Interlaboratory reference systems and centralised calibration – Prerequisites and standard procedures

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Summary

For the need of the international harmonisation and worldwide equivalence recognition of analytical data in milk recording, ICAR has undertaken the task to develop practical tools and methodologies in guidelines dedicated to laboratories to render calibration easier and cheaper as well as more efficient. Reference systems combining solid reference obtained through collaborative studies and centralised calibration are under focus and the underway description of adequate methods and procedures is presented.

Key words:*Reference, Calibration, Materials, Laboratories, Milk.*

Introduction

Genetic evaluation on milk composition has become possible only through the generalised use of rapid automated method of milk analysis. Mid infra red spectroscopic methods and fluoro-opto-electronic methods have become predominant till being the only techniques used in large milk routine testing for milk composition and somatic cell counting.

For such methods calibration of routine methods is the key operation but also the most expensive to laboratories as it requires a lot of time and competence in sample preparation and reference analysis.

Sharing calibration cost between several laboratories thus amortising over many milk sample testing appears an economical alternative for laboratories beside the possibility to optimise both calibration sample quality and harmonise reference results through same values to all the laboratories. Indeed such calibration system can be easily associated to interlaboratory studies in order to optimise of the trueness of reference values for calibration.

Promoting the implementation of robust reference established by laboratory groups and centralised calibration in so-called reference system has become a new objectives of ICAR from 2006. Recommendations as pre-requisites and optimum procedures for implementation are needed for international harmonisation.

Objectives and prerequisites

The objectives are to establish reference values for an appropriate material (milk) that can be valid for a community of laboratories (spread over a collect territory), transfer consensus reference values to the laboratories to calibrate routine methods and at end assess effectiveness of the system.

Prerequisites refer to the accuracy of routine methods, the harmonisation of laboratories, appropriate logistic conditions.

- Depending on the variation of milk composition in the collect areas and the sensitivity of methods to matrix effects, the accuracy value can become larger than in usual calibration. Before implementing centralised calibration it is of major importance to evaluate whether or not the extent of accuracy is acceptable for the intended purpose i.e. milk recording. With this respect it can be referred to experiment results presented in Kuopio (O. Leray, 2006).
- Methods, expression units (e.g. m/m, m/v, per 100 or per Kilo), criterion expression (e.g. True protein vs Crude protein) should be harmonised within the laboratory group so that calibration sample characteristics suit to every instrument equally.
- Sample preservation and transportation facilities should be adequate to analyse sample within short delays with no change in the physicochemical composition of calibration milk samples.

Means and tools

To achieve its goal, ICAR intends to produce suitable guidelines for laboratories on organising interlaboratory proficiency studies (PT) and centralised calibration (CC) and provide relevant services to countries.

International PT services are already supplied for the sake of the international reference harmonisation through a reference laboratory network according to a protocol approved by ICAR. This protocol should be detailed and become part of ICAR guidelines.

Developing/certifying international reference materials as gold standards is part of ICAR strategy beside promoting the use of national/local reference materials to relay international standard in countries either for checking reference methods or calibration.

Guidelines for proficiency studies

They will be in agreement with other general international standard on the subject which would be referred to but will additionally include specific requirements related to calibration and alternative methods thus establishing consistency with ISO 8196.

Particularly:

- The experimental design will be well stated with minimum numbers (e.g. 9 samples, 3 levels, 2 replicates) and concentration arrangement for optimised assessment (according to ISO 9622).
- Standard statistical analysis and presentation recommended using performance scores and target figures. Slope, linearity, interactions assessment will complete the statistical analysis for studies with routine methods. Examples were published in ICAR Session proceedings in Rotorua (1998) and the IDF Bulletin 342/1999.

Guidelines for centralised calibration

Guidelines will indicate protocols to:

- Evaluate the overall accuracy in a centralised calibration system.
- Define the characteristics of calibration reference material.
- Assign reference values.
- Provide indications for line adjustment in the laboratory.

Evaluation of the overall accuracy of a centralised calibration system

It can be performed by two ways, either once prior implementation of the system through an experiment provided natural conditions would not later, or through regular proficiency studies involving reference and routine methods.

Two protocols can be proposed depending on the situation:

1. In-lab experiment. It is carried out prior implementation with a unique instrument provided pre-required condition of harmonisation will be maintained later. A number of representative samples are collected in milk testing lab areas and analysed by the experimenting laboratory for both reference and routine methods. Operations are evenly repeated throughout a campaign of milk production and regional and seasonal effect are measured through ANOVA.
2. Interlaboratory studies. It is compared the reproducibility of routine methods to that of reference methods to decide whether or not centralised calibration provide equivalent laboratory bias distributions. In that case the information is general as the routine methods can be different with no relation to a well define analytical method. Recommendations of ISO 5725 are followed.

Characteristics of RMs for calibration

Adequate recommendation will be given to guarantee physicochemical quality of milk, sample preparation and batch homogeneity, preservation and shelf life, in particular with concern to the choice of the milk, milk and sample handling, chemical preservatives and sample containers.

Also indications for appropriate component arrangement and concentration range will be provided referring to optimisation of calibration and accuracy through specific designs with recombined (modified) milk samples (O. Leray, 1998, FAIR CE 1997-1999).

Assigning reference values

To limit the risk of systematic bias and get the agreement of all laboratories and parties they should not be established by a single laboratory but instead by all the laboratories of the concerned group.

The way to define reference values relates on whether or not matrix effects exist with the routine methods.

Where there is no matrix effect representativeness of calibration milk is of lower importance and focus is made only on physicochemical quality and concentration characteristics. Reference values are determined using the means of reference results of all the laboratories obtained in an interlaboratory study (Figure 1).

This is the same way also used in case of matrix effects when using milk materials well representative of the area (e.g. silo bulk milk) but choice must then be made on whether or not final calibration adjustments are locally required in laboratories with regard to laboratory biases observed. The assigned values are here used for

pre-calibration (assessing slope, linearity, inter-correction fittings) whereas calibration is completed using one or more bulk milks representative of the area (Figure 2).

When using recombined (so-called modified) milk samples, greatest interest must be given in maintaining the native physicochemical quality of milk hence representativeness may not be reached. Through the matrix effect so-prepared calibration sample are not on the average line of the population. The assigned values are then obtained through a correction from the bias between the routine and the reference method with one or more bulk milks representative of the area. Calibration can be completed or not for individual labs (as above mentioned) depending on the range of local biases and the need of milk testing purpose.

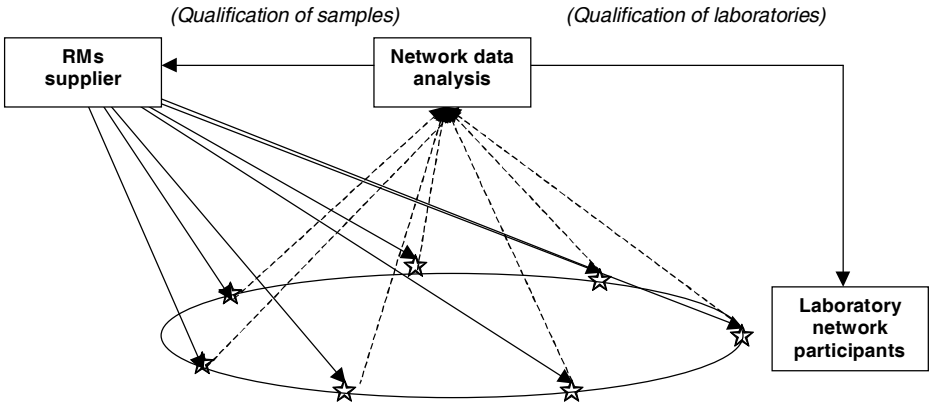

Figure 1. Scheme of assigning reference values for reference materials using interlaboratory proficiency studies.

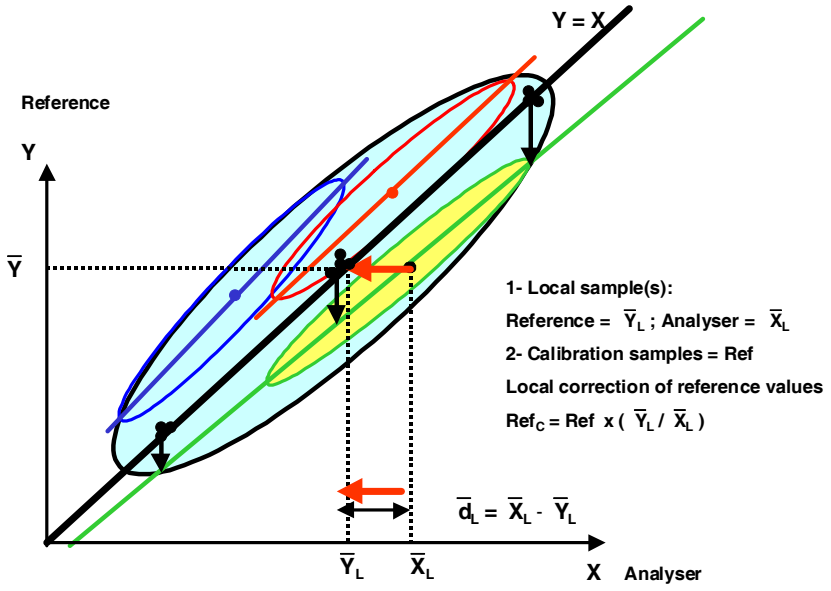

Figure 2. Correction of assigned values (Y) of calibration samples (black arrow) to reduce the laboratory bias (red arrow) analysing the yellow population.

Calibration

Recommendations for calibration operations are to follow the normal procedures of manufacturers and ISO 8196 in which centralised calibration is mentioned as a possible option. This is to 1- Check and where needed optimize instrument fittings (pre-calibration), 2- Adjust calibration, 3- Assign values for control samples.

Conclusion

Centralised calibration associated to collective determination of reference values for calibration is considered as an optimum combination to assure harmonisation of milk recording analytical data. Methodologies and technical tools have already been defined, experimented showing large efficiency. Such a combined system should serve ICAR countries to evolve towards easier and cheaper calibration systems and respond to forthcoming analytical demands of milk recording (for instance on-farm analysis).

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