SECTION 12 - GUIDELINES FOR QUALITY ASSURANCE ON DHI ANALYSIS

12.1 Field of application

These guidelines concern methods for fat, protein, lactose, urea and somatic cells determinations in individual cow, goats and ewes milk. Milk samples are in most cases preserved with chemical substances. This will be taken into account in the procedures.

They define:

- Authorised reference methods.
- Approved routine instrumental methods.
- Recommendations for sample quality.
- Recommendations for quality control of analyses.

12.2 Analytical methods

12.2.1 Reference methods

The wording "reference methods" designates the methods used to calibrate the instrumental (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 Annex 2.

Note: Reference transfers

1. 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).

2 Master instruments (indirect rapid methods) 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).

12.3 Routine (instrumental) methods

Routine methods should be either standardised methods, or methods which have received an official approval from the national DHI organisation 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 as relevant for the purpose of milk recording.

12.4 Specific recommendations for DHI milk samples

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

12.4.1 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.

12.4.2 Preservatives

Preservation of milk recording samples using chemical compounds should:

- maintain the physical and chemical properties of the milk during the period running from sampling to analysis in the usual temperature and transport conditions;
- not prevent from performing reference analysis, as the possibility of control remains to the laboratories;
- have no effect on the results of analysis with the reference methods and no or only a limited effect on the routine instrument response (a limited effect can be compensated for through calibration).
- be innocuous to DHI and laboratory staff according to local health regulations;
- be innocuous to environment according to local environmental regulations.

Notes

1. Sample preservation is promoted working with clean milking and sampling equipment, by storage of samples at cool temperatures during limited time with a minimum of handling.
2. 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:
    - o 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);
    - o some dyes which are used as colour tracer may interfere with the instrumental response (absorption of light or dye-binding with DNA). The accuracy of the sensitivity of a method may therefore be reduced. These dyes should be avoided.

12.5 Quality control for DHI laboratories

12.5.1 Quality control on reference methods

Any systematic error on 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.

12.5.1.1 External control

Every DHI routine laboratory should be involved in an interlaboratory proficiency study (IPS) 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. As far as possible, it should be aimed at establishing and maintaining a link for traceability with the international level.

The minimum frequency for participation in interlaboratory proficiency studies should be 4 times a year.

National reference laboratories should take part in international proficiency studies at a minimum frequency of once a year. Nevertheless, a more frequent participation is advised.

In the particular case of centralised calibration and control system, where only the reference laboratory performs reference methods, participation of routine laboratories in proficiency testing for reference is no longer necessary.

These trials are to be organised according to international standards, or failing that, international guidelines or agreements as indicated in section 12.5.1.

12.5.1.2 Internal control

Reference materials (RMs) are advised for use to check the exactness and the stability of reference methods used between two consecutive proficiency testing by comparison with nominal values. They will be used preferably when reference for calibration of routine methods are carried out (i.e. weekly).

They can be:

- Certified reference materials (CRMs) produced by a recognised official organisation.
- Secondary reference materials (SRMs) prepared by an external supplier.
- In-house reference materials (IRMs) prepared by the laboratory itself,where traceability is established with CRMs, SRMs or interlaboratory proficiency studies.

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 section 12.5.1.

IRMs constitute the simplest case of a single producer/user for own purposes. Therefore one is only concerned by production and quality control requirements. The laboratory then requires to meet with the demands from its own QA system, thereby referring to relevant parts of available guides.

12.5.2 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 | IDF 128 (Part II).

12.5.2.1 External control

A periodical control 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 interlaboratory proficiency studies when it has been clearly demonstrated that a single calibration can be used for all the laboratories. In the latter case, recommendations of section 12.4.1.1 and 12.5.1 are to be followed. The minimum frequency recommended is 4 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 and intercorrections.

12.5.2.2 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:

- Fat, protein and lactose (mid infra-red spectrometry): ISO 9622 | IDF 141.
- Somatic cell count: ISO 13366 | IDF 148;
- Urea (mid infra-red spectrometry): next update of ISO 9622 | IDF 141.

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

According to ISO 8196 | IDF 128, the major stages of control can be summarised as follows:

- Repeatability.
- Daily and short-term stability of instrument.
- Calibration.

In addition, checkings related to instrumental aspects are recommended in specific standards:

- Carry-over effect (all methods).
- Linearity (all methods).
- Zero-setting (all methods).
- Intercorrections (infra-red).
- Homogenisation (infra-red).

It is advised to fulfil requirements about frequencies and limits reported in Annex I.

12.6 Requirements for analytical quality control and quality assurance tools

12.6.1 Compliance with International standards, Guidelines and Agreements

12.6.1.1 Interlaboratory proficiency studies

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

At the editing date of the present documents, one can refer to:

- ISO Guide 43.
- ILAC-G13.
- International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (IDF Bulletin 342:1999).
- ISO Standard 13528.

12.6.1.2 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.

At the editing date of the documents, one can refer to:

- ISO Guide 34.
- ILAC-G9.
- ILAC-G12.

12.6.2 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 to the existence of a quality assurance system for the services production and supply as part of 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 (employed by or consultant) acting in the name of the DHI national organisation, provided that their competence and independence are guaranteed and that the audits are conducted in line with ISO and ILAC recommendations.

Note

These requirements are covered by accreditation. The latter is highly recommended in particular for specialised (inter)professional or commercial organisations dealing with large AQA lab service activities. In every case, notably for those countries where such an accreditation is not (yet) possible or for small and emerging organisations, the implementation of an in-house QA system remains the minimum base required.

APPENDIXES

SECTION 12 - QUALITY ASSURANCE ON DHI ANALYSIS

SECTION 12 APPENDIX I - RECOMMENDATIONS FOR 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.

A - Components of a quality control and recommended frequencies

Control	Frequencies	Mode
Reference methods		
• - External control	Quarterly	IPS
• - Internal control	Weekly (for each check o the mean bias)	CRMs, SRMs, IRMs
Routine methods		
• - External control	Quarterly	IPS/IEC
• - Internal control	(See b)	IRMs

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

B - Frequencies and limits for checking routine methods

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

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

Checks	Frequencies	F P L	Limits	SCC	
Instrumental fittings					
• Homogenization	Monthly	≤ 0.05 % units or ≤ 1.43 % relative	(a)	none	
• Carry-over	Monthly	≤ 1 %	(a)	(≤ 2 %)	(c)
• Linearity (curving)	Quarterly	≤ 1 % of range	(a)	(≤ 2 % of range)	(c)
• Intercorrection	Quarterly	+/-0.02	(a)	none	
Calibration					
• Mean bias	Weekly	+/-0.02 % units	(b)	+/-5 % relative	(b)
• Slope	Quarterly	1.00+/-0.02	(b)	1.00+/-0.05	(b)
		(1.00+/-0.03) (*)	(c)	(1.00+/-0.07) (*)	(c)
		(1.00+/-0.05) (**)	(c)		
Overall daily stability					
• Repeatability (sr)	Daily/every	0.014 % units	(a)	5 % relative	(a)
	Start-up	0.020 % units (*)	(a)		
• Daily/short-term stability	≥ 3/hour	+/-0.05 % units	(a)	+/-10 % relative	(b)
• Zero-setting	≥ 4/day	(+/-0.03 % units)	(c)	(≤ 5000 ¢/ml)	(c)

(a): Limit stated in ISO 9622 | IDF 141 or ISO 13366 | IDF 148
(b): Limit stemming from specifications of ISO 9622 | IDF 141 or ISO 13366 | IDF 148
(c): Tentative (indicative) limit as there is no value specified in corresponding international standards
*: Limit for first generation instruments
**: Limit for lactose

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 chosen or prepared in such a way that any outside value should be significant. 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 particular 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).

C - Recalls about checkings

- **Check on homogenisation**: 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.
- **Check on carry-over**: Successive samples with strong different of component levels may be affected by the former milk either by the residual volume of milk in the flow system or by the contamination by the stirrer and the input pipe. As an effect of a constant dilution, the error is a proportion of the difference of concentration with the previous sample. The overall carry-over effect should be minimised and, in all cases, should not exceed limits stated.
- **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).
- **Check on intercorrections**: 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.
- **Check on the mean bias**: Representative milk samples are used to check the validity of the calibration at a medium level and prevent any drift which could occur from changes in milk composition or progressive wear of instruments. Checking that the zero and the mean bias fit the stated limits provides assurance for the maintenance of the calibration on the whole range.
- **Check on the slope**: Specific sets of samples are prepared in order to cover the whole range of levels and check that the slope is within the stated limits. It is aimed at that differences between instrumental values and reference values should not be proportional to the level but constant on the whole range. The larger the range of concentrations, the bigger the error for extreme values in case of an inadequate slope adjustment.

- **Repeatability**: Repeatability check is the simplest test indicating whether or not the instrument is working properly. 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 analysing a set of 20 different individual samples in two successive runs. The estimate of the standard deviation of repeatability should meet stated limits.
- **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 fitting at a medium level. 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.
- **Zero-setting**: Rinsing the flow system and checking the "zero value" are periodically required to prevent milk matter deposit on the walls of the measurement cells and/or (depending on instruments) to detect any drift of the basic signal.

SECTION 12 APPENDIX II - INFORMATIVE

Appendix 2.1 International reference methods

Fat	
Gravimetric method (Röse-Gottlieb)	ISO 1211 | IDF 1
	AOAC 905.02 (IDF-ISO-AOAC-Codex)
Gravimetric method (modified Mojonnier)	AOAC 989.05 (IDF-ISO-AOAC)
Protein	
Titrimetric method (Kjeldahl)	ISO 8968 | IDF 20
	AOAC 991:20 (IDF-ISO-AOAC)
	AOAC 991:21
	AOAC 991:22 (IDF-ISO-AOAC)
	AOAC 991:23 (IDF-ISO-AOAC-Codex)
Casein	
Titrimetric method (Kjeldahl)	ISO 17997 | IDF 29
	AOAC 927.03
	AOAC 998.05
	AOAC 998.06
	AOAC 998.07
Lactose	
HPLC method is foreseen to provide the reference to routine methods by ISO | IDF and its international standardisation is underway (ISO DIS 22662 | IDF 198). In the meantime, standardised methods as referred to in "Part II, other methods" can be used.	
Urea	
Differential pH-method (Reference method)	ISO 14637 | IDF 195
Somatic cell count	
Microscope method (Reference method)	ISO 13366-1 | IDF 148-1

Appendix 2.2 Other methods (secondary reference)

Fat	
Butyrometric method (Gerber)	ISO 2446
	AOAC 2000.18
Babcock	AOAC 989.04
Protein	
Dye-binding (Amido Black)	ISO 5542 | IDF 98
	AOAC 975.17 (IDF-ISO-AOAC)
Dye-binding (Orange 12)	AOAC 967.12
Dumas method	ISO 14891
Lactose	
Enzymatic	ISO 5765 | IDF 79
AOAC 984.15	
Gravimetric	AOAC 930.28
Polarimetric	AOAC 896.01
Under standardisation	
High Performance Liquid Chromatography	ISO DIS 22662 | IDF 198
Differential pH-method	ISO WD | IDF (working draft)

Appendix 2.3 Standardized routine methods

Fat	
Automated turbidimetric I	AOAC 969.16
Automated turbidimetric II	AOAC 973.22
Protein	
Automated dye-binding (Amido Black)	AOAC 975.17 (FIL-ISO-AOAC)
Fat-protein-lactose	
Mid infra red (MIR) spectrometric	ISO 9622 | IDF 141
	AOAC 972.16
Urea	
(taken into account with the underway revision of ISO 9622 | IDF 141)	
Somatic cell count	
Electronic particle counter (Coulter Counter)	International standards withdrawn
Fluoro-opto-electronic methods	ISO 13366-2 | IDF 148-2
	AOAC 978.26

Appendix 2.4 Instrumental routine methods used in ICAR countries

The following list was drawn up with answers to ICAR questionnaires of 1994 and 1996, since then supplemented with new validated analysers. Methods/instruments not produced or used any longer are indicated in Italic characters.

Fat		
Turbidimetric method		
	• MilkoTester (Foss Electric, DK)	
Fat and protein		
Turbidimetric/dye-binding:		
	• MTA-PMA (Foss Electric, DK)	
Fat, protein (and lactose)		
Mid infra-red spectrometry		
	• Milkoscan (Foss Electric,DK)	102, 103, 104, 104 (A/B) 133 A, 133 B, 134 (A/B) 203 A, 203 B, 300 255 (A or B), 605 (A or B) Series 4000 (A or B) FT 120 (FTIR) FT 6000 (FTIR)
	• Multispec (Multispec ,UK)	MK 1 MK 2 Micro-null
	• Bentley (Bentley, USA)	150 2000 (A or B)
	• Lactoscope (Delta Instruments, NL)	300, 550, 750, Filter Automatic 200, Filter Automatic 400, FTIR Auto 400
	• Aegys (Anadis Instruments, F)	MI 600 (FTIR)

Urea		
Colorimetric methods		
	1-4 paradimethylaminobenzaldehyde method (DMAB)	
	Diacetyl monoxime method (DAM)	
Automated enzymatic methods		
	Conductimetry	Beckmann, BUN Analyser
	Differential pH-metry	Eurochem, CL 10, Hamilton, E.F.A.
	UV-photometry	Flow injection analysis (FIA).
	Visible-photometry	Chemspec 150 (Bentley, USA), Skalar Segmented flow analysis
Mid Infra-Red Spectrometry:		
	Milkoscan (Foss Electric,DK)	4000, FT 120 (FTIR), FT 6000 (FTIR)
	Lactoscope (Delta Instruments)	FTIR Auto 400

Somatic cell count			
• Particle counting			
	• Coultronic (UK)	Coulter Counter	
• Fluoro-opto-electronic			
• Disk cytometry			
	• Foss Electric (DK)	Fossomatic	90, 180, 215, 250, 360, 400
• Flow cytometry			
	• Anadis (F)	Somatic Cell Counter	300, 500
	• Bentley (USA)	Somacount	150, 300, 500
	• Chemunex (D)	Partec CA 11	
	• Delta Instruments (NL)	Somascope	MKII Manual, MKII Auto 200, MKII Auto 400
	• Foss Electric (DK)	Fossomatic	5000