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

NEWSLETTER 5 - May, 2014

Foreword

A long and winding road……..that was clear right from the start of the Project on a Reference System for Somatic Cell Counting in Milk. But with over 20 actively involved co-workers, it has become a very motivating journey.

Underway, we have attracted the attention of hundreds of stakeholders through presentations, articles and the website www.fil-idf.org/RSSCC. We have explained our aim to arrive at better safeguarded equivalence in somatic cell counting around the globe and how a reference system approach can contribute in this. From the replies to the questionnaires we have learned about the solid interest and the already existing structures between laboratories and other stakeholders. These interlinkages do provide important cornerstones to build a global reference system on.

The recently published 'Requirements for Reference Materials for the Calibration of Automated Somatic Cell Counters' in the Bulletin of the IDF 469/2013 list very valuable guidance. Based on provided input by Project Group members and with the valued support of the EU Reference Laboratory for Milk and Milk Products, the EU JRC/Institute for Reference Materials and Measurements has recently adopted the challenge to produce a pilot batch of a Primary Reference Material for somatic cell counting in milk. We look much forward to the outcome.

A further inspiration is the created outlook on a sound statistical model for assigning reference values to reference materials, also where the reference method has serious flaws. An outlook that also bears wider relevance for the analytical community.

The achievements thus far and the next steps with the Project will be further highlighted during the coming IDF/ISO Analytical Week and ICAR/INTERBULL Conference from 15 till 23 May 2014 in Berlin. The Project Group will meet there on Friday 16 May. The Symposium 'What can Analytics Contribute to Healthy Cows and Healthy Dairy Products?' will provide the excellent platform to update the attendees on the progress and the road still ahead. The summaries of the planned presentations are contained in this Newsletter. For more information and registration, see www.icar2014.de.

And we as a Project Group….we will carry on until we have reached our destination. This is expected to take another year or two. But yes….we are confident that we can work it out…with a little help from our friends!

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Guidelines for the use and interpretation of bovine milk somatic cell counts (SCC) in the dairy industry

By Elizabeth Berry
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The key points in the use and interpretation of bovine milk somatic cell counts are:

- Milk from most uninfected mammary glands has a normal concentration of somatic cells
- The concentration of somatic cells or somatic cell count (SCC) can be used to indicate the inflammatory status of the mammary gland
- A single SCC threshold is not suitable in all circumstances – bulk milk, cow, quarter
- At the cow level, a cut-off of around 200,000 cells/mL can be used – above this value the cow is likely to be infected. A quarter level may be used for research purposes and then a cut-off of around 100,000 cells/mL can be used – below this level the quarter is unlikely to be infected
- A single SCC should not be used to determine the infection status
- The SCC can either be measure directly or estimated through other parameters used to give an indication of the infection status
- SCC can be used to monitor mastitis or cell count programs and to calculate herd dynamics
- SCC can be used at the cow level to determine possible infection status and thus further actions – bacteriology, treatment options in lactation or at drying off, e.g. milking order, breeding or culling decisions

When interpreting SCC data, it is essential to consider the level of measurement (bulk milk, cow or quarter). The SCC can vary according to the accuracy of the method used and other factors (physiological, inflammatory and sample and processing factors). It is important, particularly at the cow or quarter level, not to rely on one value to determine the udder health status of the cow, as this status can vary within a day depending on the outcome from an inflammatory insult.

Methods and instruments used to indicate abnormal milk and cell count must be calibrated and verified to promote accuracy and precision (repeatability, reproducibility). It is essential to participate in proficiency testing as prescribed by ISO/IEC 17025:2005 and ISO/IDF standards. The importance of reference standards and materials for this testing cannot be underestimated.

Suitable reference materials and ability to participate in proficiency testing in a laboratory network structure are fundamental for this to work.

More information is in Bulletin of the IDF No. 466/2013.

Performance of the network of NRL's Milk & Milk Products for the counting of somatic cells in raw cow's milk

By Véronique Deperrois
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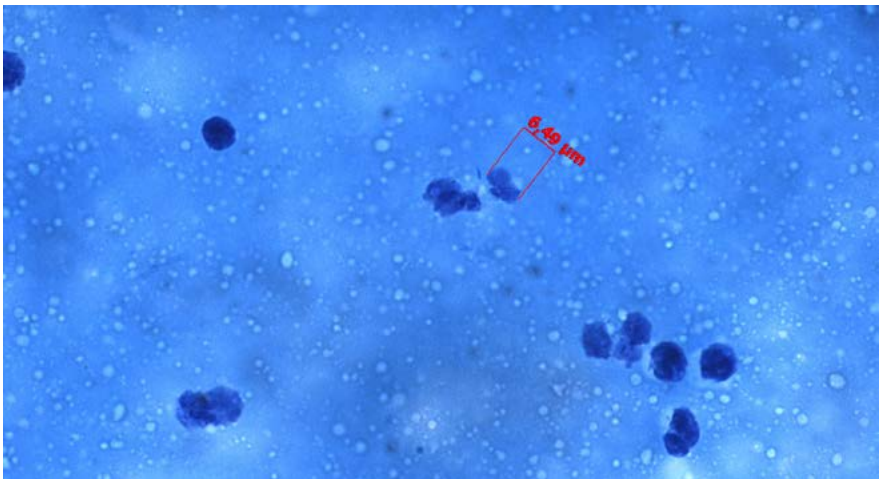
In Section IX of EC Regulation 853/2004, microbiological criteria have been fixed for raw milk (Chapter I, III) and for dairy products (Chapter II, III-criteria for the use of raw cow's milk for further processing). They include criteria on somatic cells count for raw cow's milk.
The EC Regulation 2074/2005, modified by EC Regulation 1664/2006, includes the description of testing methods for raw milk and heat-treated milk, including the reference method for somatic cell count, Standard EN ISO13366-1/IDF 148-1 as well as conditions for the use of alternative methods.
The EU Reference Laboratory for Milk and Milk Products (EURL MMP) organizes each two years an interlaboratory trial to evaluate the ability of the National Reference Laboratories (NRLs) for Milk & Milk Products to count somatic cells by the reference method EN ISO 13366-1|IDF 148-1.
Seven samples of raw cow's milk, at 4 levels of somatic cells (from 0 up to 1 000.10^3 cells.mL-1) are sent to the NRLs. These samples are analyzed by the reference method. The individual precision of the laboratories and the global performance of the network of NRLs are evaluated.
On the last two interlaboratory trials organized in 2008 and in 2010, the global performance of the network of NRLs was good, both in terms of standard deviation of repeatability (RSDr of 6-13% in 2008 and 4-8% in 2010) and standard deviation of reproducibility (RSDR of 18-21% in 2008 and 15% in 2010), and improved between the two. Most of the participants (79 % in 2008 and 85 % in 2010) showed a satisfactory individual performance, both in terms of precision (k-ratios) and trueness (z-scores).
A new inter-laboratory trial is on going.

Statistical parameters of the proficiency testing trial (10^3.mL^{-1}) (2008)

Level	n	X	$\hat{\sigma}$	s_r	RSD_r	r	s_R	RSD_R	R
Low	21	207	33	26	13 %	73	37	18 %	106
Medium	22	396	69	24	6 %	68	71	18 %	198
High	22	1020	203	103	10 %	288	215	21 %	603

Statistical parameters of the proficiency testing trial (10^3.mL^{-1}) (2010)

Level	n	X	$\hat{\sigma}$	s_r	RSD_r	r	s_R	RSD_R	R
Low	25	184	26	15	8%	42	28	15%	79
Medium	25	445	62	26	6%	71	65	15%	181
High	25	885	130	34	4%	94	132	15%	370



Comments of an ISO 13366-1|IDF 148-1 (new) user

Véronique Ninane
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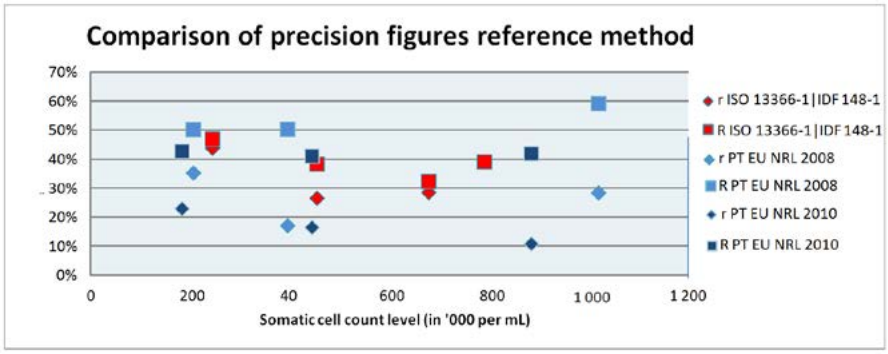
Counting somatic cells by the microscopic method, considered as the reference method in EU, becomes subjective in many situations. In particular:

- When you have to decide if the small cell you see must be counted or not. Minimum size is fixed by the standard but, despite placing in your ocular the most suited grids you find on the market, it is sometimes not easy to evaluate the dimension of the cell you see.
- When you have to determine the number of cells included in the cellular heap you see. Even when illumination has been correctly adjusted (Köhler method), it is sometimes not easy to see whether two nuclei are linked or separated.
- When you have to decide if the part of the cell you see at the border of the field must be counted or not.

With experience, you set your own judgement rules that permit to reach a relatively good level in repeatability as shown by the study reported in this Newsletter by Véronique Deperrois. It also shows that your own judgement rules are not necessarily the same as those of your neighbour: reproducibility between NRL's and compared with the values published in the ISO 13366-1|IDF 148-1 did not improve over time. There comes the need of a common reference material: to train for common judgement rules!



Collaboration with EU JRC/IRMM

After the first meeting at the EU Joint Research Centre/Institute for Reference Materials and Measurements (JRC/IRMM) in Geel (BE) in September 2012, representatives of the IDF/ICAR Project Group and IRMM met during the Annual AOAC meeting in Chicago in August 2013 to evaluate the outcome of a survey among potential users, which had been agreed upon during the first meeting.

90% of the 141 replies indicated significant interest in the development and world-wide availability of a certified reference material for SCC in milk.

To highlight the importance of this project, ICAR and IDF secretariats with the support of the EU RL for Milk and Milk Products (ANSES) wrote a recommendation letter to DG SANCO, offering the input of the expertise from the IDF/ICAR Project Group as well as eventually needed facilities with the stakeholders in the dairy networks.

Since the AOAC meeting, further scientific-technical details related to a potential CRM project were discussed, such as the suitability of developed procedures for a large scale production of reference material.

IRMM intends to formally integrate the potential project in its annual work programme.

What can Analytics Contribute to Healthy Cows and Healthy Dairy Products? – Presentation summaries

IDF/ISO Analytical Week, Berlin – Symposium – Saturday 17 May 2014

What is a reference system and why do we need it?

Harrie van den Bijgaart
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Traceability is key for a valid outcome of analytical work. This can be arranged for in multiple ways:
- by applying a defining method, i.e. the measured item is defined by a proper execution of a meticulously standardized method per se;
- by calibrating a method through the use of (certified) reference materials;
- by calibrating a routine method against a reference method, a reference method either being a defining method or another standardized method that was designated as such.

But what to do when the reference method is poor in precision and certified reference materials are lacking? How then to safeguard analytical equivalence in place, in time, and eventually between different methods applied in routine? That is where a reference system approach may serve as a complementary anchoring system to safeguard comparable and traceable measurement results for decisions in trade, in the frame of regulations and risk assessment. A few years ago, IDF and ICAR jointly took the initiative to explore the feasibility of such a reference system approach for somatic cell counting in milk. Since then, major steps have been made towards a successful implementation, as will be outlined in the following presentations.

Current Situation with the Reference System for Somatic Cell Counting in Milk –Who? What? When?

Silvia Orlandini (IDF/ISO/ICAR)
AIA-LSL ,Maccarese, Italy

This presentation summarizes the progress with the ICAR/IDF project on a Reference System for Somatic Cell Counting in Milk since 2008 and the planned actions of the Project Group.
The project has explored existing analytical reference systems in different countries for the parameter somatic cells in milk and the interest from the various stakeholders.
The approach to arrive at improved global equivalence in somatic cell counting has been presented in many ways to different audiences. This to collect information and to promote collaboration between the different stakeholders. The obtained information is being used to build up a system with secured traceability in measurements and to promote equivalence. This is the basis for creating maximal trust in its functioning.

The Implications of New Statistical Tools to Evaluate and Compare the Analytical Performance of Laboratories

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A statistical approach for the comparability of proficiency testings (PTs) by using a Quality Index PQ and of participating laboratories by using a Quality Index PL, both deriving from probabilities, is presented. Comparability of PTs and participating laboratories is part of a reference system and network structure as introduced in the framework of somatic cell counting. The approach therefore makes use of the precision parameters as reported in the international standard ISO 13366-2|IDF 148-2 and of assigned values to test materials.

Five PTs were chosen to test the approach. Beside statistical data also graphical evaluations of the PTs and laboratories are possible. Robust means of quality indices from different PTs may be used to compare laboratories over time.

Visit the website

The Project Group makes gratefully use of webspace provided by IDF. All information on the project including an up to date list of all presentations and publications can be reached through www.fil-idf.org/RSSCC.

CLICK HERE FOR MORE INFORMATION
AND PREVIOUS NEWSLETTERS

IDF (International Dairy Federation)

The mission of IDF is to represent the dairy sector worldwide by providing the best global source of scientific expertise and knowledge in support of the development and promotion of quality milk and dairy products to deliver consumers with nutrition, health and well-being.

1200 experts appointed by IDF members work on the areas of dairy farming, food standards, analytical methods, nutrition, hygiene and safety, science and technology and economics and marketing. IDF places great emphasis and importance on ensuring that the works it promotes are of the highest scientific integrity and are relevant and applicable to the dairy sector and industry as well as to international organizations, governments and legislators.

www.fil-idf.org

ICAR (International Committee for Animal Recording)

ICAR is an international non-profit body that promotes the development and improvement of the activities of performance recording and the evaluation of farm livestock.

This is achieved through:

- Establishing rules, standards and specific guidelines for the purpose of identifying animals, the registration of their parentage, recording their performance and their evaluation and publication of the findings;
- Providing incentives for concertation and collaboration in animal performance recording and evaluation within and among international organisations, public authorities and industry.

www.icar.org

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