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Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis —

Part 2: Calibration and quality control in the dairy laboratory

Lait — Définition et évaluation de la précision globale de méthodes indirectes d'analyse du lait —

Partie 2: Étalonnage et contrôle de la qualité dans les laboratoires laitiers

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 8196 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 8196-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations

ISO 8196 consists of the following parts, under the general title *Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis*:

— *Part 1: Analytical attributes of indirect methods*

— *Part 2: Calibration and quality control in the dairy laboratory*

Introduction

The main purpose of this part of ISO 8196 is to give practical details and recommendations for the calibration of instruments and quality control in routine dairy laboratories.

While ISO 8196-1 is mainly intended for experts to assess new indirect instrumental methods of analysis, this part gives guidance for routine laboratories using these methods.

Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis —

Part 2: Calibration and quality control in the dairy laboratory

1 Scope

This part of ISO 8196 gives recommendations and an example for the calibration of instruments, and for the quality control procedure in dairy laboratories.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 8196. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreement based on this part of ISO 8196 are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 8196-1:2000, *Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis — Part 1: Analytical attributes of indirect methods.*

3 Terms and definitions

For the purposes of this part of ISO 8196, the terms and definitions given in ISO 8196-1 apply, together with the following.

3.1
standardization of an instrument
experimental evaluation of the exactness of the calibration of an instrument by reference to the true values given either by a reference method or by standard materials or a standard instrument.

3.2
calibration of an instrument
adjustment of the signal from an instrument so that, at each level of the component, the mean of individual test results given by the instrument approximates closely to the true value of the component concentration

4 Calibration of instruments

4.1 General principles

This clause considers only the general principles of calibration which apply to any indirect method of milk analysis. Detailed and specific instructions for the calibration procedure, as well as for the preliminary checks concerning each group of methods, will be given in specific standards.

Even if the term “calibration” is often used for both the standardization and calibration of instruments (see clause 3), it is recommended to use these words with their proper meanings.

4.2 General procedure

4.2.1 Preliminary checks

Preliminary checks include the following.

a) Instrument checks: all functional checks and adjustment (zero setting) of the instrument specified by the standard or the manufacturer should be carried out prior to the analysis.

b) Linearity: unless otherwise stated, the relationship between the instrumental signal readings and the component concentration is linear within the specified range of concentration. Checking the linearity is a measure of how precisely the instrument follows the law of sample absorption. Normally, it is only necessary to check and adjust this on new instruments or whenever major parts (e.g. cell or servo-system) are serviced or replaced.

c) Repeatability and accuracy checks: the repeatability and accuracy of the instrument should comply with the standard specifications.

4.2.2 Standardization of the instrument

4.2.2.1 Test samples

4.2.2.1.1 Milk samples

Raw milk samples collected especially for that purpose or, when available, standard materials (milk samples or non-milk substitutes) may be used to standardize the instrument. In this part of ISO 8196, only standardization with raw milk samples analysed by the instrument and a reference method are considered.

In order to obtain the most accurate estimate of the calibration line, two major requirements should be fulfilled:

— the milk samples should cover the whole range of concentration of the component, and

— the residual standard deviation from the regression should be a minimum.

Generally this can be best obtained by pooling individual milk samples selected at different levels of concentration with a maximum of samples chosen for values close to the extremities of the range.

Sometimes, for testing of fat, the addition of cream to skimmed milk is suitable.

4.2.2.1.2 Number of samples

A minimum of 8 sets of pooled individual milk samples or at least 40 individual milk samples are required.

At least duplicate tests should be performed by reference and instrumental methods in order to reduce repeatability errors.

4.2.2.1.3 Nature of samples

Collect samples which are representative of the milk produced in the area under test. Discard colostrum and severe mastitic milk. When possible, these samples should be tested at the same age, preserved with the same chemical at the same concentration, and have undergone the same treatment as the samples which are normally analysed. This will ensure that the instrument calibration takes care of the small variations due to the travelling, preservation and storage conditions of the samples. However, before treating the samples in the form of sub-samples by the appropriate means, analyse them while fresh using the reference method.

4.2.2.2 Statistical analysis

Calculate the regression equation $\bar{y}_i = b\,\bar{x}_i + a$ from analysis data, using the least-squares method, where y_i refers to the reference method, and x_i to the instrument. See ISO 8196-1.

Calculate the residual standard deviation from the regression ($s_{y,x}$).

This value should be within the limits of the specification given by the standard for the accuracy. Then carry out the following steps.

a) Test if the slope differs statistically from 1,000 by calculating the standard deviation of b:

$$s_b = \left[{s_{x,y}}^2 \Big/ \sum (x_i - \bar{x})^2 \right]^{\frac{1}{2}}$$

The slope is correct if it is in accordance with one of the following equations:

$$b - t_{1-\alpha/2} \cdot s_b \leqslant 1{,}000 \leqslant b + t_{1-\alpha/2} \cdot s_b$$

or

$$t_{\text{obs}} = |b - 1| / s_b \leqslant t_{1-\alpha/2}$$

where t is the value of the Student distribution (see ISO 8196-1 and ISO 3534-1). It shows both the random variable (t) and a particular or observed value of this variable (t_{obs}).

b) Test the null hypothesis that the regression line goes through the centre of gravity of the sample population by calculating the standard deviation of $\bar{y}(\bar{x}) = b\bar{x} + a$

$$s_{\bar{y}(\bar{x})} = s_{y,x} \Big/ \sqrt{q}$$

where q is the number of samples.

The mean adjustment is correct if carried out in accordance with one of the following equations:

$$\bar{y}(\bar{x}) - t_{1-\alpha/2} \cdot s_{\bar{y}(\bar{x})} \leqslant \bar{x} \leqslant \bar{y}(\bar{x}) + t_{1-\alpha/2} \cdot s_{\bar{y}(\bar{x})}$$

or

$$\left[\bar{x} - \bar{y}(\bar{x})\right] - t_{1-\alpha/2} \cdot s_{\bar{y}(\bar{x})} \leqslant 0 \leqslant \left(\bar{x} - \bar{y}(\bar{x})\right) + t_{1-\alpha/2} \cdot s_{\bar{y}(\bar{x})}$$

or

$$t_{\text{obs}} = \left[\bar{x} - \bar{y}(\bar{x})\right] \Big/ s_{\bar{y}(\bar{x})} \leqslant t_{1-\alpha/2}$$

with $q - 2$ degrees of freedom and $\alpha = 0{,}05$.

c) Adjustment of the calibration will be necessary if the slope and/or the mean bias of the regression are found to be different from 1,000 and zero, respectively.

The second test (b) is equivalent to testing the mean of differences $d_i = x_i - y_i$, or mean bias $\bar{d}$ versus zero when the slope is not statistically different from 1,000, since

$$\bar{y}(\bar{x}) = \bar{y} \text{ and } d = \bar{x} - \bar{y} = \bar{x} - \bar{y}(\bar{x})$$

In that case the adjustment at the average level is correct if carried out in accordance with the following equation:

$$\bar{d} - t_{1-\alpha/2} \cdot s_d \,/\sqrt{q} \leqslant 0 \leqslant \bar{d} + t_{1-\alpha/2} \cdot s_d \,/\sqrt{q}$$

with q – 1 degrees of freedom and α = 0,05.

d) Testing the bias implies application of the test in a second step: indeed it may not be significant if the slope differs from 1,000, because s_d increases in parallel with the bias of the slope to 1,000.

If both tests (slope and mean adjustment) are negative, the intercept will not statistically differ from zero according to one of the following equations:

$$a - t_{1-\alpha/2} \cdot s_a \leqslant 0 \leqslant a + t_{1-\alpha/2} \cdot s_a$$

or

$$t_{\text{obs}} = |a| / s_a \leqslant t_{1-\alpha/2}$$

with $s_a = s_{y,x} \left(1/q + \bar{x}^2 / \text{SOS}_x\right)^{\frac{1}{2}}$

4.2.3 Calibration of the instrument

By using the appropriate control systems of the instrument, adjust the calibration slope and signal so that the observed calibration line fits the theoretical line. As some instruments allow only adjustment of the slope (assuming that the intercept is always zero), while with others a correction of both slope and intercept (backing off) is possible, no general rule can be laid down. For a correct setting of the calibration, follow the manufacturer's instructions and the specific standard.

In all cases, after the final adjustment has been made, check using milk samples that the calibration is correct both in slope and intercept, i.e. at low and high levels. It is important to ensure that the mean values obtained with both the instrument and the reference method or the standard materials are not statistically different.

4.3 Frequency of calibration control

Under routine conditions of use, the standardization of the instrument and its possible calibration readjustment are necessary only when the relationship between the instrument readings and the reference may have changed. This is likely to occur under the following conditions:

— after repair or servicing of the major components of the instrument (i.e. cell, servo, homogenizer, optical filters);

— when the properties or the composition of milk influencing the component measurement have changed under various biological conditions.

Assuming that the composition and the properties of milk remain unchanged within 1 week or 2 week periods, it is not necessary to standardize the instrument too frequently (for example every day). This procedure is costly and if carried out with too few samples or with samples not obtained specifically for that purpose, it might introduce further errors which would reflect inadequacy through a poor standardization procedure rather than real variation of the instrument calibration.

With the advent of microprocessors, it is anticipated that adjustment of the calibration will be performed automatically with a high degree of precision.

4.4 Centralized calibration

When it has been clearly demonstrated that a single calibration of the instrument can be used to analyse samples from various origins without a loss in accuracy, a centralized system of calibration is recommended. This can be done by a reference laboratory which will standardize and calibrate a reference or master instrument according to the specified method. Then, by means of suitable standard materials, the calibration of the reference instrument may be transferred to identical instruments in other laboratories.

5 Quality control in a routine dairy laboratory

NOTE This clause deals with all checks that should be performed by a routine dairy laboratory to ensure the quality of its analytical results. These recommendations can be considered as good laboratory practice.

5.1 Verification of repeatability

The first and most frequent check which has to be carried out is on the repeatability, because it is the simplest test indicating whether or not the instrument is working properly.

Check that, for repeatability conditions, the standard deviation of repeatability for the instrument is in accordance with the specification given by the standard.

Select a set of q samples (q = 20) in good physico-chemical condition and covering a wide composition range. Analyse them consecutively twice in random order so as to take into account the carry-over effect. Record the absolute difference between duplicate results (w_i) and calculate the standard deviation of repeatability s_r using the equation:

$$s_r = \left(\frac{1}{2q} \sum_{i=1}^{q} {w_i}^2 \right)^{\frac{1}{2}}$$

If a smaller number of samples is used, repeat the determination at least three times and calculate s_r using a one-way analysis of variance. The variance of error (s_e^2), or within-sample variance, is an unbiased estimator of σ_r^2.

Action should be taken if s_r is larger than the specified value.

5.2 Daily check on short-term stability of the instrument

5.2.1 Objective

This check is typical for any automatic instrumental method. Instability of the instrument signal may arise from several origins, for example electric drift, temperature variation, milk soil deposit on cell walls, etc.

Check, by analysing regularly one or more control milk samples, that the results remain within accepted tolerances, assuming that no changes in the major physico-chemical characteristics of the control milk occur during the checking period. This test is useful not only for checking the instrument stability during a working day but also from day to day between two standardizations of the instrument against the reference method.

5.2.2 Procedure

5.2.2.1 Select one milk sample of average composition or, preferably, two milk samples of low and high content using commercial pasteurized milk in which the range of fat, protein and lactose has been extended by the addition of skim milk powder, cream, etc.

Prepare carefully, under constant agitation, as many test portions as required for one or more working days. Store them with a suitable preservative at 4 °C. Good quality pasteurized milk, preserved for instance with Bronopol, can be stored safely for 2 weeks. With instruments having an homogenizer, homogenized milk can be used only if the homogenization efficiency is checked separately.

Analyse control samples on a regular basis every 40 to 60 samples.

5.2.2.2 To monitor the quality of the whole analytical procedure, including of course the instrument stability, set up a control chart according to the following principles.

a) Determine carefully the average reference content (m_0) of the control milk and the day-to-day or within-day standard deviation of reproducibility (σ_R) of the method. If unknown, σ_R may be roughly estimated as twice the value of the standard deviation of repeatability.

b) The control chart (see Figure 1) will represent:

1) a straight line in the centre corresponding to the reference value m_0;

2) a lower and an upper "confidence belt" which correspond to the $1 - \alpha$ probability of the two-sided confidence limits of the cumulative mean m of the control milk results; these limits are obtained using the formula:

$$m_0 \pm u_{1-\alpha/2} \cdot \sigma_R / \sqrt{n}$$

where

u is a particular value of the standardized normal random variable U (see ISO 8196-1 and ISO 3534-1); its value depends on the probability level ($1 - \alpha$) and the degrees of freedom;

n is the number of control samples analysed;

α is the probability of rejecting the null hypothesis ($m = m_0$) although it is correct, which would indicate the need to readjust the instrument when this is not necessary; to avoid too frequent and unnecessary adjustment, a 0,01 probability level can be used; in that case the value $u_{1-\alpha/2} = 2{,}58$;

3) a lower and an upper "individual line" which correspond to the $(1 - \alpha)$ probability of the two-sided statistical tolerance interval of individual tests; these limits are obtained from

$$m_0 \pm k_{(n,p,1-\alpha)} \cdot \sigma_R$$

where k is a coefficient determined for n number of samples and the probability level $1 - \alpha$ that the statistical interval contains at least a proportion p of the population.

NOTE For an infinite value of n, with $1 - \alpha = 0{,}99$ and $p = 99$ %, the value of $k = 2{,}58$.

c) Plot on the control chart each individual result of the control sample and the arithmetic mean (m) of results of the samples which have been analysed.

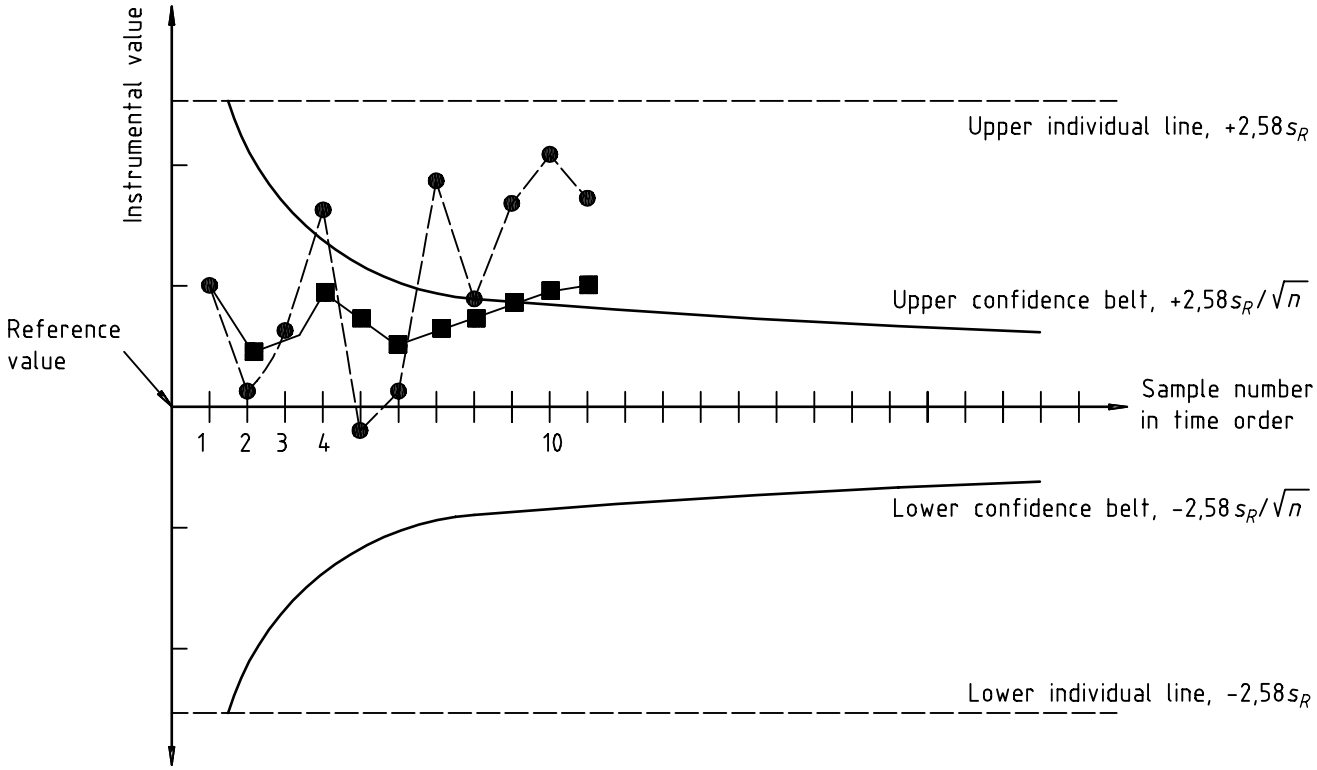
Action should be taken when:

1) the arithmetic mean (m) is, for two consecutive samples, outside the same (upper or lower) confidence belt, indicating that the instrument is drifting; this deviation should normally be in the same direction as the deviations of the individual results outside the corresponding individual line;

2) individual results fall frequently near or outside the individual lines, indicating a poor repeatability of the instrument or poor quality of the milk sample.

In each case, stop and check the instrument functions and, if necessary, readjust the calibration. The use of a microcomputer and automatic data capture system is very helpful, but in that case an automatic correction of results is strongly prohibited, in order to avoid giving "correct" results when the instrument is not working properly.

After readjustment of the instrument, make a new control chart.



Key

- - - - individual results

—— arithmetic mean of results

NOTE 1 Confidence belts relate to the arithmetic mean of the cumulative mean results $(\bar{x}_{\bar{i}})$.

NOTE 2 Individual lines relate to the individual results.

NOTE 3 Limits contain 99 % of the population with a 99 % probability.

Figure 1 — Control chart model for instrumental method of analysis

5.3 Verification of bias between laboratories

5.3.1 General

Checking differences between laboratories, regardless of their values as compared to the true value, is part of the laboratory performance checks. It is done through interlaboratory trials in which participating laboratories are asked to analyse a set of milk samples with the same (or sometimes different) type of method of analysis. Besides repeatability errors, differences between laboratories originate from the theoretical reproducibility of the method and mainly from differences in calibration. The calibration setting of instruments can be done by means of reference milk samples or by comparison with a reference method, each laboratory using its own set of milk samples. In the latter case, checking laboratory performance through an interlaboratory study may not be very meaningful if the origin of milk samples has a significant influence on the calibration of the instruments.

5.3.2 Procedure

Check laboratory bias in accordance with the method described in ISO 8196-1.

When the instrumental method is almost unaffected by the origin of milk samples, best results will be obtained by using reference milk samples for calibration.