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

NEWSLETTER 7 – JUNE 2018

Introduction

We hereby present you the 7th Newsletter on the IDF/ICAR Project on Reference System for Somatic Cell Counting in Milk. This joint project of the International Dairy Federation and the International Committee of Animal Recording aims at creating better equivalence with somatic cell counting in milk worldwide. This is relevant in light of regulatory demands on raw milk quality, for milk payment as well as for farm management and breeding decisions by dairy farmers. The project is now approaching its final phase, that is its implementation in practice.

The development of primary reference materials has well advanced by now. A first batch is expected to be launched in 2019. Next it is illustrated in this newsletter how the developed calculation model can be used to compare and rate the performance of proficiency tests and laboratories. You will also be updated on the ideas about the coordination and the safeguarding of the future functioning of the reference system. Furthermore, a brief description of the current QA system on equivalence of somatic cell counting in South-America is contained. This is one of the systems we hope to link with through this project. Contacts on a possible future implementation of the IDF/ICAR reference systems have up till now been established with regulatory and supervising bodies in Europe, US and New Zealand. All messages are on the pieces of the puzzle, but indicating that we are well underway with the final steps…

I wish you an informative read!

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Progress towards a primary reference material for somatic cell counting in milk

From mid-2015 to early 2017, a feasibility study on how to prepare primary reference materials, which shall serve as calibrators for somatic cell counting in cow's milk, was continued and concluded. The study focussed on the following issues: comparing different means of cell enrichment from milk of healthy cows, thereby using either gravity separation, centrifugation and resuspension of cells in cell-free milk, or microfiltration with subsequent resuspension of cells to the desired level. Both processes delivered comparable results as tested with different methods (direct microscopic somatic cell count (DMSCC) according to ISO 13366-1 | IDF 148-1, flow cytometry according to ISO 13366-2 I IDF 148-2, image cytometry). Stabilisation of prepared milks with low and high cell count by means of freeze-drying and spray-drying: both processes delivered milk powders with an appropriate stability over time (data currently available for a 20 months period; further testing to be done). Freeze-drying is feasible for producing a consistent batch in the small and medium-size, whereas spray-drying is preferred if a larger-scale batch (several thousands of units) shall be produced. As concerns cell integrity, there is some evidence that stabilising the milk influences the cell shape and integrity to some extent (especially freezing and heating). However, the analysis of both freeze-dried and spray-dried milks delivered comparable results when performing DMSCC, flow cytometry, or image cytometry measurements on a specific sample, indirectly indicating that the applied processes did not change the cell shapes/status to a larger extent. Furthermore, effort was invested on establishing a dedicated reconstitution protocol (addition of warm water and mixing to have the reference material (RM) ready for measurement). Processing of the candidate RMs in late

autumn 2017 was done in collaboration with an institution that has the facility and appliances for larger-scale production and the necessary technical expertise. They demonstrated that the materials are sufficiently homogeneous and that dispatch of materials to laboratories can theoretically be done at ambient temperature. Some more expert laboratories applying either ISO 13366-1|IDF 148-1 and/or ISO 13366-2|IDF 148-2 will be qualified for participation in the laboratory intercomparison envisaged for characterising the candidate RMs. Material characterisation is planned for late 2018, and the 1-year long-term stability study will finish in spring 2019. Once the materials are value assigned and are released for sale (around summer 2019), they can be used for calibration or method performance verification (as QC samples). Furthermore, it is also possible to use these primary reference materials to establish assigned values for secondary/in-house reference materials.

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Good results with trial in assessing and comparing performance of PTs and labs!

One of the most crucial elements of a reference system for establishing analytical equivalence on a global scale is the ability to statistically assess and compare different proficiency testing schemes and laboratories to each other and qualify them according to their quality of results. As part of the project, a calculation model was developed for that purpose. Basically, the calculation model is a comparison of the outcome of proficiency tests (PTs) with the validation data in ISO 13366-1|IDF 148-1:2008 (Direct Microscopic Somatic Cell Count) and in ISO 13366-2|IDF 148-2:2006 (Fluoro-opto-electronic counting). For more information, see text box below.

In order to test the statistical approach in practice, it was applied in the characterisation of the Agroscope-Suisselab Somatic Cell Count Standard (SCCS), a reference material on two levels (approx. 200,000 and 400,000 cells/ml). The characterisation process serves to establish the assigned value of the reference material. Approximately 30 labs from different countries and using different methods participate in the characterisation. Participation varies from round to round, some labs participate each time, others e.g. once a year. The labs consider the characterisation process as a PT.

In addition to the classical evaluation there is a long-term evaluation using the probabilistic comparison and assessment model. Each level in each round is handled as an own PT and also groups of labs can statistically be treated separately, assigning an own quality index P_Q to them. Each set of data sent by the lab (from each operator and/or each instrument) is handled as an independent "lab", assigning an own lab quality index P_L to each of them. P_Q and P_L may range from 0 to 1, with 1 indicating perfect performance.

The calculation model was published open access in 2016 and can be downloaded from https://link.springer.com/article/10.1007%2Fs00769-016-1207-y (*Probabilistic Comparison and Assessment of Proficiency Testing Schemes and Laboratories in the Somatic Cell Count of Raw Milk*).
A more comprehensive description was presented during the ISO/IDF Analytical Week in 2014 and can be ordered from the authors (*The Implication of New Statistical Tools to Evaluate and Compare the Analytical Performance of Laboratories*).

The long-term evaluation of the PTs so far shows three major effects:

- as labs in routine measurement are separated on two distinct concentration levels (see also Fig. 2) the quality indices are zero, meaning that the reproducibility standard deviations are much inferior to what is written in ISO 13366-2|IDF 148-2;
- looking at a subgroup of labs in the AFEMA network (www.afema-ev.de) only, the quality indices P_Q are between 0.75 and 0.95, showing successful PTs which are within the limits of ISO 13366-2 | IDF 148-2;
- labs performing DMSCC have bigger problems with low levels (P_Q near zero) than with high levels (P_Q between 0.15 and 0.65), but in general the outcomes of these PTs are not successful when compared to ISO 13366-1 | IDF 148-1.

The evaluation of the labs in a single PT shows two levels as mentioned above. The P_L values are between 0.15 and 0.97 for the majority of the labs (most of them are also linked to other PT schemes), whereas a minority of labs shows values of nearly zero. AFEMA labs and others show very often satisfying values. This is partly due to the fact that the assigned value of the PT (and reference material) is calculated from the routine instruments, using AFEMA data with an equal weight for each laboratory.

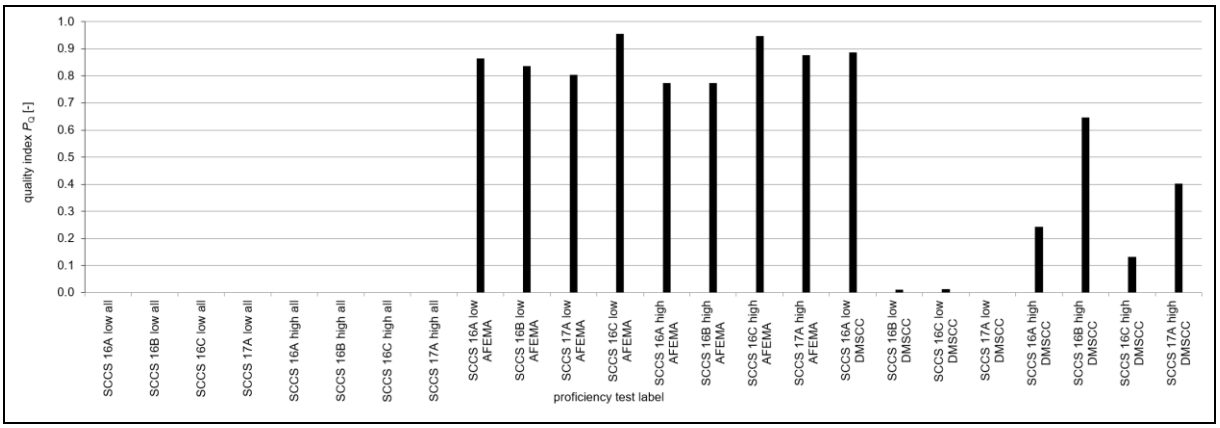

Fig. 1: Assessing PTs using the probabilistic comparison and assessment model and Agroscope-Suisselab SCCS, levels low and high. The first eight P_Q values on the left include all routine measurement data. Values 9 to 16 include the routine measurement data of the AFEMA labs. Values 17 to 24 are data from labs using DMSCC.

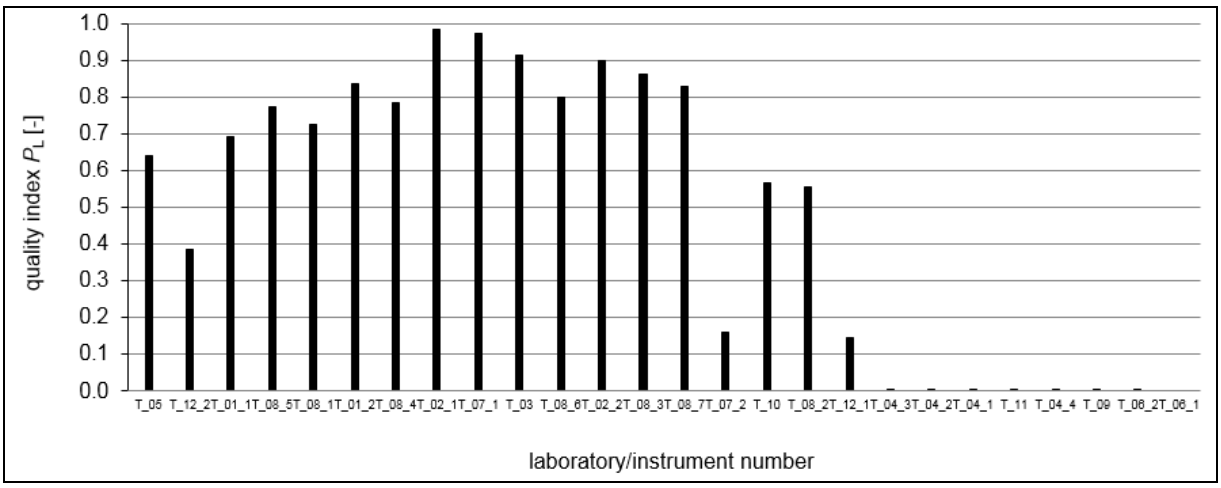

Fig. 2: Assessing labs using the probabilistic comparison and assessment model for Agroscope-Suisselab SCCS, level high, January 2017, rising means from left to right. Results show that labs using the routine method measure on two levels. The first 18 values from the left show P_L values above zero (AFEMA labs and others, often linked to other PT schemes) because the assigned value of the PT is calculated from the routine measurement data of the AFEMA labs with an equal weight for each lab. Values 19 to 26 are (almost) zero and are from labs measuring at a higher level.

Long-term evaluation of the labs (or operator or instrument, if known) so far shows some labs performing repeatedly very successful with median P_L values above 0.7 and others repeatedly performing less successful with median P_L values less than 0.5. However, it has to be kept in mind, that the use of the calculation model should include data from different proficiency testing organizers. An evaluation from the perspective of one organizer only delivers interesting data but is not an objective picture of how labs (operators, equipment) perform!

Taken together, the use of the probabilistic comparison and assessment model seems to work well in daily routine, delivering promising values for the assessment of PTs and labs.

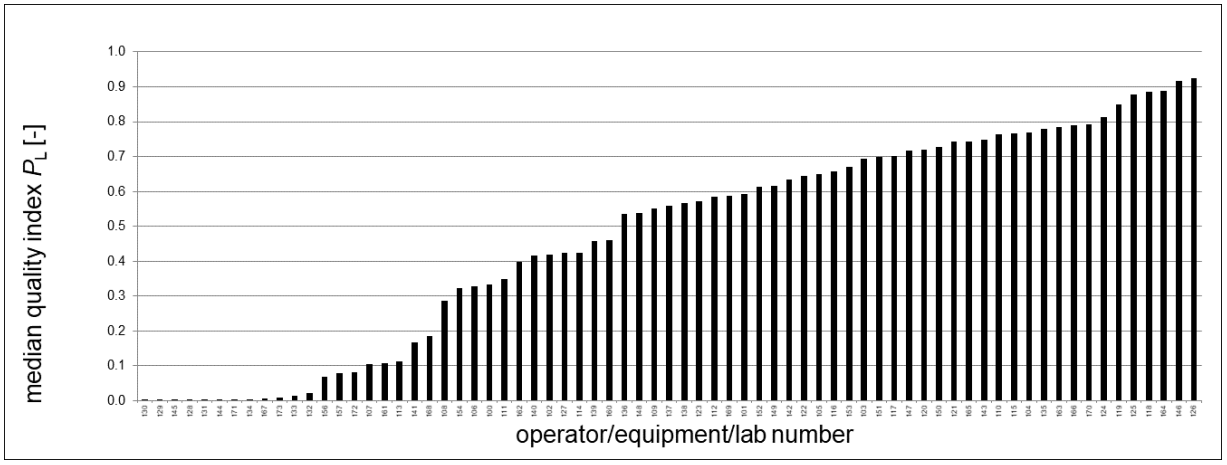

Fig. 3: Long-term assessment of labs (or operators or equipment, if known) using the probabilistic comparison and assessment model and lab median data, showing labs performing repeatedly very successful with median P_L values above 0.7 and others repeatedly less successful with median P_L values less than 0.5. It has to be borne in mind that the use of the calculation model should include data from different proficiency testing organizers to get an objective picture of how labs perform!

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NeuBo – how to implement a worldwide reference system for somatic cell counting?

When this joint IDF-ICAR project started, most of the colleagues involved in the action team were reasoning about the question “Will it be possible at all to put all pieces of the puzzle together to establish a reference system?” Today, more than six years later, we know “yes, it is!”

But how can such a sophisticated system be implemented on a worldwide scale? Without any doubt, this huge undertaking needs a huge will of cooperation of many stakeholders. And in addition, cooperation needs a structure!

What should such a structure look like? When analysing all the stakeholders and their interests as well as the present structures available, it became clear that such a structure has to be completely neutral and able to work on a worldwide scale. And it became clear that such a structure does not exist yet, it has to be created. A neutral body – NeuBo!

First, the mission of NeuBo has to be clear. A draft presented by the action team reads: “Promoting and supporting optimal equivalence in analytical results for the dairy sector worldwide through the application

of international standards and guidelines, reference materials, reference systems and participation in proficiency testing in an integrated, robust, neutral and non-commercial structure."

In practise, NeuBo should start with implementing the Reference System for Somatic Cell Counting by coordinating the characterisation of a primary reference material and with monitoring and safeguarding the functioning of the reference system. This will include the creation and maintenance of a database with information provided by participants in the frame of confidentiality contracts.

In addition, NeuBo should provide a communication platform involving all stakeholders in the dissemination of information and promoting traceability in agro-food on a global scale as well as offering advice and neutral consultancy services; working towards harmonisation of reference materials and proficiency testing schemes should also be a task of NeuBo.

Still, NeuBo is only a concept. Therefore, one question is obtruding: How can we initiate NeuBo? During the last months we talked to some important stakeholders to make them familiar with the NeuBo concept and to seek consent about the mission. We will pursue with this activity. At the same time we are checking possible legal forms for NeuBo under European conditions. More details will be presented during the next months.

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Example of QA management on SCC in South America

INTI Lácteos is the Dairy Research Center of the National Institute of Industrial Technology in Argentina. For more than 25 years, it has been offering an integrated system with provision of reference materials and proficiency tests (SICECAL - REDELAC). Both tools are key for assuring the quality of the results and for the monitoring of the performance of the Argentine and South American laboratories. Within the offer of this integrated system, the provision of reference material (bimonthly) and proficiency testing (semi-annual) in somatic cell count are two of the tools with greater reach, reaching about 40 laboratories throughout South America. Both services operate with a working range of between 50,000 cells/ml and 1,000,000 cells/ml. In addition to the monitoring and evaluation of the laboratories, INTI Lácteos provides continuous technical assistance in quality assurance according to the needs of each of them, with the aim of improving their performance and making their results accurate, reliable, comparable and traceable to a reference. This has resulted in noticeable improvement of their performance over the years. In Argentina, this system is used as a monitoring tool for the laboratories authorized for the single settlement system for the payment of milk on quality, SCC being one of the regulated parameters.

With the intention of strengthening these tools at the regional level and connecting the largest number of laboratories in South America, in 2015 the Latin American and Caribbean Laboratories Network (REDLAT) was created. The aim is to harmonize analytical methodologies, to safeguard the comparability and traceability of analytical measurements, the exchange of experiences from the technical field and the provision of training and technical assistance related to the improvement of the compositional and hygienic quality of milk.

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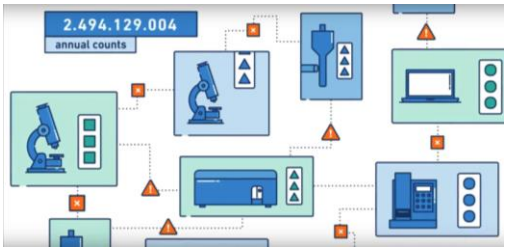
Contact with regulatory and supervising stakeholders

After earlier contact with the former EU Reference Laboratory for Milk and Milk Products, the project has recently been presented to regulatory and supervising bodies in other geographies. We are happy to note the constructive interest of the US Department of Agriculture, the US Dairy Herd Improvement Association as well of the New Zealand Ministry of Primary Industries. All have indicated willingness to look into the options to link their safeguarding systems for equivalence in somatic cell counting to the IDF/ICAR reference system, when being implemented. Members of the Action Team will continue to pursue contact with regulatory and supervising entities in outer countries in order to promote the aimed at global adoption of the system to the widest extent feasible.

Video on the project

In order to better explain the purpose and the approach with the project to a wider audience, the key points are captured in a short-animated video. This can be accessed through here. We encourage you to share this video with interested stakeholders.



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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 organisations, government 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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