
Discussion and conclusion

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The following is a summary of the main pieces of successive discussions either just after each presentation or at the end of each half session. It was established from taken brief notes and related talk memories and reformulated for better understanding with appropriate context recalls.

ICAR Reference Laboratory Network is used as a vehicle to broadcast and help to implement harmonised analytical practices worldwide in the ICAR sphere. It was shown that not all ICAR members organisations involved in dairy production had adhered to the system by appointing a reference laboratory. As well it was demonstrated how important the role of these laboratories can be in anchoring local routine laboratories to a universal reference and providing indication on the true uncertainty of their results. It is reminded that comparable equivalent results are a strategic point in milk recording with regard to genetic evaluation and trade.

The actual issue is nowadays on how to increase the participation of ICAR MOs in the reference laboratory network and provide them access to the international reference. The chairman explained that at first in 1994 it was a volunteer demarch proposed to organisations to enter an analytical quality assurance system and accompany a general QA trend made to harmonize quality levels and face the upcoming globalization of trade. Progressive implementation and development expansion was expected throughout time through repeated promotion and communication through conferences like today. This has been the normal upgoing dynamic observed until a plateau was reached in 2003 that is needed to pass through.

The question arose about the fact that this is no more the only original QA interest of individual MOs but the ICAR collective interest which is addressed. Hence one is wondering whether a clearer and more ostentatious step is required possibly by making participation mandatory. Such an option is felt well in line with the ICAR QA policy developed with the ICAR audit system and delivery of a Certificate of Quality. This is retained as a further proposal to ICAR Board.

Part 1

**ICAR Reference
Laboratory Network -
Objectives & and
stage of progress
(O. Leray)**

International survey on SCC: Situation of lab network organisation and practices (S. Orlandini)

PT scheme interlinkage and international laboratory anchorage (O. Leray)

The joint IDF-ICAR project on Reference System for Somatic Cell Counting derives from the ICAR international interlaboratory experience. It is to describe and organize in an optimal way an interlaboratory activity in order to assess laboratories and proficiency testing schemes, characterise reference materials and determine international reference values. A final goal is to distribute trustworthy reference values through international standard reference materials.

A survey done by the joint IDF/ICAR working group allows results permitted having a global view on existing regional laboratory networks or PT schemes and the established cross-linkages and international anchorage (ICAR) worldwide. It is proposed that those replies taking part in the questionnaire (>200 replies!) on somatic cell counting should also be encouraged/invited to participate as new participants in an international interlinked system. Motivate them to do so!

It is also noted that tools have been developed for implementing a reference system based on traceable competence of participating laboratories. But getting them implemented is not such easy and will take quite some efforts from all parties involved. Indeed PT scheme bridging and international anchorage have been for long only theoretical and were concretized only from 2010 using the model here presented with taking example of somatic cell counting although it is general and valid for all methods.

At the present stage the laboratory network for SCC is only virtual based on existing regional networks and interlinkage. Actually it is to be implemented but few laboratories practice regularly the reference methods (DMSCC). Regular lab training and performance evaluation in DMSCC and in-house calibration in automated SCC is to be organized. For all the needed interlinking there is still a considerable and indispensable role for secondary reference materials.

It is mentioned that handling, transport and storage can influence/affect strongly the quality and the durability of reference materials and that these various dimensions must be under control. Reference materials should meet criteria as listed in ISO Guide (RMs Series 30 to 35). Additional guidance is in a document being finalized by the Project Group. Transportation is covered therein. There are objective tools available to assess the suitability of applying procedures for handling, transport and storage. Suitable means of preservation seem to be in the application of preserving agents, deep-freezing and/or lyophilization of reference material. However, all these options bear to some degree of risk. These risks will be outweighed. Special attention is given to custom obstacles and the need for a meticulous preparation of shipment and accompanying documents. Identification and prior contact with key services / persons in countries may be needed in some countries. Being asked for further clarification on the presented PT scheme interlinkages, the speaker stresses that the real picture was presented. Many interlinkages can be identified and provide a robust basis for further intensification.

More knowledge is acquired about reference materials for somatic cell counting and clearly the future will be with improved practices and results. Trust and objectiveness are needed around labs. No one is willing to deviate so there is a need to define the system to assist the lab and avoid discrepancy and achieve compliance. In that frame also ICAR Board is thinking about more stringency, this to be able to trace compliance in practice.

Participants were asked on their interest in taking part in a reference system approach. US experience was shared: 4 labs participate in national proficiency testing, partly linking up to an international proficiency testing scheme. It is well

agreed with the principle of a reference system approach and the striving to obtain better equivalence. But the difficulty is in how to bring this all closer together. Who is going to say you are out?

Answers given were that this is the aim of the project to implement an unique worldwide accepted source of true values (consensus) with growing the whole project together and seeking authoritative approval and coordination. The application of the interlinkage / anchorage to SCC as presented is an example of how it can work.

As a conclusion the need is expressed for a concrete structure to handle these issues. That brings a clear assignment for the follow-up work to do. This was not further contested.

An update is given on the milk recording analysis system which is run in Ireland and its integration in the ICAR AQA system. Ireland joined the ICAR system in 1996 and nominated Teagasc Moorepark to the ICAR reference laboratory network.

The Irish DHI milk analysis system is organised with four dairy laboratories anchored to the Technical Services Laboratory of Teagasc MoorePark. The latter has implemented a centralized calibration through reference materials for MIR milk analyzers and somatic cell counting. Beside regular proficiency testing trials are run. Labs are characterized through three colours white (correct), yellow and red according to three ranges and occurring biases. Like in soccer, yellow means a warning and is asking for attention, a red card will result in serious demand for a real extra effort so to comply again. Statistical compliance evaluations are made on occurrence frequencies of both colours.

Referring to the former presentation it is recognized that the used reference values can be biased. However this can only be within accepted limits of error thanks to applied QA and anchorage to the ICAR ref lab network. Reference values are produced by Teagasc Moorepark with reference methods.

The speaker mentions that sampling error is a renewed critical issue especially with regard to the carry-over effect between successively milked animals. For animal health monitoring through milk recording samples, carry-over may be even more relevant.

Individual protein composition is of a great interest for nutritional, functional, and technological issues, similarly as fatty acid compositions hence determining possible levers to influence protein composition at the farm level is particularly important. High throughput phenotyping methods are sought through and FTMIR is foreseen as a first attempt.

The reference method applied was LC-MS, thereby identifying at least 25 different molecules. Details can be found in the publication of Miranda et al. Concerning the statistical techniques there were several alternatives evaluated like the Penalization Method, Ridge regression, LASSO and Elastic Net. However, that did not bring any improvement as compared to PLS.

**ICAR Inter
Laboratory
Proficiency Testing:
Ireland Update -
Review 2002-2011
(M. Burke)**

Part 2

**Determination of
milk protein
composition by mid
infrared spectrometry
(M. Ferrand)**

Critical points with the application are a robust sample set with accurate reference method results, gain in accuracy by counteracting proteolysis and a harmonization system between labs. Harmonization system can be the same as for fatty acid composition in milk in the Phénofinlait programme as presented in Bourg-en-Bresse (FR) in 2011.

To a question on the actual value for breeding in general beside the individual cow level, the speaker answered that value was for both, but she can't get in more detail. At present, the protein FTMIR project has not yet had a follow-up in France.

Prediction of milk coagulation properties by FTMIR (M. Cassandro)

Much interest is given to predict directly technological parameters on individual animal milk with high throughput mid infrared analysis and open the door to genetic evaluation on cheese making ability. FTMIR is used to bypass the determination of different parameters usually measured and combined separately. The goal is to obtain directly the key useful information needed for the targeted purpose.

Possible correlations between coagulation properties of the milk and other traits were not evaluated yet. Nevertheless there is indeed a significant correlation with the casein content (itself tightly correlated with cheese yield) but in the model there are more factors of interest involved to enable predicting milk clotting characteristics at cheese making. A Foss-supplied calibration model for the MilkoScan FT6000 is applied to extract spectral data in routine.

Merging of spectral datasets from different MIR instruments (J. Fernandez)

Available spectral mid infrared information is getting larger and larger with technology improvements. The ranges of possible parameters prediction and the sophistication of new parameters or investigated information are increasing thus making utilisation spread over larger scales not restricted to the interest of a sole laboratory but to all the laboratories of which results may have to be compared. The predicted characteristics are getting more qualitative (beside quantitative determination) to move forward to expert systems and have access to immediate decision with the associated statistical risk of error. This induces costs significantly higher to establish the reference than in classical laboratory analysis since the latter more often results from expensive surveys and expert diagnoses. New strategies are to develop collaborative programmes involving several partners and laboratories where the huge work load can be suitably shared as well as the outcomes. The core tools to build such a system are spectral data bases which then can be used to create new analytical tools for the sake of all the partners. This is the case for the Phénofinlait programme in France and Optimir and RobustMilk programmes at the European scale undertaken from 2008.

Since there are different devices and devices brands / manufacturers one major issue lies in establishing an exact correspondence between mid infrared spectra of each FTMIR spectrophotometer type and brand so as to obtain identical predictions whatever the device and location with the same milk sample. Such a task has been undertaken since 2008 and subsequent progress from different sources was presented last year in Bourg-en Bresse (FR) 2011. Last developments were made since then in the Optimir programme as here presented.

As a first remark the chair appreciated that the standardization and merging of spectral datasets had seemingly found a solution. However a subsidiary remaining issue will be the harmonization of databases for merging and consolidation where

different spectral references are used. The speaker indicated that these are first results obtained only in fat prediction and next confirmation should be made for more sensitive milk components such as fatty acids.

About changes brought in the instruments the speaker mentioned a quite stable behaviour of FTMIR instruments in normal conditions but upon repairs or servicing there is a necessity to check and re-establish the equivalence of the spectrum again as normal.

The question was raised on the absoluteness of the master instrument as reference provider, i.e. to which golden reference standard (re reference spectrum) is it anchored. The possibility to use deep frozen milk reference materials to standardize instrument spectra and allow long term preservation and wide broadcasting was mentioned. The positive outcomes of Phénofinlait programme in spectrum and data harmonisation using deep frozen milk samples and orthogonal fat, protein, lactose arrangement were mentioned (ICAR 2011) thus indicating the way yet opened. Similar experimental milk sample set design is used in Optimir.

The discussion came to the manufacturer standard solution (Equalizer, Foss) and its equal validity for qualitative and quantitative analytical approaches that was confirmed by the speaker and Foss attending representatives.

The chair reminded the utmost influence of homogenization quality on biases between instruments based on the observations made for fatty acids in the Phénofinlait programme with Milkoscan FT6000 prior standardized with the Equalizer solution. He emphasized on that any reference material should take into account the homogenization effect by having a fat globules suspension able to react similarly to milk fat. He noted that using a milk posed the problem of the the stock and reproducibility (milk is variable) and that the optimum would be a chemically reconstituted solution with standard fat emulsified phase that can be exactly reproduced at each preparation. This would serve to align all the spectrophotometers answers. Thereafter a new issue for scientists and standardizers should be to define the standard spectrum target.

Question was then raised about the spectra alignment with Bentley devices and whether or not a standard solution similar as Equalizer is used. Bentley representative indicated there is no such solution used since instruments are very stable and regular checks prior running analysis are made with water.

In the end the potentiality of orthogonally designed sample sets already used for fat, protein and lactose in mid infrared calibration (IDF 141:2000) is pointed out for fatty acids and individual protein with regard to large range coverage and compensation of eventual problems in intercorrection. It is anticipated that good accuracy can be obtained from robust global calibrations.

Globalization by bringing significant changes in society and economy results in new challenges for the dairy analytical sector and new strategies for international organisations such as ICAR.

The presentations gave examples of new (innovating) approaches to handle analytics at a broader level in order to assure analytical harmonisation and equivalence worldwide. These on-going developments are processed within a network of partner experts and organisations and thanks to regular information in similar conferences and meetings in ICAR and IDF. A number of international experts are attending and will relay relevant requests, suggestions and ideas to

Conclusion

other workshop such the next ICAR analytical brainstorming workshop and technical bodies (e.g. ICAR and IDF-ISO working groups) for the sake of the dairy community.

In this way the chairman Olivier Leray invited the participants to join the brainstorming workshop scheduled the next day so as to continue and refine the thinking in a still more interactive session and indicate that some reporting can be made the next week at the IDF-ISO Analytical Week 2012 in Tel-Aviv.

To conclude the chairman warmly thanked the speakers and attendees and invited them all to attend the next conference meeting of ICAR Reference Laboratory Network in Berlin during the next Biennial ICAR Session 2014. Christian Baumgartner (organising committee) provided some information on the event and pointed out that there will be a joint organisation of ICAR session and IDF-ISO Analytical Week 2014 in succession with an overlapping part common to ICAR and IDF for persons with shared interest. He expressed his hope and wish that it could meet the launching of the IDF-ICAR SCC Reference System.