THE GLOBAL STANDARD
FOR LIVESTOCK DATA

Section 12 - Guidelines for Milk Analysis

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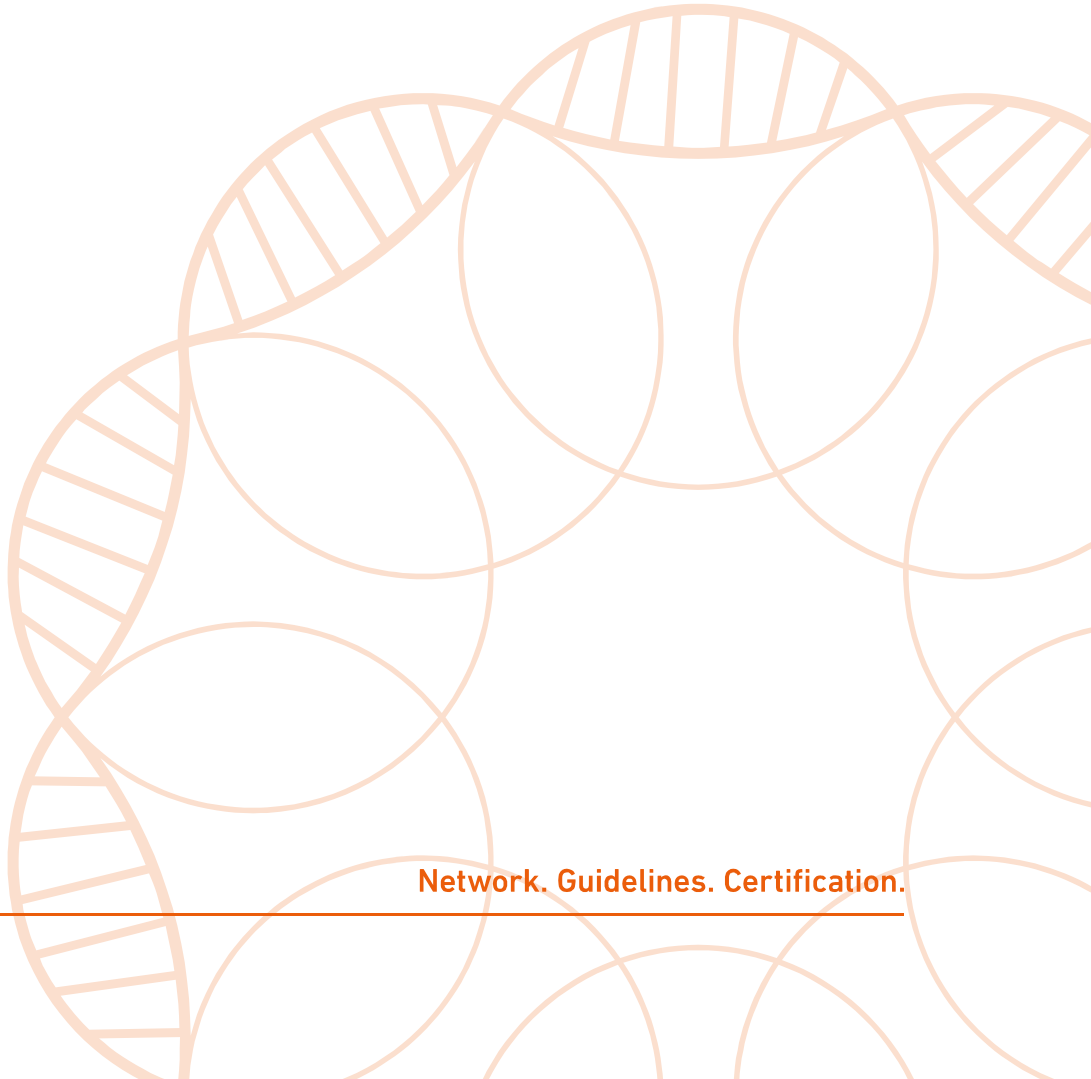

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Tables

Change Summary

Date of Change	Nature of Change
August 2017	Reformatted using new template.
August 2017	Table of contents added.
August 2017	Heading numbers and heading text edited for clarity and removal of redundant text.
August 2017	Captions added to all tables. Tables added to table of contents.
August 2017	Cross references updated and automated.
August 2017	Stopped track changes and accepted all previous changes.
August 2017	Moved the file to the new template (v2017_08_29).
September 2017	Corrections to tables 1 and 2 in Section 7, from Gavin Scott (12TH Sept, 17).
October 2017	Hyperlinks have been corrected as well as the date of the version.
August 2018	Revision and update of the whole text by ICAR Milk Analysis Sub-Committee.
September 2018	Approved by ICAR Board with insertion of reference to Section 11 in chapter 4.
October 2018	Prepared for consideration by ICAR General Assembly.
December 2018	Minor formatting of Table 2 to improve readability and finalised for publication.
January 2019	Add link to Procedure 1 in chapter 3 Routine (instrumental) Methods.

1 Field of application

These guidelines concern methods for fat, protein, lactose, urea and somatic cell count determinations in individual cow, goat and ewe milk. Milk samples are in most cases preserved with chemical substances. This will be taken into account in the procedures. These guidelines define:

a. Authorised reference methods.

b. Operation of validated routine methods.

c. Recommendations for controlling sample quality.

d. Recommendations for quality control of analyses.

2 Reference methods

The wording "reference methods" designates the methods used to calibrate the routine methods.

The reference methods should be internationally standardised methods (i.e. ISO, IDF, AOAC methods), although practical arrangements are permitted (see note below). The reference methods are listed in clause 8 (Appendix 2. Methods) below.

Note: Reference transfers

a. Rapid chemical methods can be used instead of a more time consuming reference method as far as results have shown to be equivalent to those from reference methods (i.e. Gerber method for fat, Amido Black method for protein, enzymatic method for lactose).

b. Master instruments may be used to produce "reference values" for other instruments and for other laboratories in case of a system with centralised calibration. Instrumental values may be considered equivalent to the values of the method used as reference for the calibration. Application of a centralised calibration concept must take into account sensitivity of routine methods to matrix effects (milk composition).

3 Routine (instrumental) methods

Routine methods should be methods which are fit for purpose on the basis of a performance evaluation by an expert laboratory and using a standardised protocol, or methods approved at the international level by ICAR. With this respect, conditions and procedure of evaluation, as well as requirements for ICAR approval, are defined in a standard protocol approved by ICAR (Procedure 1 of Section 12 of the ICAR Guidelines - as here) as relevant for the purpose of milk recording.

4 Specific recommendations for controlling the quality of DHI milk samples

Refer to Section 11 for guidelines on devices for collecting milk samples and sample sizes.

The quality of the sample is the first major requirement for a consistent analytical result. Good quality samples are a prerequisite to establish whether analytical quality requirements are met.

4.1 Bottles

In general terms, vials and stoppers must be suitable for their purpose (to bring milk without loss or damage to laboratories). For instance, a too large empty volume above the milk may facilitate churning during transport, especially with non-refrigerated milk. A too small empty volume above the milk may give rise to problems with mixing. Fat loss may occur with imperfectly tight stoppers.

4.2 Preservatives

Preservation of milk recording samples using chemical compounds should:

a. Maintain the physical and chemical properties of the milk during the period between sampling and analysis under the locally applicable temperature and transport conditions.

b. Not prevent from performing reference analysis, as the possibility of comparative analysis remains to the laboratories.

c. Have no effect on the results of analysis with the reference methods and no or only a limited but consistent effect on the reference method and routine method responses. A limited but consistent effect can be compensated for through calibration and/or applying a fixed correction.

d. Be innocuous to DHI and laboratory staff according to local health and safety regulations.

e. Be innocuous to environment according to local environmental regulations.

Notes

a. Sample preservation is promoted by working with clean milking and sampling equipment, by storage of samples at cool temperatures during limited time with a minimum of handling.

b. Appropriate preservatives are mentioned in relevant standards with guidance (ISO 9622 | IDF 141 and ISO 13366 | IDF 148). Nevertheless, in general care must be taken for:

- the preservative excipient: depending on the excipient - generally salts - various effects can be observed for applied formulations where none exists in the pure form (case of potassium dichromate and bronopol in milk by mid infra red spectrometry);
- some dyes which are used as colour tracer may interfere with the instrumental response (absorption of light or dye-binding with DNA). The accuracy or the sensitivity of a method may therefore be reduced. These dyes should be avoided.

5 Quality control in DHI laboratories

5.1 Quality control on reference methods

Any systematic error with the reference method leads to an overall systematic error on routine results. This type of error, which may exist between laboratories within a country (or organisation) and between countries co-operating within international frameworks such as ICAR, justifies performance evaluations at both levels, national and international.

5.1.1 External control

Every DHI routine laboratory should participate or otherwise be linked in with an interlaboratory proficiency testing (IPT) scheme. Proficiency testing should be organised preferably by a national reference or pivot laboratory appointed for that by the national DHI organisation. The reference laboratory will provide analytical precision traceability by its regular participation in international proficiency trials.

Note:

In situations where there are not sufficient laboratories to implement a national scheme, the laboratory can join PT schemes organised by a national or an international PT provider or the national DHI PT scheme of a neighbouring country.

The minimum frequency for participation in interlaboratory proficiency testing should be 2 times a year.

National reference laboratories should take part in international proficiency tests at a minimum frequency of twice per year. A more frequent participation is advised.

These trials are to be organised according to international standards, or failing that, international guidelines or agreements as indicated in this section.

5.1.2 Internal control

If available, reference materials (RMs) are advised for use to check the exactness and the stability of reference methods used by comparison with nominal values. They will be used preferably when reference analyses for calibration of routine methods are carried out.

These can be:

a. Certified reference materials (CRMs) produced by a recognised official organisation.

b. Secondary reference materials (SRMs) prepared by an external supplier.

c. In-house reference materials (IRMs) prepared by the laboratory itself, where traceability is established with CRMs, SRMs or interlaboratory proficiency tests.

Whatever the choice made by the laboratory, CRMs and SRMs are to be produced and provided in QA conditions and according to international standards, or failing that, international guidelines or agreements as indicated in clause 5.1.1 above.

5.2 Quality control on routine methods

Routine methods provide the results effectively used for DHI purposes and, therefore, their consistency has to be checked.

For this, reference is made to the standard ISO 8196-2 | IDF 128-2.

5.2.1 External control

A periodical check of the accuracy must be applied by an national expert laboratory, either through individual external control (IEC), by comparison of routine methods to reference analysis on samples representative of the laboratory area, or through participation in interlaboratory proficiency testing when it has been clearly demonstrated that a single calibration can be used for all the laboratories. In the latter case, recommendations in clause 5.1.1 above are to be followed. The minimum frequency recommended is 2 times a year.

Repeatability and suitability of calibration are the main parameters to be checked. Depending on the experimental design, additional aspects can be evaluated such as sample preservation

and instrumental parameters such as linearity, intercorrections (with MultiLinear Regression (MLR)-based calibration models) and intra-laboratory reproducibility.

5.2.2 Internal control

Irrespectively of the parameter, an internal quality control on routine methods has to be carried out in routine testing at the laboratory.

In general the standard ISO 8196 | IDF 128 does not define limits to fulfil for each method and/or milk component. Therefore specific standards have to be applied where they exist:

a. Fat, protein, lactose and urea (mid infra-red spectrometry): ISO 9622 | IDF 141.
b. Somatic cell count: ISO 13366-2 | IDF 148-2.

Preparation of control or pilot samples, used for monitoring instrument stability, should be made under quality assurance (i.e. quality control for homogeneity and stability), thereby referring to relevant indications of international standards/guides for reference materials.

According to ISO 8196 | IDF 128, the major checks in quality control are on:

a. Repeatability.
b. Daily and short-term stability of instrument.
c. Calibration.

In addition, checkings are recommended for:

a. Carry-over effect (all methods).
b. Linearity (all methods).
c. Zero-setting (all methods).
d. Intercorrections (with MLR-based calibration models).
e. Homogenisation (infra-red).

It is advised to fulfil requirements about frequencies and limits as in clause 7 below.

6 Requirements for analytical quality control and quality assurance tools

6.1 Interlaboratory proficiency tests

Interlaboratory proficiency trials are to be organised in quality assurance conditions, according to international standards, or failing that, international guidelines or agreements:

a. ISO 17043.
b. ILAC-G13.
c. International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (IDF Bulletin 342:1999).
d. ISO Standard 13528.

6.2 Reference materials

Reference materials used for DHI analytical purposes are to be produced in quality assurance conditions, according to international standards, or failing that, international guidelines or agreements:

a. ISO 17034

b. ILAC-G9.

c. ILAC-G12.

6.3 Choice of AQA service suppliers

Choice of Analytical Quality Assurance (AQA) service suppliers - i.e. proficiency testing and reference material - by DHI laboratories is to be made in tight relation with the overall DHI AQA system.

Services suppliers should operate under quality assurance and be able to provide documented proof of that.

Service suppliers should submit themselves to a periodical independent audit, i.e. a third party, in order to have the conformity of its QA system judged. These audits can be carried out by accreditation assessors, commissions of user representatives, experts acting on behalf of the national DHI national organisation, provided that their competence and independence are guaranteed and that the audits are conducted in line with ISO and ILAC recommendations.

7 Appendix 1. Analytical quality control in milk testing laboratories

It is to be expected that meeting these requirements will provide a satisfactory minimum quality level for analytical measurements, as well as comparability between laboratories and countries. If the following scheme cannot be immediately applied, it should be considered as a target.

7.1 Components of quality control and recommended minimum frequencies

Table 1. Components of quality control and recommended minimum frequencies.

Control	Frequencies	Mode
Reference methods		
- External control	Half-yearly	IPT
- Internal control	Weekly (check of mean bias)	CRMs, SRMs, IRMs
Routine methods		
- External control	Half-yearly	IPT/IEC
- Internal control	(see 7.2)	IRMs

IPT: Interlaboratory Proficiency Testing.
CRMs: Certified Reference Materials.
IEC: Individual External Control.
SRMs: Secondary Reference Materials.
IRMs: In-house Reference Materials.

7.2 Frequencies and limits for routine methods

Frequencies and limits stated hereafter in Table 2 are for a part defined in existing ISO | IDF standards or are derived from contained recommendations. Other values are indicative as they are not defined in a standard. Experience will show whether or not the latter ones are suitable for all laboratories.

Limits stated below in Table 2 are proposed as "action limits" for internal instrument management. They should only be considered as targets to users and not be used for external evaluations for which other (larger) values can appear more suitable.

Table 2. Minimum frequencies and limits for checking routine methods.

Checks	Frequencies	F P L Limits		SCC Limits	
Instrumental fittings					
Homogenization	Monthly	≤ 1.0 % relative	(a)	Not applicable	
Carry-over	Monthly	≤ 1 % relative	(a)	≤ 2 % relative	(b)
Linearity (curving)	Quarterly	≤ 2 % of range	(a)	≤ 2 % of range	(b)
Intercorrection	Quarterly	±0.02 % units	(a)	Not applicable	
Calibration					
Mean bias	Weekly	±0.02 % units	(c)	±5 % relative	(c)
Slope	Monthly	1.00±0.03	(c)	1.00±0.05	(c)
Overall daily stability					
Repeatability (sr)	Daily/every start-up	0.014 % units	(a)	6% relative at 150 000 cells/ml 5 % relative at 300 000 cells/ml 4% relative at 450 000 cells/ml 3% relative at ≥750 000 cells/ml	(b)
Daily/short-term stability	≥ 3/hour	±0.05 % units	(c)	±10 % relative	(c)
Zero-setting	≥ 4/day	±0.03 % units	(c)	≤ 8 000 cells/ml	(b)

(a): Limit stemming from ISO 9622 | IDF 141
(b): Limit stemming from ISO 13366-2 | IDF 148-2
(c): Indicative limit as there is no value specified in corresponding international standards

Note 1:

In case calculated values are out of limits but do not differ from a statistical point of view, adjustments in instrumental settings are not justified. Therefore, representative and/or adequate sample sets should be used in such a way that any outside value is significantly different. Relevant aspects in this are type and number of samples, number of replicates and level of concentration.

Note 2:

a. Milk with high fat and protein concentrations (milk of buffaloes, ewes, and specific cow and goat species). Because of variable high fat and protein contents, reliable limits for repeatability and short-term stability can be determined by multiplying limits for cows by the ratio of buffaloes (or ewes) average level versus cows average level.

b. Goats milk: Limits can be the same as for cows milk in case of similar fat and protein content. In case of high fat and protein contents, one will operate according to a).

Note 3:

The criteria are calculated according the ICAR protocol on milk analyser evaluation and ISO 8196-3 IDF 128-3, available here.

7.3 Checking

a. **Check on homogenisation (only applicable with IR instruments):** In infra-red analysis, the natural size of fat globules strongly affects the measurement of fat, therefore a fat size reduction is applied through an homogenisation before the measurement. Inefficient homogenisation results in poor repeatability and drifts of the signal.

b. **Check on carry-over:** In case of successive samples with strong differences of component concentrations, the result for a sample may have been affected by the former milk sample, e.g. by the residual volume of milk in the flow system or by the contamination by the stirrer and the pipette. The error is a proportion of the difference of concentration with the previous sample. The overall carry-over effect should be minimised, should not exceed limits stated and can be corrected for in routine operation by applying carry-over compensation factors.

c. **Check on linearity:** Specific sets of samples are prepared in order to cover the whole range of concentration and check that the instrumental measurement is proportional to the concentration of the component measured. The percentage of the bending can be estimated by the ratio (range of the residuals observed) x 100 /(range of the levels).

d. **Check on intercorrections (with MLR-based calibration models):** Specific sets of samples are prepared in order to create independent modification in respective components and verify that changes in one particular component do not affect significantly the measurement of the other components. Intercorrections are set in order to compensate the natural interactions due to a incomplete specificity of methods. The larger the range of concentrations of the correcting channel, the bigger the potential error due to an inadequate intercorrection adjustment for the corrected channel.

e. **Check on the mean bias:** Representative milk samples are used to check the validity of the calibration at a medium level and to indicate whether any drift has occurred due to changes in milk composition or progressive wear of instruments.

f. **Check on the slope:** Specific sets of (calibration) samples are prepared in order to cover the whole range of levels and check that the slope is within the stated limits. The larger the range of concentrations, the bigger the error for extreme values in case of an inadequate slope adjustment.

g. **Repeatability:** A repeatability check is indicating whether or not the instrument is working stable. Repeatability is evaluated at the start-up of each instrument on the basis of 10 times replicate analysis of one (control) milk sample. During routine testing a regular test can be made by replicate analysis of the control sample. The estimate of the standard deviation of repeatability should meet stated limits.

h. **Daily and short-term stability:** Every day and regularly along a working day, the so-called control samples (or pilot samples) are used to check instruments

functioning at one or more concentration levels. Differences observed against assigned values should not exceed the stated limits +/-L. It is advised to complete the control using the calculation of the cumulative mean of the n successive differences which should not exceed the limits +/-L/√n, see ISO 8196|IDF 128.

i. **Zero-setting:** Rinsing the flow system and checking the "zero value" are periodically required to check for fouling on the walls of the measurement cells and/or (depending on instruments) to detect any drift of the basic signal.

8 Appendix 2. Methods

8.1 International reference methods

Table 3. International reference methods.

Fat	
Gravimetry (Röse-Gottlieb)	ISO 1211 | IDF 1
Gravimetry (modified Mojonnier)	AOAC 989.05
Crude (or total) Protein	
Titrimetry (Kjeldahl)	ISO 8968 | IDF 20, parts 1 and 3 AOAC 991:20 AOAC 991:21 AOAC 991:22 AOAC 991:23
Casein	
Titrimetry (Kjeldahl)	ISO 17997 | IDF 29, parts 1 and 2 AOAC 998.05 AOAC 998.06 AOAC 998.07
Lactose	
HPLC	ISO 22662 | IDF 198
Urea	
Differential pH-method	ISO 14637 | IDF 195
Somatic cell count	
Direct microscopic somatic cell count	ISO 13366-1 | IDF 148-1

8.2 Secondary international reference methods

Table 4. Secondary international reference methods.

Fat	
Butyrometric method (Gerber)	ISO 19662 | IDF 238 AOAC 2000.18
Babcock	AOAC 989.04
Protein	
Dye-binding (Amido Black)	AOAC 975.17
Lactose	
Enzymatic	ISO 5765 | IDF 79 AOAC 984.15
Differential pH-method	ISO 26462 | IDF 214

8.3 Standardized routine methods

Table 5. Standardized routine methods.

Fat	
Automated turbidimetry I	AOAC 969.16
Automated turbidimetry II	AOAC 973.22
Protein	
Automated dye-binding (Amido Black)	AOAC 975.17
Fat-protein-lactose-urea	
Mid infrared (MIR) spectrometry	ISO 9622 | IDF 141 AOAC 972.16
Somatic cell count	
Fluoro-opto-electronic methods	ISO 13366-2 | IDF 148-2 AOAC 978.26