ARTICLE

QUALITY PROVISIONS FOR THE PRODUCTION OF Cecalait® SECONDARY REFERENCE MATERIALS (SRMs)

For many years, Actilait (technical institute for milk and dairy products) has been proposing external reference materials (SRMs) for the calibration or control of methods in chemistry and microbiology, under the trademark Cecalait®.
Within the context of the quality control of analytical methods, secondary reference materials are complementary tools to proficiency tests to ensure the quality of the laboratories' determinations.
These secondary reference materials must therefore be produced according to a series of very strict quality provisions:
- From the outset, for milk SRMs, the milk must be selected and undergo treatments to avoid any physical alteration in order to ensure its quality and good preservation.
- The determination of the assigned values is the key point of the process. It is carried out by "expert" laboratories, rigorously monitored by the quality surveillance committee (CSQ). Moreover, a series of complementary technical dispositions ensure the quality of the assigned values sent to user laboratories.
- Finally, the express transport in well-defined conditions concludes this process.

A reference material is defined as a material (sample) one or more of whose properties are sufficiently established and defined.

Many types of reference materials exist:
- external reference materials (MRE), whose assigned value per analyte is determined by an interlaboratory test,

- internal reference materials (MRI), whose assigned value is established by the user (comparison with the MRE value, pure solutions...).

The Cecalait® reference materials proposed by Actilait are external reference materials (MRE). Two types of samples are proposed according to their purpose:

① **Calibration of a measuring instrument:** In this case, the samples are often multi-level, covering the totality of the range of application of the method. They are used to establish the calibration function (most often according to a linear model) between the instrument and the reference. Within the context of quality control associated with the adjustment, the laboratory will then proceed with a verification step (with or without specific samples) and an examination of the statistical parameters of the calibration (slope, standard deviation of deviations, residual standard deviation of regression...).

Concerning Cecalait® SRMs, this is the case for amido black, somatic cells, lipolysis (copper soap), urea, cryoscopy and infrared (median and high range (pre-calibration)) samples.

② **Quality control of measurements*:*** In this case, the samples are mainly mono-level. Following the analysis of the material by the laboratory, the results obtained are compared with the reference value and the laboratory interprets the deviation observed in relation to the tolerance (Shewhart-type control charts, comparison with a tolerance fixed beforehand, calculated according to the principle of the critical difference at 95 %).

Concerning Cecalait® SRMs, are included according to the matrix or the domain:

- *Milk: chemical method (Gerber, Kjeldahl, dry matter…),*
- *Butter and cheese: composition parameters (dry matter / moisture, fat, nitrogen, chloride…),*
- *Microbiology: microorganisms at 30 °C, coagulase positive Staphylococcus and E. coli.*

The production and determination of these samples are subjected to a series of measures throughout the various phases of the process.

The process can be split up into 3 main stages:

- Sample preparation,
- Determination of assigned values,
- Dispatch to clients.

1- Sample preparation:

In general, the physico-chemical samples are prepared in-house either from milk (preserved with bronopol) or dairy products, or externally in pilot industrial plants. The microbiological samples are prepared with milk and then freeze-dried to ensure their stability.

♦ *Chemistry:*

• Concerning the samples prepared with raw milk, the bacteriological quality of the milk has to be good and it must not have undergone any physical treatment that may deteriorate it, such as pumping, cooling or reheating... In practice, it will always be from small quantities of milk, from a single milking without any cooling and directly taken from the cheese dairy when the milk is delivered.

Moreover, all the pre-treatment operations must not deteriorate the milk or its components. With suitable equipment it is therefore possible to carry out the following operations: siphoning, separation of compounds by micro- or ultra-filtration.
Supplementary stabilisation treatments can, if necessary, be applied for certain criteria (for example heat treatment for lipolysis samples).

• For the other matrixes (butter, cheese), the samples supplied by pilot plants must be received in their final format. The pilots have been chosen to ensure the homogeneity of the batches sent to the laboratories (test performed in the sample feasibility phase).

• The principle of the distribution in vials is to ensure the quality of the milk (in particular fat) and guarantee the homogeneity of the batch produced. For milk samples, the initial mixtures are distributed in vials under constant magnetic agitation and without any air being incorporated by siphoning. The vials used in this case are provided with screw-caps (triple seal) to ensure air tightness and are filled to the brim to avoid churning.

♦ *Microbiology:*

• For the microbiological samples, the chosen treatment has to fulfil 2 objectives: guarantee the homogeneity between vials and obtain samples representative of a natural sample (diversity of the flora) to ensure the transferability of the results obtained to the routine samples.

• For the preparation of SRMs for the enumeration of microorganisms at 30°C, raw milk from several producers, with a natural, varied flora, is used. Concerning the SRMs for the enumeration of staphylococci and *E. coli*, the samples are prepared with sterilised milk contaminated with both of these strains and associated flora, isolated from dairy products.

• The freeze-drying diagram and the rehydration protocol have been studied so as not to deteriorate the bacteria and to enable their revivification before analysis.

- Homogeneity controls

Homogeneity tests are performed with routine analysers: infrared analyser for milk (for fat), flow cytometry germ counter for the microbiological parameters, cell counter for somatic cells. These controls are not systematically carried out on all the batches produced.

2- Determination of assigned values:

The assigned (reference) values are obtained by a specific interlaboratory test integrating 4 or 5 expert laboratories (according to the criteria).
These expert laboratories have been chosen because they have ISO 17025 accreditation, where possible, for the criteria under consideration, and are monitored yearly according to very strict specifications:

- Participation in at least two proficiency tests per year (of which at least 1 Cecalait®) with a minimum performance level of 75 % (3 tests out of 4 within the accepted tolerances).
- Inclusion of the laboratory's results in the calculation of the assigned values in at least 75 % of cases.

Each year the Cecalait® quality surveillance committee of Actilait decide on the renewal or not of the expert laboratories on the basis of their results over two rolling years in relation to the specifications.
In case of non-renewal the laboratory is informed and a new expert laboratory is sought for the criterion considered.

For the determination of the assigned value, the expert laboratories receive the samples for analysis. A series of quality provisions are integrated according to the criteria and the matrixes:

- Analysis in duplicate (Gerber, dry matter, nitrogen…),
- Analysis of month M-1 batch (with blind codification of month M and M-1 samples),
- Analysis of sample stability (fat by extraction),
- Analysis of pure solutions (Kjeldahl…),
- Instrumental verification of the results (lipolysis, somatic cells...).

The tolerances are accurately formalised for each procedure (maximum deviation between duplicates, tolerance according to M-1 samples, % recovery of pure solutions…).

Firstly, laboratories are selected on the basis of the quality controls above. The mean of their results is then calculated after elimination if necessary of any results "detrimental to the symmetry".
In the case of certain calibration samples (lipolysis, urea, somatic cells), a linearization of the different contents is realised according to the dilution factors used (mixture of a "rich" base and a "poor" base).

To be validated as the assigned value, the calculated mean has to fulfil two additional criteria: a minimum number of results taken into account and a maximum range (standard deviation of the series). These provisions, "minimum number of results" and "maximum standard deviation", are formalised for each SRM in the quality documentation.

If not, (failure to meet one or more of these provisions), the samples are sent to the expert laboratories again for analysis (2^{nd} analytical series).
The assigned value is then calculated as above, integrating the results of both analytical series.

Following this determination, a certificate indicating the assigned value(s), the associated uncertainty (in the majority of cases) and the use-by date is issued for this SRM.

3- Dispatch to clients:

Specific methods of packaging exist according to the matrixes and criteria of the SRMs:

- insulated boxes with ice or not.

These provisions are accurately established for each type of SRM (type of box, presence or not of ice and number of ice packs according to the box).

The samples are then sent by "express" for delivery the next day in the majority of European Union countries and within 2 to 3 days for other countries.

Only compliance with all these provisions guarantees the quality of the samples and the associated assigned values sent to the laboratories. Thus their use will effectively contribute to the process of controlling the quality of the results.