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

Part 2: Calibration and quality control in the dairy laboratory

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

Partie 2: Calibrage et contrôle qualité dans les laboratoires laitiers

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 8196-2|IDF 128-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This second edition of ISO 8196-2|IDF 128-2 cancels and replaces the first edition (ISO 8196-2:2000), which has been technically revised.

ISO 8196|IDF 128 consists of the following parts, under the general title *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis*:

- *Part 1: Analytical attributes of alternative methods*
- *Part 2: Calibration and quality control in the dairy laboratory*
- *Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis*

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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ISO 8196-2|IDF 128-2 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

All work was carried out by the Joint IDF-ISO Action Team on *Automated methods* of the Standing Committee on *Quality assurance, statistics of analytical data and sampling* under the aegis of its project leader, Mr. O. Leray (FR).

This edition of ISO 8196-2|IDF 128-2, together with ISO 8196-1|IDF 128-1 and ISO 8196-3|IDF 128-3, cancels and replaces IDF 128:1985, which has been technically revised.

ISO 8196|IDF 128 consists of the following parts, under the general title *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis*:

— *Part 1: Analytical attributes of alternative methods*

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

— *Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis*

Introduction

The main purpose of this part of ISO 8196|IDF 128 is to provide practical details and recommendations for the calibration of instruments and quality control in routine dairy laboratories, including the checking of compliance with a specification value or limit.

ISO 8196-1|IDF 128-1 is mainly intended for users to assess alternative methods of analysis and gives guidance for routine laboratories using these methods.

This part of ISO 8196|IDF 128 relates directly to ISO 8196-1|IDF 128-1 for the definition of the relevant performance characteristics, for the quantitative evaluation of the overall accuracy and the establishment of relevant standard limit values to comply with in analytical quality assurance as described. The general concepts apply to all analytical methods, but special emphasis is given to rapid physicochemical methods which are currently in use for the compositional testing of milk.

ISO 8196|IDF 128 (all parts) only specifies the single linear regression model as a simplified approach to allow users to determine equivalence of an alternative method with a reference method. However, the linear regression approach is valid as a determination of method equivalence only in limited circumstances or if a high correlation between the results of the reference method and the routine method is achieved. If a high correlation is not achieved, recourse should be made to other data handling and measurement error modelling techniques. Although these techniques are referred to, they are not specified in ISO 8196|IDF 128 (all parts).

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

Part 2:
Calibration and quality control in the dairy laboratory

1 Scope

This part of ISO 8196|IDF 128 gives guidelines for the calibration of instruments and quality control procedures for milk analysis in dairy laboratories.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8196-1|IDF 128-1:2009, *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis*

ISO 8196-3|IDF 128-3:2009, *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis*

3 Terms, definitions, and symbols

3.1 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8196-1|IDF 128-1 and the following apply.

3.1.1
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

3.1.2
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 closely approximates the true value of the component concentration

IMPORTANT — Even if the term "calibration" is often used for both the standardization and calibration of instruments (see Clause 4), the use of these words according to the definitions in 3.1.1 and 3.1.2 is strongly recommended.

3.2 Symbols

b	linear regression coefficient or slope
$\bar{d}$	average of differences d_i or mean bias
d_i	difference between means of duplicates of x_i and y_i
$\bar{d}_{\text{rel}}$	relative mean bias
$L_{\bar{d}}$	limit of the mean bias $\bar{d}$
$L_{\bar{d}\text{rel}}$	limit of the relative mean bias
m_0	reference value for the control chart
n	number of replicates
P_{xy}	sum of products of x and y
q	number of samples
r	repeatability limit
r_{xy}	correlation coefficient
R	reproducibility limit
S_d	sum of squares of d
S_x	sum of squares of x
S_y	sum of squares of y
s_b	standard deviation of slope
s_{x_0}	standard error of estimate x_0
s_y	estimate of standard deviation of y
s_{yx}	estimate of the standard deviation of accuracy
t_{obs}	observed value of the Student t-test
$t_{1-\alpha/2}$	t-value of the Student distribution for a two-sided probability $1-\alpha$
$t_{1-\alpha}$	t-value of the Student distribution for a one-sided probability $1-\alpha$
$u_{1-\alpha/2}$	value of the standard normal distribution for a two-sided probability $1-\alpha$
$\bar{x}$	arithmetic average of $\bar{x}_i$
$\bar{x}_i$	mean of duplicates of x_i
$\bar{y}$	arithmetic average of $\bar{y}_i$
$\bar{y}_i$	mean of duplicates of y_i
$\bar{y}(\bar{x})$	predicted values from $\bar{x}$ by linear regression.
ν	degree of freedom
σ_r	standard deviation of repeatability
σ_R	standard deviation of reproducibility
σ_y	standard deviation of y
σ_{yx}	standard deviation of accuracy
$\sigma_{yx\text{rel}}$	relative standard deviation of accuracy

4 Calibration of instruments

4.1 General principles

This clause only considers the general principles of calibration which apply to any alternative method of milk analysis.

Detailed and specific instructions for the calibration procedure, as well as for the preliminary checks concerning each group of methods, shall be given in specific documentation.

The calibration here described is based on the assumption of an existing linear relationship between the alternative method and the reference method. The utilization of an ordinary least-squares (OLS) linear regression model with the reference method as dependent variable (y-axis) is recommended as practical and reliable:

a) the measured residual standard deviation is structurally the lowest and is independent of the exactness of calibration, hence it can be used for calibration control as a method accuracy characteristic (see ISO 8196-1|IDF 128-1);

b) prediction of true values from alternative method values and calculation of associated errors is simplified;

c) the differences in measurement results as compared to applying other linear regression models are not significant.

4.2 General procedure

4.2.1 Preliminary checks

4.2.1.1 Instrument checks: all functional checks and adjustments (zero setting) of the instrument specified by the relevant International Standard or the manufacturer should be carried out prior to the analysis.

4.2.1.2 Linearity: unless otherwise stated, the relationship between the instrumental signal readings and the component concentration is linear within the specified range of concentration. Normally, for alternative methods, it is only necessary to check and adjust linearity on new instruments or whenever major parts (e.g. cell or servo-system) are serviced or replaced.

4.2.1.3 Interference corrections (inter-corrections): where instrumental compensations for interfering components are applied to optimize accuracy, these should be checked and eventually adjusted prior to the analysis.

4.2.1.4 Repeatability and accuracy checks: the repeatability and accuracy of the instrument should comply with the specifications of the relevant International Standard.

4.2.1.5 Linearity and interference corrections on a single matrix. In order to check both linearity (4.2.1.2) and interference corrections (4.2.1.3), a set of samples can be prepared from a single milk matrix in such a way as to cover the concentration range of a component while maintaining others constant. For linearity, the component range relates to the measurand and the alignment of measurement results is checked against the theoretical mixing ratio or the relevant concentration calculated from prior analyses. For interference correction, the range concerns the interfering component, and the absence of related induced bias for the measurand requires checking.

4.2.2 Standardization of the instrument

4.2.2.1 Test samples

4.2.2.1.1 General requirements

Milk samples collected especially for that purpose or, when available, standard materials with similar characteristics may be used to standardize the instrument.

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

a) the samples cover the whole range of concentration of the component;

b) the residual standard deviation from the regression is minimal.

Depending on the measurand, this can best be 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 or by separation and recombination of milk components or by spiking and/or aqueous dilution. Prior to use, the equivalence of the alternative calibration samples for the component should have been validated by comparison with usual milk samples.

4.2.2.1.2 Nature of samples

For any calibration, it is of utmost importance that calibration samples are representative of the samples routinely tested, that is same types of milk (i.e. milk from individual animals, herd bulk milk, silo milk, processed milk), submitted to the same treatment and preservation, and originating from the same collection areas at the same periods of time. The period during which the calibration samples remain valid is to be established experimentally, taking into account changes in milk production factors related to season and regional animal feeding practices.

Discard milk of evident poor physical quality so as to guarantee homogeneous intakes and proper functioning of the instrument (suitable milk flow).

The reference method should be applied to the fresh calibration sample, i.e. before eventually treating the samples in the form of sub-samples.

4.2.2.1.3 Range of measurand

Samples collected should cover the whole range of concentrations for the component of interest. The larger the range used for calibration, the more accurate the adjustment of the calibration line. In that respect, it is recommended that the standard deviation of reference results, s_y, complies with Condition (1):

$$s_y \geqslant 5 s_{yx} \quad (1)$$

or, equivalently, the correlation coefficient, r_{xy}, complies with Condition (2)

$$r_{xy} \geqslant 0{,}98 \quad (2)$$

Alternatively, where the accuracy and/or the measurand range cannot meet the recommendation, appropriate alternative calibration samples (4.2.2.1.1) might be considered.

NOTE Equivalent to sample pooling (4.2.2.1.1), pooling of individual data can serve to produce a suitable virtual calibration data set where sufficient correlation cannot be achieved by physical means (see ISO 8196-3|IDF 128-3:2009, 5.2.2.2.3.2).

4.2.2.1.4 Number of samples

4.2.2.1.4.1 The number of samples depends on the objective of the laboratory, the accuracy of the method and the heterogeneity of the sample population for the measurand.

4.2.2.1.4.2 Select the number of samples with respect to composition representativeness for main components.

Define the number of samples needed with respect to the various origins of the test samples and the degree of their representativeness reached by assembly. For instance, in the analysis of composition, the numbers of representative samples recommended for use as a minimum are listed in Table 1.

Table 1 — Origin and number of representative samples

Milk type	No. representative samples
Individual animal	100
Herd bulk	40
Bulk	6
Processed	Set of milk samples produced from the original bulk milk or the bulk material used in the process, for appropriate levels of the measurand

Otherwise, a set of alternative calibration samples of equivalent representativeness prepared from at least nine equidistant measurand concentrations over a range relevant for the commingled milk samples can be used.

NOTE A minimum number of nine concentration levels for alternative calibration samples allows for a regular coverage for main milk components (i.e. fat, protein and lactose) and at the same time sufficient component combinations to check instrument fittings (linearity, interferences).

4.2.2.1.4.3 Ensure the exactness of calibration throughout the range of concentration, i.e., as specified in 4.2.2.1.4.4 and 4.2.2.1.4.5.

4.2.2.1.4.4 For the mean level, choose the minimum sample number so that any calibration bias (so-called mean bias $\bar{d}$) exceeding the limits $\pm L_{\bar{d}}$ set by the user is statistically significant for a risk α of error. From this prerequisite it follows that the limits of uncertainty of the estimated bias should not be larger than the calibration limits stated.

From the standard deviation of accuracy, σ_{yx}, estimated by s_{yx} in a former evaluation (see ISO 8196-1|IDF 128-1 and ISO 8196-3|IDF 128-3) and the previously defined limits of calibration bias $\pm L_{\bar{d}}$, the number q should fulfil Conditions (3):

$$L_{\bar{d}} \geqslant \frac{u_{1-\alpha/2}\,\sigma_{yx}}{\sqrt{q}} \quad \Leftrightarrow \quad q \geqslant \frac{u^{2}_{1-\alpha/2}\,\sigma^{2}_{yx}}{L^{2}_{\bar{d}}} \quad \Leftrightarrow \quad q \geqslant \frac{3{,}84 \times \sigma^{2}_{yx}}{L^{2}_{\bar{d}}} \tag{3}$$

or using the limits $\pm L_{\bar{d}\mathrm{rel}}$ of the relative mean bias $\bar{d}_{\mathrm{rel}}$ and the relative standard deviation of accuracy $\sigma_{yx\mathrm{rel}}$:

$$q \geqslant \frac{u^{2}_{1-\alpha/2}\,\sigma^{2}_{yx\mathrm{rel}}}{L^{2}_{\bar{d}\mathrm{rel}}} \quad \Leftrightarrow \quad q \geqslant \frac{3{,}84 \times \sigma^{2}_{yx\mathrm{rel}}}{L^{2}_{\bar{d}\mathrm{rel}}} \tag{4}$$

where

$u_{1-\alpha/2}$ is the value of standard normal distribution for a probability level $1-\alpha$;

with

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

$$\bar{d}_{\text{rel}} = \bar{d} \times 100/\bar{y}$$

$$\sigma_{yx\text{rel}} = (\sigma_{yx}/\bar{y}) \times 100$$

$$\alpha = 0{,}05$$

EXAMPLE

Fat:	σ_{yx} = 0,07 % mass fraction fat	with $L_{\bar{d}}$ = 0,02 % mass fraction fat accepted	$\Rightarrow q \geqslant 49$
Somatic cell count:	$\sigma_{yx\text{rel}}$ = 10 %	with $L_{\bar{d}\text{rel}}$ = 3 % accepted	$\Rightarrow q \geqslant 43$

4.2.2.1.4.5 For the calibration line, the limits for the relative uncertainty of slope b should not exceed the maximum value δb_{rel} defined beforehand by the user for the slope bias, so that any error in excess of this is significant. Thus, with a sample population already known with regard to σ_{yx} and σ_y or the usual correlation coefficient, r_{xy}, the number q should fulfil Conditions (5):

$$q \geqslant u_{1-\alpha/2}^2 \times 100^2 \left(\frac{\sigma_{yx}^2}{\sigma_y^2 - \sigma_{yx}^2} \right) \frac{1}{\delta b_{\text{rel}}^2} \Leftrightarrow q \geqslant 38\,400 \left(\frac{\sigma_{yx}^2}{\sigma_y^2 - \sigma_{yx}^2} \right) \frac{1}{\delta b_{\text{rel}}^2} \tag{5}$$

or equivalently

$$q \geqslant u_{1-\alpha/2}^2 \times 100^2 \left(\frac{1}{r_{xy}^2} - 1 \right) \frac{1}{\delta b_{\text{rel}}^2} \Leftrightarrow q \geqslant 38\,400 \left(\frac{1}{r_{xy}^2} - 1 \right) \frac{1}{\delta b_{\text{rel}}^2} \tag{6}$$

with

$$\delta b_{\text{rel}} \geqslant u_{1-\alpha/2}\, \sigma_b \times 100/b = u_{1-\alpha/2} \times 100\, [\sigma_{yx}/(\sigma_y \sqrt{q})]$$

$$\alpha = 0{,}05$$

EXAMPLE

Fat:	σ_y = 0,5 % mass fraction	σ_{yx} = 0,07 % mass fraction	(r_{xy} = 0,990 1)	with a limit δb_{rel} = 4 % $\Rightarrow q \geqslant 48$
Free fatty acids:	σ_y = 0,5 mmol/100 g	σ_{yx} = 0,15 mol/100 g	(r_{xy} = 0,953 9)	with a limit δb_{rel} = 5 % $\Rightarrow q \geqslant 152$

4.2.2.1.5 Number of replicates

Perform sample analysis at least in duplicate.

Where the standard deviation of repeatability of the alternative method is significantly larger than the standard deviation of repeatability of the reference method and the standard deviation of accuracy, the number of replicates can be increased so as to get standard deviations of mean results that at minimum are equivalent for both methods.

Thus the number of required replicates is obtained from Condition (7):

$$n_{\text{alt}} \geqslant n_{\text{ref}} \left(\frac{\sigma_{\text{alt}}}{\sigma_{\text{ref}}} \right)^2 \tag{7}$$