leave farmers and other stakeholders some time to accommodate for the expected change in counting level.



Qlip is using the EC JRC CRM for method performance verification of the microscopic reference method and for assigning reference values to secondary reference materials that are used to verify, and where necessary to adjust, the calibration settings with routine methods. Since early 2021 the Qlip secondary reference materials for somatic cell counting are provided with two reference values, one still based on the in-house microscopic counting according to ISO 13366-1|IDF 148-1, the other one assigned according to the guidance in IDF Bulletin 508 (2021) and so fully traceable to the EC JRC CRMs. The Qlip materials are accompanied by an explanation on the background and guidance on the application of the provided reference values.”



## **New IDF Bulletin n°510 – Inventory, Evaluation and Perspectives on methods for determination of Somatic Cell Count**

*By Thomas Berger (Agroscope, CH) and Daniel Schwarz (FOSS, DK) (co-chairs of the former IDF Action Team S15)*

IDF Action Team S15 entitled “Improvement of the reference method for somatic cell counting” has been working on an inventory regarding methods for somatic cell counting, evaluated the performance of the different methods available, defined criteria on a new reference method for somatic cell counting, and developed recommendations on methods with the potential of becoming a new reference method. The work of AT S15 has been completed with the publication of the Bulletin paper and it has been decided within IDF that the AT S15 will be integrated into AT S09.

### Summary of the Bulletin paper

Somatic cell count (SCC) represents the total number of somatic cells in milk and is used as an indicator of udder health and milk quality worldwide. The parameter is used for regulatory purposes as well as for the management of animal health on farms. The current microscopic-based reference method for SCC has been described as tedious, cumbersome and challenging to work with. Hence, the objective of this work is to define the criteria for a reference method, map all available methods for determination of SCC and to recommend one or two candidates that could potentially be used as a new reference method. The criteria for a reference method defined in this work were that the method needs to be robust, highly reproducible, well-established in the field of cell counting, open and adjustable, and to measure the same as the current method but with better performance.

Numerous methods for the determination of SCC are available. They were described in detail and categorized as follows: 1) Microscopic counting (manual), 2) Microscopic counting (automated), 3) Flow cytometry (dedicated, open systems), 4) Flow cytometry (adjustable, open systems), 5) Image cytometry (fluorescence microscopy, dedicated, open systems), 6) Image cytometry (fluorescence microscopy, adjustable, open systems), 7) Impedance flow cytometry, and 8) Other, indirect methods.

To be able to compare the performance of the different methods, a feasibility study was performed. The results revealed that the current reference method is not optimally fit for purpose. The results further indicated that automated microscopy, open and adjustable variants of flow and image cytometry, respectively, are potential candidates for the reference method.

However, detailed protocols on how such methods could be used routinely for reference testing purposes do not yet exist. In a next step, such protocols need to be developed and subsequently proficiency and ring trial data are to be generated so that the actual performance of the methods can be determined.

[The IDF Bulletin can be ordered from the IDF online catalogue.](#)



## Agroscope

# Antoinette (DNA and Antibody Stained Total and Differential Somatic Cell counting in Milk using Flow Cytometry) project – Somatic cell count and differentiation in raw milk using flow cytometry

By Jérôme Widmer, Thomas Berger, Lotti Egger (Agroscope, CH)

### Introduction

For the milk producers, mastitis is probably one of the main problems. Mastitis is a common inflammation of the cows udder with negative consequences for milk production and milk quality. Apart from the veterinary issues that it generates, the economic loss is significant. Actually, mastitis is expressed by the total number of cells in the milk (above 350,000 cells/mL). 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, especially macrophages). This is the reason why Agroscope established a new method that allows to count and differentiate the two cell populations without centrifugation steps that may enrich some cell types due to the complexity of the milk matrix.

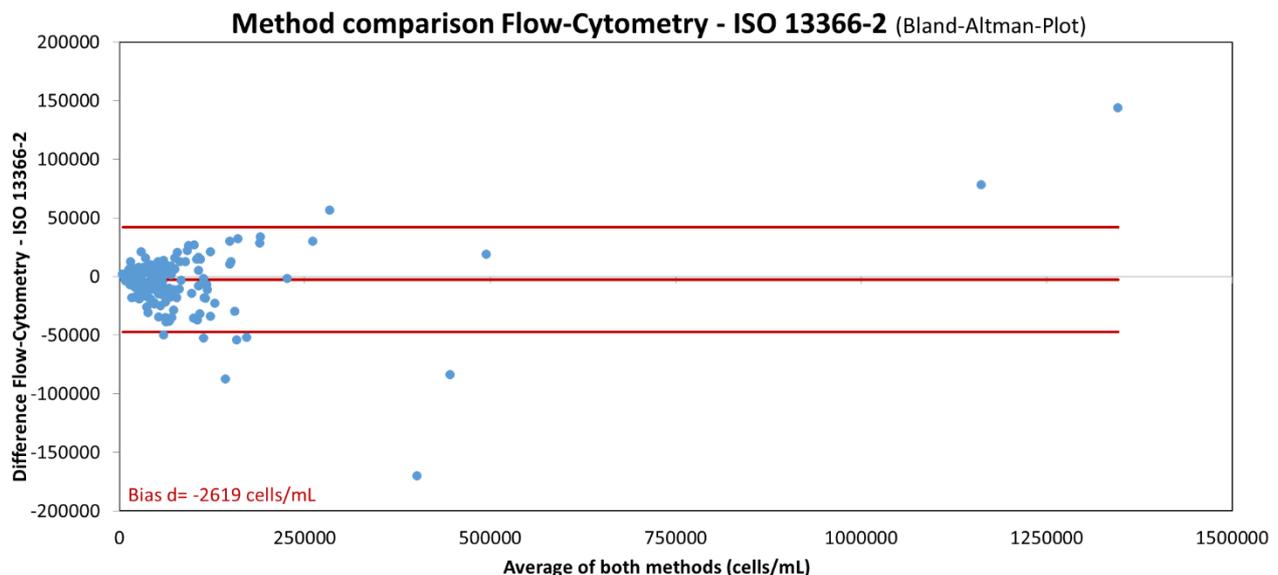


Fig. 1 – Method comparison between Flow cytometry (open method) and the ISO 13366-2|IDF 148-2 method for total cell counts, using a Bland-Altman graphic (Bland & Altman, 1986).

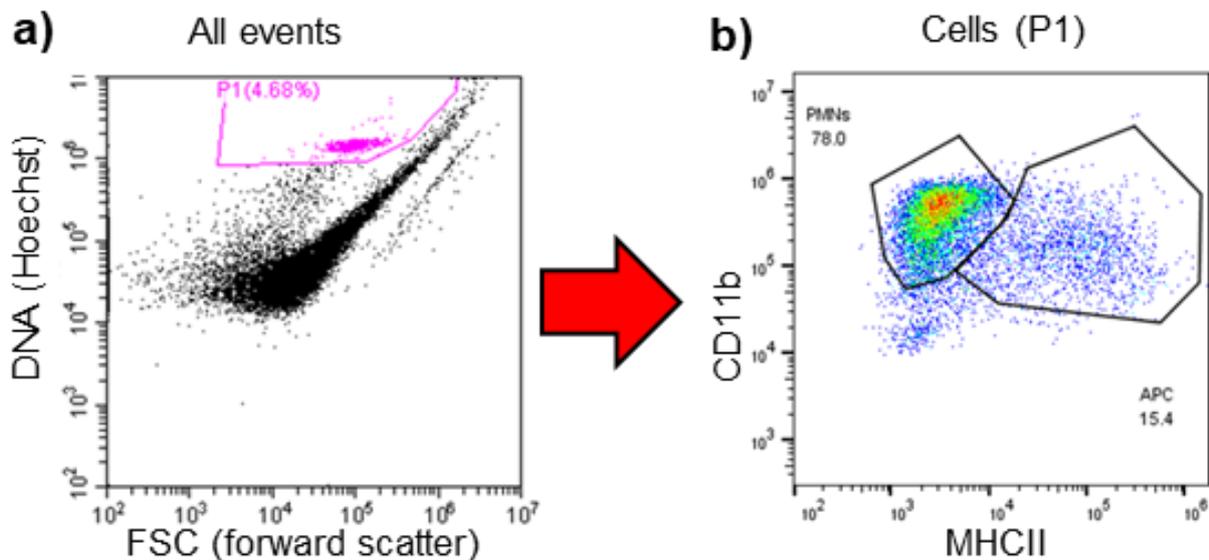


Fig. 2 – a) Total cell count (P1) based on DNA staining (Hoechst channel). Negative events are remaining MFG after clearing. b) In gate 1 (P1) only, CD11b and MHCII are compared, APC are expressing MHCII, their population is shifted to the right.

### Method

Milk contains milk fat globules (MFG) with a similar size distribution 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). Total cell counts are performed based on DNA staining (Hoechst, blue). Its ability to enter both live and dead cells was determinant, especially for a method that must be capable of measuring fresh milk as well as milk containing preservatives or dried samples. Statistical analysis performed on 240 individual samples analysed with the new open flow cytometry and the ISO 13366-2|IDF 148-2 has shown a good comparability between the two methods (bias: - 2,619 cells/mL Flow-ISO 13366-2|IDF 148-2) and a CV of 5.8 % (Fig. 1). To differentiate the PMN from APC cells, the surface markers CD11b and MHCII were selected. Both cell types express CD11b (green) but only APC are expressing MHCII (red) (Fig. 2).

### Summary

Until now precise somatic cell counts in raw milk are performed on dedicated automated counters 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 cytometric measurements. Those washing steps can lead to the enrichment of some cell populations due to the complexity of the matrix milk. Indeed, as PMNs are engulfing MFGs, their relative density is changing and they can be floating in the cream or being entrapped 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 the total number of somatic cells in the milk but also to give a precise value for the two populations of cells without loss or selection of cells.



# MIAMi (Microscopic Image Analysis in Milk) project – Applying modern microscopic techniques for somatic cell counting in milk



By Silvia Orlandini

The standardized reference method to count somatic cells in milk is ISO 13366-1|IDF 148-1 Milk — Enumeration of somatic cells —Part 1: Microscopic method (Reference method). However, it is only limitedly applied in laboratories and comes with a poor precision. The MIAMi project is designed to develop and implement a microscope method with modern technologies that will improve method precision and provide better traceability of counting results through storage of the digital images of the microscopic fields. The project was first presented during the ISO/IDF Analytical Week 2018 in Dublin (IR) and found support from the different stakeholders in the analytical area that are fully aware of the difficulties to apply the microscope method as it is standardized nowadays.

Milchprüfing Bayern (DE) and Qlip (NL) have agreed to jointly invest in the development of automated microscopy/image analysis as an alternative for the current manual method. They have purchased the required microscopes and materials for their two laboratory locations and are putting expertise and labour in method development. Successively Page & Pedersen, Milkotronic and Foss have materially supported in the project development during 2019 and 2020.

Currently, two microscopes with automated XY movement and Z axis movement for focusing are located at Milchprüfing and at Qlip. The microscopes are operated with software that can do automatic counting of the fluorescent cells.

At the beginning of the project, the samples were dyed with methylene blue (MB) and ethidium bromide (EtBr) according to ISO 13366-1|IDF 148-1 and smeared on object slides. These samples were counted manually (MR) and automatically (AMR) from the acquired images by five technicians. Figure 1 shows the favourable repeatability with the AMR procedure: AMR sr% < MR sr%.

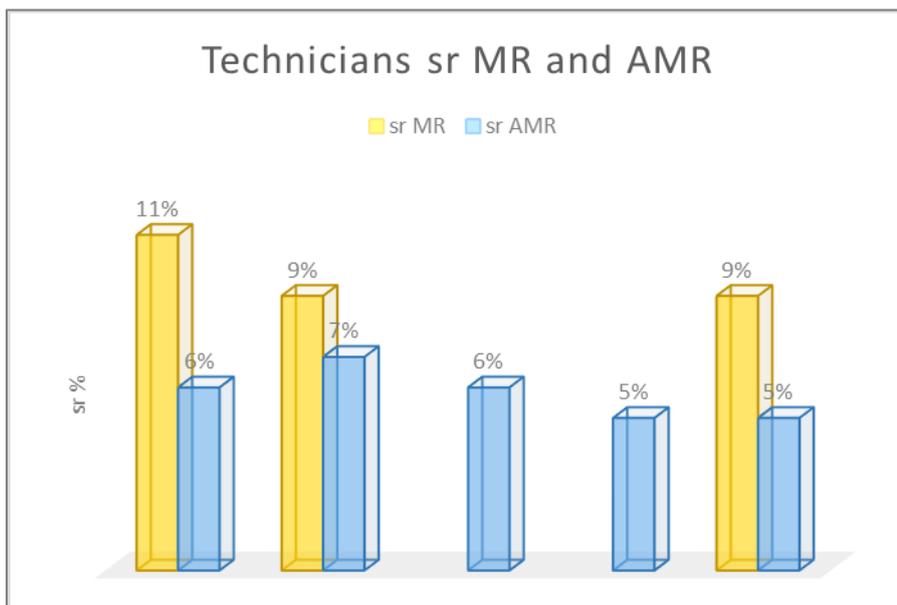


Fig. 1 – Comparison of the relative standard deviation of repeatability with MR and AMR



Successively, samples were placed in a counting chamber with a depth of 20  $\mu\text{m}$  to optimize the image acquisition in a single focus plane. For comparison, samples were prepared as dry smear or as liquid milk and dyed with EtBr and Yo-Pro1. This latter solution improved the quality of the images and produced a more homogeneous background as compared to EtBr.

During 2020, in two different phases, 24 samples were analysed at Milchprüfing by one technician, in two replicates with the different procedures for a total of 336 results.

The preliminary results of relative repeatability and relative difference from the anchor/reference values are presented in figure 2 and figure 3. The obtained relative repeatability values for all procedures tested are much better than the  $r\%$  reported in ISO 13366-1|IDF 148-1. In the whole range of 140,000 to 1,100,000 cells/mL  $r\%$  was lower than 8%. These values are much closer to the  $r\%$  values in ISO 13366-2|IDF 148-2 (11%) than in ISO 13366-1|IDF 148-1 (36%). It is thereby to be noted that the involved technician showed excellent consistency in manual counting,  $r\%$  for MR was at the same favourable level as with AMR.

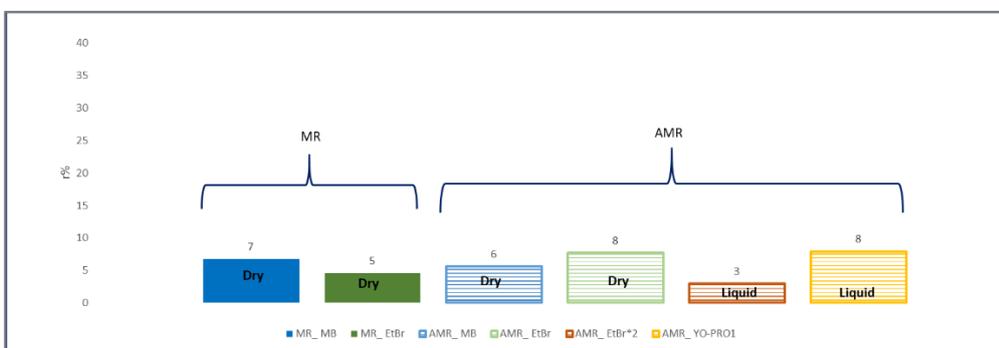


Fig. 2 – Mean relative repeatability for each method tested in a range of concentration 140 000 - 1 100 000 cells/mL

The anchor/reference values of the test samples were calculated according to IDF/ICAR Bulletin 508 and therefore traceable to EC JRC CRM SCC. The relative bias from the anchor/reference value was below 5% for all the methods tested, whereby results for liquid milk showed lower bias than those for dried smears.

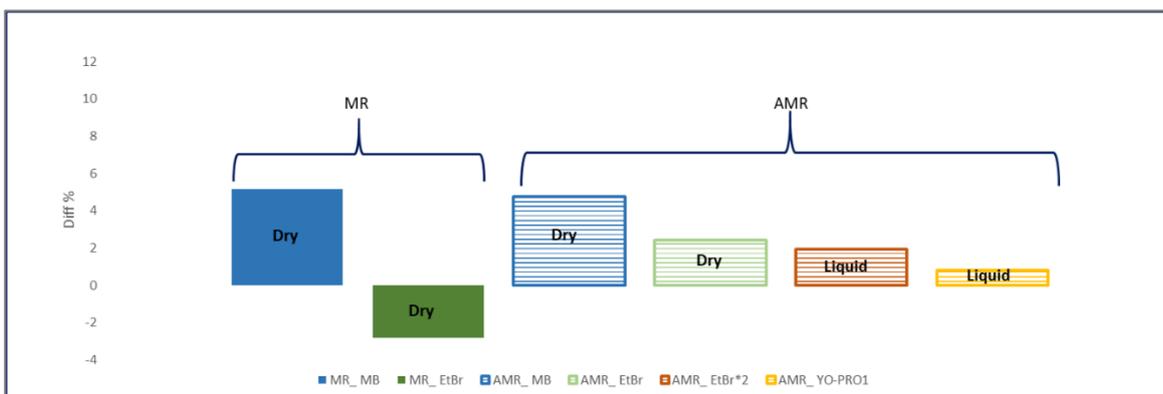


Fig. 3 – Mean relative difference (Diff %) from anchor/reference value



The preliminary results of MIAMi project show that the precision and accuracy of the implemented microscope technique using digital images are favourable to ISO 13366-1|IDF 148-1 and in the same order of magnitude as with ISO 13366-2|IDF 148-2. The use of fluorescent dyes EtBr and Yo-Pro 1, a counting chamber and liquid milk offer attractive options to analyse the images automatically (AR). The results obtained with AR are still under further evaluation.

The project will continue to feed the database with additional results from both laboratories to arrive at a more solid statistical underpinning and to elaborate data on method reproducibility. The encouraging results obtained in this preliminary phase provide good perspective to improve the precision of the microscopic reference method with applying other counting techniques and digital imaging.

Tab. 2 Abbreviations used

SRM	Secondary Reference Material
MB	Methylene Blue ISO 13366-1 IDF 148-1
EtBr	Ethidium Bromide ISO 13366-1 IDF 148-1
EtBr <sup>2</sup>	EtBr concentrated
MR	Manual Reading. Microscope reading using the ocular*objective magnification. The technician count the cells directly form the microscope fields
AMR	Automatic Manual Reading. The technician count the cells from the images acquired with a digital camera on the screen.
AR	Automatic Reading. The software counts the cells from the images using the software

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## Outlook on the work of the joint IDF/ICAR Action Team S09

By Vesela Tzeneva (NIZO, NL) and Daniel Schwarz (FOSS, DK)

The globalization of the analytical results plays a major role in free and fair trade of milk and dairy products. The worldwide equivalence of analytical results cannot always be ensured by “only” producing standardised analytical methods but may also need reference materials for a better safeguarding. Being one of the most frequently executed measurements, routine methods for SCC are clearly superior to the reference method in terms of analytical performance. With the lack of certified reference material and the use of secondary reference materials from various sources, laboratories have through the years adopted various solutions for anchoring their counting level. It was clear that a reference system approach was needed to optimally safeguard equivalence of the results for somatic cell count obtained with different methods and in different laboratories.

In 2011, the IDF/ICAR Action Team S09 was established with the aim to develop a reference system for somatic cell counting, recognised and adopted as such by regulatory bodies and competent authorities worldwide. The focus laid on the development of a workable reference system and plans for its implementation.

The first aim of the Action Team was successfully completed in the beginning of 2020 by the launch of the new primary reference material for somatic cell count by the EC Joint Research Centre.

Several laboratories already evaluated the application of the primary reference material in practice and kindly shared their experience in this newsletter. To further support the evaluation and implementation of the new primary reference material the IDF Bulletin N° 508/ 2021 was published.

In the meantime, new initiatives have been started to improve the reference method for total somatic cell count and to describe a new reference method for differential somatic cell counting. Two projects are currently running on these topics, ANTOINETTE and MIAMi, and the progress of the work was described above.

By launching the new reference material one goal of the AT S09 has been accomplished. However, its practical application has just begun. The latest developments underline the need of organised support for further acceptance and proper implementation of the primary reference material. Beyond that, the current reference method for somatic cell counting is under evaluation and the need for a method that also covers differential cell counting is growing, as described in IDF Bulletin N° 510/ 2021.

In the future, the joint IDF/ICAR Action Team S09 will continue to safeguard the equivalence of somatic cell counting around the world by supporting the application of the primary reference material.

In the short run, Action Team S09 will also offer a platform for communication of the achievements obtained within ANTOINETTE and MIAMi projects. The Action Team will encourage and facilitate the collaboration between the project teams to ensure and promote the best possible solution in terms of improving the reference methods for somatic cell counting.

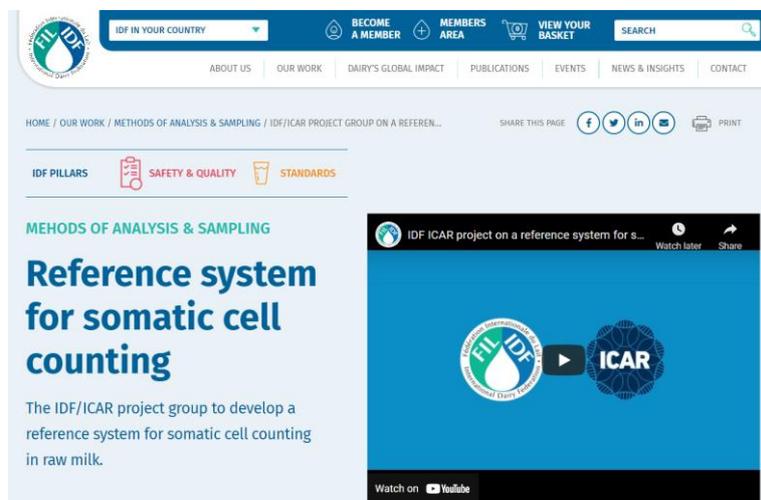


## Members of the joint IDF/ICAR Action Team S09:

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## New webpage for the IDF/ICAR project

[IDF/ICAR project group on a reference system for somatic cell counting - IDF - IDF is the leading source of scientific and technical expertise for all stakeholders of the dairy chain \(fil-idf.org\)](#)



## IDF (International Dairy Federation)

*Helping nourish the world with safe and sustainable dairy*

The IDF is the leading source of scientific and technical expertise for all stakeholders of the dairy chain. Since 1903, IDF has provided a mechanism for the dairy sector to reach global consensus on how to help feed the world with safe and sustainable dairy products. A recognized international authority in the development of science-based standards for the dairy sector, IDF has an important role to play in ensuring the right policies, standards, practices and regulations are in place to ensure the world's dairy products are safe and sustainable.



[www.fil-idf.org](http://www.fil-idf.org)

## ICAR (International Committee for Animal Recording)

ICAR is the recognised global standard for livestock recording. Since its inception in 1951 ICAR has promoted the development and improvement of animal identification, performance recording and evaluation in farm animal production. This is achieved through the establishment of guidelines and standards, specific for the purpose of identifying animals, the registration of their parentage, recording their performance and evaluating their genetics, (including their bearing on animal health, care, productivity, food safety and the environment). Through its global network of some 170 professionals in ICAR's 14 sub committees and working groups, these guidelines are published and maintained for all on [www.icar.org](http://www.icar.org).



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