Guidelines on On-farm Milk Analysis

Foreword

The present document was elaborated by a joint working group of representatives from different working parties of ICAR so as to cope with different aspects related to milk analysis on the farm for milk recording purposes. This working party on On-farm Milk Analysis (WP OMA) was created in Summer 2007 and held its first meeting on 27 November 2007 when the programme of work was adopted. The document was reviewed and commented in June 2008 in Niagara Falls-USA and was amended with the comments and complements received since.

The present document is modular in several aspects dealt with in ICAR working parties, such as identification, milk measurement, milk analysis, milk recording data, etc. It contains the identified elements needed to account for on-farm analysis in milk recording. Those elements should be further included at their proper places in existing ICAR guidelines.

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0. Introduction

For many decades milk recording analysis is performed in specialised milk testing laboratories equipped with automated instrumentation for rapid testing. Those laboratories have implemented quality control and quality assurance procedures according to international standards as ISO/IEC 17025 and ISO 9001, proof of which can be given through accreditation and/or certification by competent bodies.
The last decade has shown the advent of portable analytical devices and analytical modules for direct in-line/on-flow analysis on-farm. As with centralized analysis, these analytical units are to be adequately managed in terms of quality control and quality assurance when delivering data for an official milk recording system.
On-farm analysis is generally being developed and implemented with direct farm management purposes in mind. However, use by/for official milk recording is envisaged. It is therefore essential that analytical devices can respond to both the needs for individual and collective interests.
An analytical quality assurance (AQA) frame has been designed by ICAR for milk analysis in laboratories. This frame defines general recommendations to be followed by organisation to get ICAR approval or certification for milk analysis.
In the forthcoming situation official milk recording data will be obtained from different analytical systems in different conditions, ICAR should therefore complement the existing AQA system so as to assure quality and precision of recording data in working with various analytical systems.
A frame is needed as a safeguard for quality to the users and as a practical tool to manufacturers who can find adequate technical indications or targets. It should be defined by ICAR through adequate guidelines dealing with the respective aspects related to milk recording analysis.
Under these circumstances, the ability of the equipment to meet with the performance criteria and clear recommendations for its practical application and proper quality assurance measures are a task for the manufacturers of the analytical devices, milk producers are responsible for a proper use, milk recording organisations for supervision on the execution of routine analysis in different places.

1. Outline

On-farm analytical devices are generally integrated with systems for animal identification, milk measurement and sampling.
Although made at first for farm milk production management, collected data are also expected to be exported and used for collective purposes or connected to other external systems making necessary compatible records under standard formats.

The introduction of on-farm analysis comes along with a higher frequency of milk analysis. As a consequence, the methods of lactation calculation have to be adapted. Specific recommendations are needed with the implementation on automated milking systems (AMS), more particularly to assure the representativeness of the outcome.
Devices developed for on-farm analysis should be robust for tougher conditions than in a laboratory with regard to temperature, humidity, shocks, etc. The strive for more robustness and stability at a lower price than in the laboratory may end up in lower performance in terms of precision.
A somewhat lower performance as compared to laboratory instruments is acceptable with more frequent analysis since estimate uncertainty can be reduced by averaging. However, situations with a systematic bias should be avoided. So, it is essential to define the accuracy limits for compositional analysis in milk recording.
The frame to draw should provide proper elements for ICAR agreement including on-farm measurement system validation and minimum quality control and quality assurance procedures necessary to provide sufficient accuracy in milk recording data.
Similarly to classical former devices, the on-farm analytical devices should be accounted for in a quality assurance system for milk production and genetic evaluation through compliance with international ICAR guidelines.
Therefore this document defines:

1- Various possible situations with on-farm analysis,
2- Acceptable limits for precision and accuracy for on-farm analytical devices,
3- Conditions to fulfil for evaluation and ICAR approval,
4- Conditions and check limits for quality control
5- Compatibility with existing systems (identification, data record/transfer, lactation calculation)
    a- Identification
    b- Animal data recording
    c- Lactation calculation
    d- Milking machine parameters

2. Terms and definitions

2.1. Milk analyser

Analytical device specifically dedicated to the analysis of milk. It is generally used for instrumental automated methods in laboratories and by extension applies also to milk analytical devices installed on-farm.

2.2. On-farm milk analyser

Milk analyser installed on the farm that is used either to detect or to quantify various components or characteristics in milk.

Note :Milk analysis so performed can be considered as the result of the direct measurement of a representative sample of the whole milking performed through a specific sampling device or the result of the integration of successive serial in-line measurements of milk component(s) in weighted proportion to the total quantity of milk produced.

2.3. At-line milk analyser

Milk analyser installed beside a production line that is used once a representative sample of the whole milking is obtained. Such devices are likely to be located close to the milking unit but not exclusively. They can have similar characteristics as those used in laboratories and in extreme cases be an element of an on-farm laboratory. The number of analysers is independent of the number of milking units but related to the number of samples to be analysed.

Note: Also called off-line analysers

2.4. In-line milk analyser

Milk analyser installed in the production line (i.e. milk pipeline). Analysis may be performed during the milking process (real time) or at the end on a representative aliquot sample of the whole milking (differed time).

Note: Also called on-line analysers

2.5. Real time milk analyser

Milk analyser that analyses milk in real time during milking using sensors in contact with milk flow.
Repeated milk scanning combines composition (concentration), flow rate and time measurements in order to provide estimates of component quantities and concentrations at the end of every individual milking.
It may be either an in-line analyser or a single multiplexed at-line analyser connected to milking units through individual in-line sensors and a connection network (e.g. wires or optical fibres).

2.6. Accuracy

Extent of correctness of an estimate obtained with the analytical method. Also called overall accuracy, it is expressed through a standard deviation that combines both random error (precision) and systematic error of the method. The part independent from calibration and precision errors, so-called 'accuracy of estimate', is a characteristic of alternative methods of analysis. Overall accuracy enables estimating the measurement uncertainty.

2.7. Measurement uncertainty

Uncertainty (so-called expanded uncertainty) of measurement is related to overall accuracy of the method. It expresses the range of occurrence of a result through its standard deviation (standard uncertainty) and a coverage factor k for a given probability (usually k=2 for a 95% probability). It is presumed that the resulting error is normally distributed.

2.8. Natural day-to-day variation

Usual variation of a production parameter (e.g. milk yield, composition) observed between days in normal production conditions, in the absence of sudden interferences (e.g. health, feeding), for an individual animal. It is characterised by the between days (so-called day-to-day) standard deviation for the production parameter, where measured with reference methods for sampling and analysis (i.e. manual sampling and chemical analysis)

Note: It is normally estimated through extensive measurements with, per animal, significant numbers of successive day-to-day records throughout representative periods of time of one or more lactations. Statistical analysis should exclude strong erratic deviation obviously different from the average trend of residuals, significant shifts related to changes in herd (e.g. feeding, housing) and compensate for the natural drift of the average trend specific to each animal that occurs during lactation. Robust standard deviation estimates can be calculated from meta-analysis of data of a number of animals and representative lactation periods.

3. Quality assurance - Requirements for the purpose of official milk recording

The milk recording chain should be set under control with formal engagement of different partners as shown in Figure 1. Respective commitments refer to recommendations/requirements described in ICAR guidelines. This scheme is also valid to the case of off-farm analysis for which recommendations already exists.

3.1. Manufacturers

Manufacturers should propose analytical devices responding to minimum characteristics defined by ICAR.
Characteristics to comply with are:

3.1.1. Adaptation to milking environment

- Robustness (shock and water proof)
- Ruggedness (response sensitivity to environment factors)
- Dimensions, shape, positioning (no hampering, harmless, sanitary construction)
- Temperature variation (extreme temperature proof)

3.1.2. Analytical characteristics

- Repeatability
- Day-to-day stability (reproducibility)
- Accuracy
- Selectivity or matrix effects (interactions, interference)

3.1.3. Facilities

- Calibration setting and control
- Milk sampling
- Setting automation
- Sample/animal identification
- Recording/exporting data

3.2. Milk recording organisation

3.2.1. Quality assurance system for on-farm analysis

A milk recording organisation should commit for implementing analytical quality assurance in compliance with recommendations given in relevant ICAR guidelines.

3.2.2. Approval of on-farm milk analyser

On-farm analytical devices should have been evaluated according to recommendations in a relevant ICAR protocol and be approved before being used.

3.2.3. Approval of reference material providers

Reference material providers should be either accredited/certified or at least work under quality assurance with regular audit of the milk recording organisation.

3.3. Milk producers

3.3.1. Commitment in quality control of analysis

A milk producer should commit for implementing quality control in compliance with recommendations given in relevant ICAR guidelines.

3.3.2. Commitment for manufacturer servicing

A milk producer should commit for implementing regular servicing on on-farm devices according to manufacturer’s recommended procedures.

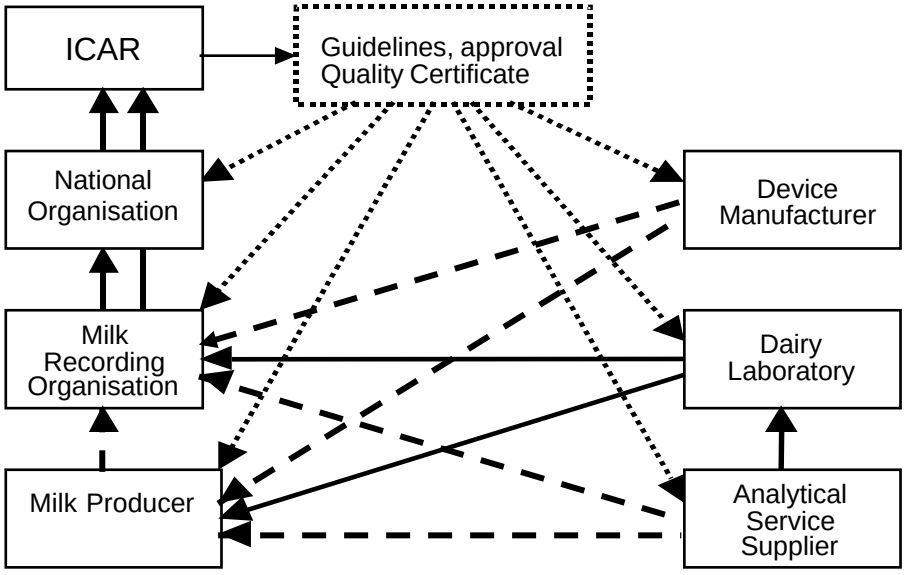

Figure 1. Contributors in the analytical process in milk recording and the chain of commitments for AQA
Arrows indicates the direction of commitments: actually existing (continuous), needed for OMA purposes (broken) and where ICAR guidelines should rule (dotted)

4. Definitions of different situations

Three different situations are identified with regard to sampling and milk analysis that defines three analytical devices categories with specific recommendations/requirements:

4.1. In-laboratory analysis

As related to the actual situation of milk recording analysis it is already covered by ICAR's guidelines and analytical quality assurance system of ICAR. Sampling and analysis are separated and respective measurement devices must fulfil limits for accuracy stated by ICAR.
The existing guidelines for milk recording analysis provide basic conditions for analytical data quality in laboratories and constitute a reference frame to be met by alternative analytical systems in order to maintain consistence and compliance throughout space and time in milk recording analysis.

4.2. On-farm/at-line analysis

Sampling and analysis are performed separately. Sampling devices can be used as well in off-farm analysis as in the classical (former) system and should therefore fulfil the same requirements and accuracy limits as already stated in ICAR guidelines.
At-line analysers allow more frequent milk analysis by farmers. Therefore a lower accuracy at the level of the individual result can be accepted. Specific conditions and accuracy limits have to be established for that.
In case that an at-line analyser is used to replace the laboratory system at a usual record frequency, for instance where the sample transportation to laboratories is impractical, it should comply with the ICAR guidelines for laboratory analysers.

4.3. On-farm/in-line analysis

Also here, in case compositional analysis is performed more frequently than in the classical system, a lower accuracy for the individual result will suffice. Specific conditions and relevant accuracy limits are to be established for that.

5. Bases and conditions of equivalence with the classical system

5.1. Objectives

To establish limits for accuracy of composition analysis that provide sufficient measurement precision:
- for milk producers to manage day-to-day milk production
- for milk recording organisation to maintain sufficient accuracy in estimating genetic indicators.

To establish consistence and correspondence between different measuring systems with regard to measurement uncertainty that enables comparison within time and space.

5.2. Maximum limits for composition measurement accuracy

5.2.1. Rationales

The accuracy of the analytical device must allow an adequate monitoring of significant day-to-day production changes. Compositional information of interest is that outside the regular natural variation related to normal physiological and milking conditions. Therefore the accuracy of the analytical device should be better than the natural day-to-day fluctuation of the measured criteria to achieve statistical significance.
The variation in fat concentration is used to calculate maximum statistical limits for precision and accuracy from that stated for laboratory analysers. The calculated values serve to establish limits for the evaluation of new milk analysers and quality control in routine testing.

5.2.2. Natural day-to-day (or between day) fat content variation

Regular natural day-to-day variation is expressed through the standard deviation σ_{BDC}. Maximum acceptable limits established from convergent experiment observations are $L\sigma_{BDC}$ = 0,25 g/100g or when expressed as a confidence interval $\pm 2.L\sigma_{BDC} = \pm 0,5$ g/100g.

5.2.3. Statistical bases

5.2.3.1. Measurement error

Every individual milk composition result C can be defined as:

$C = T + e_{BDC} + e_S + e_A$

where
T = True unknown value
e_{BDC} = between days error of milk composition (natural)
e_S = error of the sampler (sampling error)
e_A = error of the analyser (analytical error)

Which results in the breakdown of the variance as

$$\sigma_C^2 = \sigma_{BDC}^2 + \sigma_S^2 + \sigma_A^2 \qquad (1)$$

where $\sigma_S^2 + \sigma_A^2$ expresses the overall error of measurement

Further subscripts $_{L.}$ and $_{F.}$ differentiate same parameters for at-laboratory and on-farm analysis respectively.

5.2.3.2. Maximum acceptable analytical error σ_A

The error of measurement at farm should be lower or equal to the error resulting from natural day-to-day variation:

$$\sigma_{FS}^2 + \sigma_{FA}^2 \leq \sigma_{BDC}^2 \quad \Leftrightarrow \quad \sigma_{FA} \leq (\sigma_{BDC}^2 - \sigma_{FS}^2)^{1/2} \qquad (2)$$

where
σ_{BDC} = between days standard deviation of concentration,
σ_{FA} = the standard deviation of analytical measurement at farm
σ_{FS} = the standard deviation of sampling at farm.

From relation (2) the upper limit of σ_{FA} is $L\sigma_{FA} = (L\sigma_{BDC}^2 - L\sigma_{FS}^2)^{1/2}$ (3)

a- At-line measurement : From the limit $L\sigma_{LS} = L\sigma_{FS}$ in Table 1, the limit is $L\sigma_{FA}$ = 0,23 g/100 g
b- In-line measurement : With sampling $\sigma_{FS} = 0$, $L\sigma_{FA} = L\sigma_{BDC}$ and the limit $L\sigma_{FA}$ = 0,25 g/100 g

NOTE Sampling error may exist somewhere also with in-line analysers but at the end it is included into the analytical error hence it is set to zero in the formula.

5.2.3.3. Statistical limits for instrument evaluation and quality control

The limits of statistical parameters used for instrument evaluation and quality control are calculated through multiplying the limit for laboratory analysis by a correspondence factor or equivalence factor (FE) defined as the ratio between the limit of the standard deviation of analytical measurement at the farm, $L\sigma_{FA}$, and the limit of the standard deviation of analytical measurement at the laboratory, $L\sigma_{LA}$:

$FE = L\sigma_{FA} / L\sigma_{LA}$

thus giving

a- At-line measurement : FE = 2,3 lowered down to 2
b- In-line measurement : FE = 2,5

NOTE For at-line analysers FE is lowered down to 2 in order to comply with the limit even in case of possible underestimation of accuracy of sampler and/or analyser devices when they are found at the specified limits. Samplers and analysers may be supplied by different manufacturers, therefore the responsibilities in limiting the overall measurement error are shared between them.

This differentiates three classes of analytical devices for milk recording with different accuracy requirements and different level of agreement for use:

Category 1:	Laboratory milk analyser	FE=1
Category 2:	On-farm milk analyser at-line	FE=2
Category 3:	On-farm milk analyser in-line	FE=2,5

Calculated limits for statistical parameters relevant for method characterisation and quality control are reported in Tables 3 and 4 respectively.

5.2.3.4. Minimum number of recordings for uncertainty equivalence in composition recording

This paragraph relates to the uncertainty of composition estimate C. It compares on-lab and on-farm analysis in order to determine the minimum number of independent recordings per animal needed on-farm that would achieve, by averaging, the same uncertainty in composition estimate as with one record, or, equivalently, the minimum record number ratio of on-farm analysis to laboratory analysis required for a lactation.

From equation (1) applied to on-farm and laboratory analysis, lower or equal error in estimating animal data on-farm is achieved through

$$\sigma_{FC}^2 / n_{FA} \leq \sigma_{LC}^2 / n_{LA} \quad \Leftrightarrow \quad (\sigma_{BDC}^2 + \sigma_{FS}^2 + \sigma_{FA}^2) / n_{FA} \leq (\sigma_{BDC}^2 + \sigma_{LS}^2 + \sigma_{LA}^2) / n_{LA} \qquad (4)$$

or

$$\sigma_{FC}^2 / N \leq \sigma_{LC}^2 \quad \Leftrightarrow \quad (\sigma_{BDC}^2 + \sigma_{FS}^2 + \sigma_{FA}^2) / N \leq (\sigma_{BDC}^2 + \sigma_{LS}^2 + \sigma_{LA}^2) \qquad (5)$$

with $N = n_{FA}/n_{LA}$

N is the number of on-farm recordings needed to compensate the lower accuracy of a single recording as compared to laboratory analysis. It is calculated from (4) through

$$N \geq [(\sigma_{BDC}^2 + \sigma_{FS}^2 + \sigma_{FA}^2)/(\sigma_{BDC}^2 + \sigma_{LS}^2 + \sigma_{LA}^2)]$$

By setting values at their limits and combining (3)

$$N \geq (2.L\sigma_{BDC}^2)/(L\sigma_{BDC}^2 + L\sigma_{LS}^2 + L\sigma_{LA}^2)$$

From existing limits (Table 1), $N \geq (2.0{,}25^2 / (0{,}25^2 + 0{,}10^2 + 0{,}10^2) = 1{,}5$

Thus with analytical devices fulfilling the limits stated, **N=2 recording on-farm** is sufficient to provide uncertainty of the average result equivalent to the outcome of laboratory testing.

Throughout the whole lactation, the required number of milk recordings in order to achieve equivalence is given by multiplying the usual total number by a factor of **1,5**.

Table 1 – Limits of measurement error (L_) derived from ICAR guidelines

Components of error	Limits of standard uncertainty
- Composition analysis (F, P, L): *from ICAR Guidelines for DHI analyses and ISO 8196-2 :*	$L\sigma_{LA}$ = 0,103 g/100 g $L\sigma_{LA} = (L\sigma_R^2 + L\sigma_{y,x}^2)^{1/2}$ $L\sigma_R = 0{,}025$ *and* $L\sigma_{y,x} = 0{,}10$ g/100 g
Sampling error on fat concentration *from ICAR Guidelines for sampling devices and ISO 8196-2 :*	$L\sigma_{LS}$ = 0,103 g/100 g (2,5 %) $L\sigma_{LS} = (L\sigma_d^2 + L\sigma_d^2)^{1/2}$ $L\sigma_d^2 = 0{,}05/2 = 0{,}025$ *and* $L\sigma_d = 0{,}10$ g/100 g
- Day-to-day variation of composition (% fat) *from experiments*	$L\sigma_{BDC}$ = 0,25 g/100 g or ± 0,5 g/100 g
- Milk record composition (fat) estimates *from equation (1)*	$L\sigma_{LC}$ = 0,38 g/100 g $L\sigma_{LC} = (L\sigma_{BDC}^2 + L\sigma_{LS}^2 + L\sigma_{LA}^2)^{½}$

6. Evaluation protocol for ICAR approval

The general principle of two subsequent test phases remains:

6.1. Phase 1 – Test bed in-lab evaluation

The first part of the ICAR document related to Phase 1 is relevant for on-farm analytical devices minding adjusting limits of compliance for accuracy stated in Table 3.

Some parts may not be relevant for some devices; therefore they are used only where justified by the instrument principle. Specific approaches are needed for in-line real time devices and specific complementary requirements are foreseen for in-line real time devices with regard to consistence of sensor signal and final results.

6.2. Phase 2 – On-farm evaluation

Items pertaining to preservation and milk ageing are not relevant for in-line analysers but can be so for at-line analysers in case milk analysis is delayed after the milking. The paragraph Practical convenience is valid for all devices.

Specific facilities are necessary to allow proper representative milk sampling for reference analysis. For in-line analysers, milk pipetting/intake device to sensors should permit to check analytical response for calibration in quality control checking. There are parts of the requirements to manufacturers (6.1.3) as they are indispensable for a proper analyser monitoring.

6.3. Particularities of in-line/real time analysers

Analytical characteristics are assessed for each instrument (per milking unit) and for the whole milking system in the parlour (including all the analytical devices). Every milking device must comply individually to acceptable limits as well as the system with regard to overall accuracy. If individual milkings show similar

precision and accuracy figures, the merging is possible in order to produce average values that characterise the whole system.

Precision (repeatability and reproducibility): A direct evaluation of precision is hampered in natural milking conditions since animal milking cannot be replicated with qualitatively and quantitatively identical milk production (and identical milk release), hence cannot be analysed twice.

Indirect strategies may be used as, for instance, implemented at the sensor level to measure intermediate elements of precision and calculate final precision figures as indicated in the following. Their application is much dependent on the principle and facilities of the devices.

NOTE Use of artificial udder and adequately preserved milk may be an option to evaluate precision of parts or the whole measuring system. Extreme care is then necessary in preserving milk integrity and imitating natural milk release conditions (temperature, fat gradient). Options may be prior hand milking recycled twice or identical milk portions of fresh commingled milking. Artificial udder material should not retain any part of milk or milk component (negative internal wall slope, unwettable coating).

Accuracy: Comparisons to relevant reference methods allow determining accuracy characteristics according to ISO 8196.

6.3.1. Preliminary fittings

Preliminary fittings are generally specific for off-line milk analysers. Nevertheless, these characteristics should also be checked on individual sensors for in-line real time devices. Same test procedures as for off-line analysers remain valid for in-line devices where milk portions can be analysed at the sensor level using an adequate intake device. Appropriate adjustment is the responsibility of the manufacturer.

6.3.2. Repeatability

Milking the same milk cannot be performed twice per cow, therefore repeatability is measured at the sensor level with milk samples homogeneously sampled during the milking. It is associated to a complementary check for result consistency by comparing the mean sensor result to the value recorded during the milking. The standard deviation sr_s of the ranges between duplicates gives the repeatability standard deviation of the sensor while the standard deviation sd of differences provides the repeatability standard deviation sr of the instrument through $sr = (sd^2 - sr_s^2/2)^{½}$
This figure is made for all the devices by averaging the repeatability variance in order to obtain the repeatability standard deviation of the system.
The values obtained are compared to limits stated in table 3.

6.3.3. Reproducibility

Milking the same milk cannot be performed twice per cow, therefore reproducibility is measured at the sensor level with milk samples homogeneously sampled during the milking. It is associated to a complementary check for result consistency by comparing the mean sensor result to the value recorded during the milking.
The representative sample is analysed in duplicate on every device (sensor) of the system. Then to calculate from duplicate results the repeatability sr_s of all the sensors, the standard deviation of means of duplicates $s_{\bar{x}}$ and the standard deviation between devices $s_a = (s_{\bar{x}}^2 - sr_s^2/2)^{½}$

For all the devices the reproducibility standard deviation of the individual device sR_d is obtained through $sR_d = (s_a^2 + sr_s^2)^{½}$

The repeatability standard deviation of the system is obtained by averaging the reproducibility variances.

a- Within milking (system) reproducibility The same milk samples are repeatedly analysed by all the sensors of the system during the same milking.

b- Between milking (device) reproducibility Every milk sample is stored under appropriate conditions (temperature, preservative) and re-analysed on the same sensor for 10 successive milkings.

The values obtained are compared to limits stated in Table 3.

6.3.4. Accuracy

The accuracy of every instrument in the parlour and the total accuracy of the system should be evaluated through comparison with results obtained with a relevant reference method by a competent (accredited) laboratory. Accuracy standard deviation is calculated according to the ICAR protocol for milk analyser evaluation (or ISO 8196).

Since only one measurement per recording is possible, the accuracy measured covers all the sources of errors (repeatability, within-device reproducibility, accuracy of estimates, trueness (bias)).

Biases between analysers can be approached by re-analysing the same milk samples at the sensor levels but this does only provide information on the sensor calibration without covering milk measuring/sampling.

6.3.5. Carry over

A real time analysis system is not subjected to carry over effect, due to the large amount of milk passing through the system.

6.4. Fitting facilities

6.4.1. Milk sampling device

A sampling system should allow representative milk sampling needed for:

- possible other analysis of components or characteristics not measured with the on-farm device
- quality control comparisons .

The minimum sample volume should allow the performance of quality control analysis, that includes minimum duplicate milk re-testing through the device or performing appropriate chemical analysis as reference for calibration. It should not be lower than 30 ml.

6.4.2. Intake piping device for external sample

The analytical device should allow analysing samples from external sources so that calibration check/adjustment will be possible through known (reference) samples.

The maximum volumes consumed per test should allow re-testing milk obtained from the sampling device. It should preferably not exceed 10 ml.

Otherwise the manufacturer should provide appropriate alternative procedures for quality control and calibration.

6.4.3. Periodical rinsing and zeroing

Cleaning/rinsing process for the flow system should exist to be performed at a chosen frequency in order to avoid milk component layer accumulation on sensors and maintain stability in the instrument response.

Zero check/adjustment should be applicable before every herd milking and at a chosen frequency during testing series.

6.4.4. Security levels for adjustment

- 1st level of access: Open to milking operator (with possible locking-unlocking for security). Simple adjustment with standard pilot sample(s) before the milking (analysis, validation vs assigned values, testing),

- 2nd level of access: To secure specific fittings (e.g. calibration), a part of the device interface can be kept locked. It can be open to the milking operator and service engineers under conditions.

Any other relevant recommendations of existing ICAR guidelines shall be fulfilled.

6.4.5. Robustness / ruggedness

- humidity, water: The device should be waterproof or in any case should resist to humidity / water conditions in the place of functioning (milking parlour, AMS, other),

- temperature: The device should function within the range of temperatures that prevail in the location of functioning (milking parlour, AMS, other). Analytical response should not be influenced by temperature conditions/variations.

- acids/alkalis: The device should be insensitive to possible exposure of chemicals (e.g. detergents) used in the place of functioning.

- physical shocks, vibrations: The device should be insensitive or protected against possible physical shocks (mishandling, animal, etc) or vibrations (e.g. pump) in the place of functioning.

- size and shape: The device should be adapted to the milking device and environment (milking parlour, AMS) so that milking operation can be carried easily with no physical hampering. Small size and smooth shapes avoiding angles where clothes can catch on are preferable.

- effect of milking machine: The same parameters as for milk yield recording devices should be investigated and should not influence significantly recording results in the range of their usual variations. (e.g. flow rate, vacuum, position on the pipeline, etc).

Any other relevant recommendations of existing ICAR guidelines shall be fulfilled.

6.4.6. Cleaning

Cleaning of the analytical device should be performed at the end of milking. Recovery of initial zero values is an adequate indicator of the appropriateness of cleaning and rinsing of the system. Cleaning of derivations for sampling and milk measurement control should be achieved with the whole milking system. Special emphasis is given on possible growth and accumulation of micro-organisms (avoid dead corners) and further contamination of milk from insufficient cleaning.

Any other relevant recommendations of existing ICAR guidelines shall be fulfilled.

6.4.7. Servicing

Servicing operations should be well documented and either provided by manufacturers or made possible by users minding specific training. Easy and quick replacement of entire parts of the system should allow resolving problems in real time without hampering the whole milking.

Any other relevant recommendations of existing ICAR guidelines shall be fulfilled.

7. Quality control and calibration

7.1. General recommendations

Implementation of quality control is mandatory for official milk recording and in every other case where recorded data are used for a collective purpose. Otherwise it is strongly recommended for the sole farm management use.

Components of quality control listed in ICAR Recording Guidelines (Section 12) are applicable at appropriate frequencies in places were analyses are performed according to Table 2.

Table 2 – Component of quality control of DHI analysis

Control	Frequencies	Mode
Reference methods: - External control - Internal control	 Quarterly Weekly (calibration check)	 IPS CRMs, SRMs, IRMs
Routine methods: - External control - Internal control	 Quarterly According to Table 4	 IPS/IEC IRMs/ECMs

IPS : Inter-comparison Proficiency Study (at-lab and on-farm devices)
IEC : Individual External Control
CRMs : Certified Reference Materials
SRMs : Secondary Reference Materials
IRMs : In-house Reference Materials (control, monitoring, calibration)
ECMs: External Control Materials (service suppliers)
External quality control is implemented by a competent body, thereby linking to systems which are implemented with professional laboratories.

7.2. Internal quality control on on-farm analysers

Internal quality control is meant here to assure official milk recording data for genetic performance meaning that any analytical data used for official milk record should be surrounded by appropriate quality checks. For official milk recording purposes, the following recommendations constitute requirements. For any other purposes they constitute a guidance to users.

7.2.1. Nature, frequencies and limits

On-farm analysers shall be used for a recording frequency twice or more the frequency with a laboratory system. If they are used at the same frequency as in a laboratory system, they should fulfil the accuracy limits for laboratory analysers and have been validated as an analyser of Category 1.

Internal quality control follows the general scheme designed for laboratory analysers provided to fulfil appropriate minimum frequencies and maximum limits for checks relevant with the category of instrument (Table 4).

7.2.2. External reference material

Checks must be rapid and easy, making use of known samples for calibration and internal checks. Where no specific reference values are needed (i.e. control samples, carry over), samples can be prepared from local farm milk.

7.2.3. Internal quality control implementation

7.2.3.1. Instrumental fittings

Provided recommendations are only indicative as some facilities and sources of deviation - such as homogenisation and carry over - may not exist in the instrument. Indications of manufacturers are to be followed. Adequate procedures can be found in the ICAR protocol for milk analyser evaluation according to ISO 8196.

For in-line real time analysers automated check facilities should be installed in the device in order to facilitate and shorten check operations before milking. It should include adequate recording of obtained data for quality control traceability and further maintenance by the manufacturer.

7.2.3.2. Zero setting and stability of calibration line

For at-line analysers, the stability of the calibration line should be checked at the beginning of every analytical session using known materials at low and medium levels of the components.

The nature of the check material depends on the device and the choice of the manufacturer. For instance:
- The medium material can be a long term preserved milk or a standard liquid or a solid material (e.g. filter) giving results at a similar average level as milk.
- The low (zero) level material can be pure water or a standard zero solution or a solid material (e.g. filter).

Concentration target values are those determined by simultaneous analysis with calibration samples or by comparison with the former control milk sample until the next calibration.

During checking, materials are to be analysed minimum in duplicate and mean values obtained should comply with the tolerance interval stated in Table 4 for zero setting and daily calibration.
For real time analysers similar automated tests with sensors should be installed in the device to assure users about the stability of the system. Permanent stable material can be installed as integral part of the device.

7.2.3.3. Repeatability and daily stability

For at-line analysers, perform duplicate analyses of a control sample (7.2.3.3) at the beginning and the end of every analytical session:
- The range between duplicates should not exceed the values r stated in Table 4 for repeatability.
- The range between the four replicates of the analytical session should not exceed the values R stated in Table 4 for reproducibility.

Periodical summaries and calculation of repeatability and reproducibility standard deviations throughout a rolling period (e.g. last 20 sessions) can provide deeper information on the regularity of the method and elements to estimate the measurement uncertainty of the on-farm device. sr and sR values should comply with relevant limits in Table 4.

For real time analysers similar automated tests with sensors should assure users about the stability of the system.

7.2.3.3 and 7.2.3.4 can be conducted together.

7.2.3.4. Calibration and accuracy

For at-line analysers same procedures as for laboratory analysers can apply. Calibration should be periodically checked using milk sample sets with known reference values appropriate for the method. They can be milk sampled at the farm and analysed with reference methods or adequate samples provided by external suppliers and recognised by the milk recording organisation.

For in-line real time analysers calibration can only be checked using milk samples analysed later on with appropriate methods, which can be either a reference method or a milk analyser suitably calibrated. However it cannot be performed in short delays due to required milk sampling and reference analyses performed by a competent party (e.g. accredited laboratory).

Calibration should be checked and adjusted minimum quarterly for at-line analysers and yearly for in-line analysers. Slope and bias values of the calibration line should be within the limits in Table 4.

Accuracy should be checked against reference methods minimum yearly and comply with accuracy limits for individual animals in Table 3.

NOTE Checking calibration of sensors is not easy on-farm and cannot provide the total information of the device calibration in case final results combine test scans and milk quantity measurements. It should be reserved to maintenance.

7.2.3.5. Measurement consistency

For specific in-line real time analysers that combine a number of measurements, assessing consistence between the final result and the response of the scanning sensor allows checking proper functioning of the measuring system, including milk flow rate, milk composition, milking time combined in the final result.

At a yearly frequency, every milking of every animal is sampled with the sampling device of in-line analysers and re-analysed through the analytical sensor used for calibration.

The difference d_C between the measurement results and the result of the sensor should not exceed the reproducibility value R of Table 4 and the average of n differences be outside $\pm 2.(sR^2 + sr^2)^{1/2} / \sqrt{n}$.

Table 3 – Precision and accuracy limits for test bed evaluations of milk analysers in milk recording

Component		**Fat**	**Protein**	**Lactose**	**Urea**	**SCC**
Units		g/ 100 g	g/ 100 g	g/ 100 g	mg/ 100 g	10^3 cells/ml
Range	Total			4,0 - 5,5	10,0 – 70,0	0 – 2000
	Low					0-100
	Medium	2,0 - 6,0	2,5 - 4,5			100-1000
	High	5,0 - 14,0	4,0 - 7,0			> 1000
Sample number	Animals (Na)	100	100	100	100	100
	Herds (Nh)	5	5	5	5	5

Milk analytical devices		**Laboratory**			**On-farm At-line**			**On-farm In-line**		
Equivalence Factor	**FE**		**x 1**			**X 2**			**x 2,5**	
Component		**F-P-L**	**Urea**	**SCC**	**F-P-L**	**Urea**	**SCC**	**F-P-L**	**Urea**	**SCC**
Units		g/ 100 g	mg/ 100 g	percent	g/ 100 g	mg/ 100 g	percent	g/ 100 g	mg/ 100 g	percent
Repeatability								[a]	[a]	[a]
Standard deviation *(sr)*	- Total range			4%			8%			10%
	- Low			8%			16%			20%
	- Medium	0,014	1,4	4%	0,028	2,8	8%	0,035	3,5	10%
	- High	0,028	2,8	2%			4%			5%
Within lab reproducibility										
Standard deviation *(sR)*	- Total range			5%			10%			13%
	- Low			10%			20%			25%
	- Medium	0,028	2,8	5%	0,056	5,6	10%	0,069	6,9	13%
	- High	0,056	5,6	2,50%	0,056	5,6	5%	0,070	7,0	6%
Accuracy										
Animal sample SD *(sy,x)*	- Total range			10%			20%			25%
	- Low									
	- Medium	0,10	6,0		0,20	12,0		0,25	15,0	
	- High	0,20			0,20 [b]			0,25 [b]		
Calibration [c]										
Mean bias *($\bar{d}$)*	- Total range		± 1,2	± 5 %		± 2,4	± 10 %		± 3,0	± 13 %
	- Medium	±0,05			±0,10			±0,13		
	- High	±0,10			±0,20			±0,25		
Slope *(b)*		1±0,05	1±0,10	1±0,05	1±0,10	1±0,10	1±0,10	1±0,13	1±0,10	1±0,13

[a] Where relevant i.e. for in-line differed time analysis.
[b] No larger tolerance by the usual factor 2 for sheep and goat to maintain accuracy with no more numerous records.
[c] Compared to manufacturer calibration.

Table 4 – Quality control – Minimum frequencies and maximum limits (tentative)

Milk analytical devices	Laboratory			On-farm At-line			On-farm In-line		
	Frequencies	Limits F P L	Limits SCC	Frequencies	Limits F P L	Limits SCC	Frequencies	Limits F P L	Limits SCC
Units		g /100 g	percent		g /100 g	percent		g /100 g	percent
Instrumental fittings				[a]			[a]		
Homogenization	monthly	0,05 (1,43 %)	None	*yearly*	*0,05 (1,43 %)*	*none*	*Not relevant*		
Carry-over	monthly	1 %	2 %	*yearly*	*1 %*	*2 %*	*Not relevant*		
Linearity (curving)	quarterly	1 % of range	2 %	*yearly*	*2 %*	*4 %*	*yearly*	*2,5 %*	*5 %*
Intercorrection	quarterly	± 0,02	None	*yearly*	*± 0,05*	*none*	*yearly*	*± 0,05*	*none*
Consistency (n samples)							yearly	± 0,14/√n	± 35%/√n
Calibration									
Mean bias	weekly	± 0,02	± 5 %	quarterly	± 0,04	± 10%	quarterly	± 0,05	± 13%
Slope	quarterly	1,00±0,02	1,00±0,05	quarterly	1,00±0,04	1,00±0,12	quarterly	1,00+/-0,05	1,00+/-0,13
		1,00±0,05 [b]			1,00±0,05 [b]			1,00±0,05 [b]	
Daily stability									
Repeatability limit (r)	start-up	0,04	14%	start-up/end	0,08	28%	*start-up/end*	0,10	35%
Repeatability SD (sr)	start-up	0,014	5%	20 sessions	0,028	10%	*20 sessions*	0,035	13%
Daily/short-term belt	3/hour	± 0,05	± 10%	3/hour	± 0,10	± 20%	*not relevant*		
Reproducibility limit (R)		0,07	14%	session	0,14	28 %	*session/day*	0,17	35 %
Reproducibility SD (sR)		0,025	5 %	20 sessions	0,05	10 %	*20 sessions*	0,06	13 %
Zero-setting	4/day	±0,03	5000 SC/ml	start-up	±0,03	10000 SC/ml	*start-up*	±0,03	13000 SC/ml

[a] Where relevant depending on the instrument
[b] Limit for lactose

NOTE For species with significantly higher concentration in fat and protein (i.e. sheep, buffalo, particular goat and cow breeds), it is appropriate to adjust those limits in proportion of respective mean levels hence to multiply by average species/average cow. For sheep a factor 2 is found suitable.

8. Requirements related to milking systems (Recording Devices SC)

8.1. Evaluation of in-line real time analysers

8.1.1. General

The accuracy of in-line analysers should be evaluated in the condition of configuration and with associated devices as distributed by the manufacturer.

The milking population used for evaluation should be representative for the largest population (with regard to milk production and composition) the analyser is intended for so as to illustrate that high animal performances can be properly measured.

Since a same milking cannot be performed twice per animal:
- Neither repeatability nor reproducibility (between days and between devices) checks can be implemented on-farm for routine quality control hence are of less interest to users. Since then their evaluation can be performed only in the evaluator laboratory through adapted methodologies and remains optional,
- Accuracy measurement should include random errors of repeatability, between consecutive days and between devices reproducibility.

Where adapted procedures, for instance use of preserved milk or substitutes to mimic replications, are to be used, it should have been prior clearly demonstrated that these adequately reproduce the milking conditions with fresh milk, so as not to introduce possible deviation or misinterpretation.

In any aspect of the evaluation, approved reference procedures and methods for representative milk measuring and sampling (whole milking in the bucket according to ICAR) and analytical methods (ISO | IDF methods) should be applied.

8.1.2. Evaluation of the effect of the milking machine on accuracy

8.1.2.1. Laboratory tests:

The role of laboratory tests is to ensure that the tested device is not influenced by the milking machine and flow rate of milk.

That means the influence of:
- milk flow rates on results of a milk of known composition, for instance 1, 3, 5, and 9 kg/min at a given milking vacuum and air inlet at the claw,
- different vacuum levels such as 40, 45 and 50 kPa at a given milk flow rate and air inlet,
- different air inlet such as 0, 8, 12 and 20 l/min at a given milk flow rate and vacuum level,
- tilting (except otherwise stated by the manufacturer). If a maximum tilting is stipulated it should be tested for accuracy of the device at a given milk flow rate, vacuum level and air inlet,

In addition, according to ISO standard 5707 real time analysers should not cause any vacuum drop greater than 5 kPa at a milk flow of 5 kg/min beneath the teat during milking for cows. Thus it is to measure the vacuum drop due to the device compared with no device fitted on the LMT 5 kg/min.

NOTE For application to other species with different milk yield and composition, such as goat, sheep and buffalo, other tests involving different parameters shall be carried out.

8.1.2.2. Field tests:

Field test are necessary in order to ensure that accuracy is the same whatever is the milk flow and the milk composition. Tests should be carried out at least on four devices in two different farms as described for milk meters.

9. Data Records and Data Management

Because of many different well-established data transfer standards at national level, it is not possible for ICAR to define an international standard. Different data transfer protocols (XML, CSV, ADIS, etc.) as well as different national data dictionaries are used. ICAR will only confine these standards with defining the necessary content of the data records. Existing international standards like ISO, ICAR or ISOagriNET should be used. Therefore, ICAR gives only definitions how to handle data without submitting a statement about transfer protocols and data dictionaries.

Information can be transmitted on farm (e.g. from the analyzing unit to a processing computer, e.g. from a processing computer to a herd management computer, etc.) or between business partners like farmers, milk laboratories, milk recording organizations and IT centres. For data management and data transfer, each milking has to be reported during the sampling period with one data record. Data items like farm ID, animal ID, date, time, session milk yield and abnormal end of the milking must be included for each milking during this sampling period. In addition, an average 7 day milk yield as calculated by the farm management software should be reported.

Minimum data transfer requirement is to transmit the information registered in table 1. These information are necessary to calculate a 24 hour, a 48 hour, a 96 hour, etc. milk yield as regulated in national or international guidelines (mandatory items) (example 1). If milk content values are broken down by an analysing unit, the items presented in table 2 must be added to the data record as defined in table 1 (optional items) (example 2). In addition, milk sample bottles can be used to control the results of the installed analysing unit by official laboratory results. In this case, the milk sample bottle has to be identified clearly to combine the results of the installed analysing unit with the results of this bottle. One data record must then include the mandatory information of table 1, the results of the installed analysing unit as defined in table 2 and one of the unique identification alternatives of a milk sample bottle as described in table 3 (optional and conditional items) (example 3).

Generally, the manufacturers of analysing units have to ensure that all information is transferred using one record for each animal and each milking.

Table 1: Entity of on-farm analysis of milk content, mandatory items

item	**data type** [1)]	**length**	**decimal**	**Description**
farm ID	N	15	0	farm identification number (official (in law) farm identification number or farm number given by milk recording organization)
animal ID	N	15	0	official (in law) animal identification number on national or regional level [2)]
date	N	8	0	(starting) date of milking YYYYMMDD (year, month, day) (20071127 = 27th November 2007)
time	N	6	0	starting time of milking hhmmss (hour, minute, second) (140145 = 14:01:45)
session milk yield	N	3	1	individual milk weight (in kg), given by the animal during the milking (178 = 17.8 kg)
abnormal end of the milking	AN	1	0	T or F, T = True, F = False (if false, then normal milking, if true, then milking was aborted)
7 day milk yield	N	3	1	7 day average, as calculated by the management software (in kg)
… ANALYSIS …				[3)]

[1)] data type: N = numeric, AN = alpha numeric

[2)] animal ID in accordance with ISO standard 11784 are composed of a country code (a) and a national identification code (b)

(a) 'country code' means a 3-digit numeric code representing the name of the country in accordance with ISO standard 3166

(b) 'national identification code' means a 12-digit numeric code to identify an individual animal at national level; if the national identification code is less than 12 digits, the space between the national identification code and the country code shall be completed with zeros

[3)] Each value wich is detected in the analysing unit should be submitted following the configuration of Table 1. Table 2 gives an overview about feasible analysis values.

Table 2: Examples for analysed values, optional items

item	**data type**	**length**	**decimal**	**Description**
fat percent	N	4	2	fat percent (in %), (0421 = 4.21 %)
protein percent	N	4	2	protein percent (in %), (0389 = 3.89 %)
lactose percent	N	4	2	lactose percent (in %), (0485 = 4.85 %)
somatic cell count	N	5	0	somatic cells in thousand, (00195 = 195,000)
urea	N	3	0	urea (in ppm), (224 = 224 ppm)
… OTHERS …				[4)]

If more samples per cow per recording day are analysed, results for each sample must be reported. These can be presented either as single results or as an average of n samples.
If one sample per cow is fatcorrected according to national or international standards, the corrected values are reported.

[4)] Other items (other values) have to be authorised by ICAR to define a data transfer standard.

The possibility for submitting bottles to a milk testing laboratory must be taken into account (e.g. control of the analysing units, e.g. official milk recording, etc.). The bottles must be identified clearly. This unique identification can be achieved by using

a) a bar code or
b) a data chip (e.g. RFID) or
c) a sample bottle ID or
d) a unique number for sample box including the sample bottle number.

For this reason, the record should be extended as described in tables 3a up to 3d (alternative).

Table 3a: Example for identifying milk sample bottles using bar code, optional item

item	**data type**	**length**	**decimal**	**description**
bar code	N	10	0	bar code

or

Table 3b: Example for identifying milk sample bottles using a data chip, optional item

item	**data type**	**length**	**decimal**	**description**
RFID	N	?	0	data chip

or

Table 3b: Example for identifying milk sample bottles using a unique sample bottle ID, optional item

item	**data type**	**length**	**decimal**	**description**
sample bottle ID	N	?	0	individual ID of each bottle

or

Table 3d: Example for identifying milk sample bottles using sample box number and sample bottle number, optional items

item	**data type**	**length**	**decimal**	**description**
sample box number	N	6	0	number of sample box
sample bottle number	N	4	0	bottle number within the sample box

Example 1: Minimum data transfer requirement – recording the milk yield

Two cows (DK 1 12 321 51235 and AT 05 1235 4123) at farm 276031239512354 were milked at 27th November 2007 around 2:00 p.m. (automatic milking system). Fat, protein, lactose, somatic cell count and urea were not (!) analysed automatically by the installed analysing unit. No milk samples in bottles collected. The data records must include:

Animal DK 1 12 321 51235:

```
farm ID                  276031239512354
animal ID                208011232151235
date                     20071127
time                     140145
session milk yield       178
abnorm. end milk. sess.  F
7 day milk yield         532
```

Animal AT 05 1235 4123:

```
farm ID                  276031239512354
animal ID                040000512354123
date                     20071127
time                     141852
milk yield               106
abnorm. end milk. sess.  F
7 day milk yield         213
```

Example 2: Analysing unit is producing milk content results, no 'official' collection of milk sample bottles

Twp cows (DK 1 12 321 51235 and AT 05 1235 4123) at farm 276031239512354 were analysed by an on-farm analysing unit at 27th November 2007 around 2:00 p.m. (automatic milking system). Fat, protein, lactose, somatic cell count and urea were analysed automatically by the installed analysing unit. No milk samples in bottles collected. The data records must include:

Animal DK 1 12 321 51235:

```
farm ID                  276031239512354
animal ID                208011232151235
date                     20071127
time                     140145
milk yield               178
abnorm. end milk. sess.  F
7 day milk yield         532
fat percent              0421
protein percent          0389
lactose percent          0485
somatic cell count       00195
urea                     220
```

Animal AT 05 1235 4123:

```
farm ID                  276031239512354
animal ID                040000512354123
date                     20071127
time                     141852
milk yield               106
abnorm. end milk. sess.  F
7 day milk yield         213
```

fat percent	0409
protein percent	0372
lactose percent	0475
somatic cell count	08918
urea	190

Example 3: Analysing unit is producing milk content results, 'official' collection of milk sample bottles

Two cows (DK 1 12 321 51235 and AT 05 1235 4123) at farm 276031239512354 were analysed by an on-farm analysing unit at 27th November 2007 around 2:00 p.m. (automatic milking system). Fat, protein, lactose, somatic cell count and urea were analysed automatically by the installed analysing unit. Milk sample bottles with bar code identification were collected. The data records should include:

Animal DK 1 12 321 51235:

farm ID	276031239512354
animal ID	208011232151235
date	20071127
time	140145
milk yield	178
abnorm. end milk. sess.	F
7 day milk yield	532
fat percent	0421
protein percent	0389
lactose percent	0485
somatic cell count	00195
urea	220
bar code [5)]	5863252147

Animal AT 05 1235 4123:

farm ID	276031239512354
animal ID	040000512354123
date	20071127
time	141852
milk yield	106
abnorm. end milk. sess.	F
7 day milk yield	213
fat percent	0409
protein percent	0372
lactose percent	0475
somatic cell count	08918
urea	190
bar code [5)]	9371535180

[5)] Instead of bar code identification of the sample bottles it is possible to use data chips, sample bottle IDs or sample box number including sample bottle number.

10. **Requirements related to animal identification (Animal Identification SC)** (see 8)

11. **Requirements related to lactation calculation (Lactation Calculation WG)** (under development)

12. **Requirements related to recording data (Animal Recording Data WG)** (see 8)