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Milk — Enumeration of somatic cells —

Part 2: Guidance on the operation of fluoro-opto-electronic counters

Lait — Dénombrement des cellules somatiques —

Partie 2: Lignes directrices pour la mise en œuvre des compteurs fluoro-opto-électroniques

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Foreword

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

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

ISO 13366-2 | IDF 148-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This edition of ISO 13366-2 | IDF 148-2 cancels and replaces ISO 13366-2:1997 and ISO 13366-3:1997, which have been technically revised.

ISO 13366 consists of the following parts, under the general title *Milk — Enumeration of somatic cells*:

— *Part 1: Microscopic method (Reference method)*

— *Part 2: Guidance on the operation of fluoro-opto-electronic counters*

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

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

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ISO 13366-2 | IDF 148-2 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Action Team on *Automated methods*, of the Standing Committee on *Quality assurance, statistics of analytical data and sampling*, under the aegis of its project leaders, Mrs S. Orlandini (IT) and Mr H.J.C.M. van den Bijgaart (NL).

This edition of ISO 13366-2 | IDF 148-2 cancels and replaces IDF 148A:1995, methods B and C of which have been technically revised.

ISO 13366 consists of the following parts, under the general title *Milk — Enumeration of somatic cells*:

— *Part 1: Microscopic method (Reference method)*

— *Part 2: Guidance on the operation of fluoro-opto-electronic counters*

Milk — Enumeration of somatic cells —

Part 2:
Guidance on the operation of fluoro-opto-electronic counters

1 Scope

This part of ISO 13366|IDF 148 gives guidance on the operating conditions for counting somatic cells, in both raw and chemically preserved milk, using fluoro-opto-electronic somatic cell counters in which either a rotating disc technique or flow cytometry is applied in the counting section.

The guidance is applicable to the counting of somatic cells in raw cow milk. The guidance is also applicable to raw milk of other species, such as goat, sheep and buffalo, if the specified prerequisites are met.

2 Normative references

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

ISO 8196-1|IDF 128-1, *Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis — Part 1: Analytical attributes of indirect methods*

ISO 8196-2|IDF 128-2, *Milk — Definition and evaluation of the overall accuracy of indirect methods of milk analysis — Part 2: Calibration and quality control in the dairy laboratory*

ISO 13366-1|IDF 148-1, *Milk — Enumeration of somatic cells — Part 1: Microscopic method (Reference method)*

ISO Guide 34, *General requirements for the competence of reference material producers*

ISO Guide 43-1, *Proficiency testing by interlaboratory comparisons — Part 1: Development and operation of proficiency testing schemes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8196-1|IDF 128-1, ISO 8196-2|IDF 128-2 and the following apply.

3.1
reference method
method described in ISO 13366-1|IDF 148-1 for the counting of somatic cells

3.2
somatic cells
those cells that show more than a threshold intensity of fluorescence due to the staining of DNA in their nuclei

NOTE The number of somatic cells is expressed in cells per millilitre.

4 Principle

Fluoro-opto-electronic counters contain functions for the uptake of reagents and test sample, a mixing section and a counting section. In the mixing section, the test sample is mixed with a buffer and a stain solution. Part of the resulting mixture is transferred to the counting section and put onto an object plane. Each stained particle observed with a fluorescence microscope produces an electrical pulse that is filtered, amplified and recorded. The resulting pulse height distribution is electronically processed, whereby discrimination is made between noise signals and pulses that are attributed to stained somatic cells. The discriminator level can either be fixed or dynamic.

In the mixing section, closely controlled volumes of test sample and buffer/stain solutions are dosed and mixed. Mixing can take place in a cup, a mixing chamber, a centrifuge, a sample loop or in the tubing leading to a flow cell.

In the counting section, either disc cytometry or flow cytometry can be applied. In the case of disc cytometry, a thin film of the mixture is brought through a nozzle to the top of a vertical rotating disc. This rotating surface acts as a moving object plane for a fluorescence microscope. When using flow cytometry, part of the mixture is placed in the high-speed flow of a surrounding sheath liquid in a capillary flow cell. Through acceleration, the mixture forms a thin string in which the somatic cells are dynamically focused and aligned. This string then passes the objective of a fluorescence microscope.

Some instruments contain two channels in the counting section. In terms of analytical quality assurance, such a situation should be considered equivalent to working with two separate units, so the performance should be evaluated separately for each channel.

5 Factors affecting the results of measurements

5.1 Sample bottles

Sample bottles should be fit for use; i.e. to transfer test samples from the point of sampling to the laboratory without loss or damage.

Care is to be exercised that sample bottles are leak-proof and that a proper empty volume is left. Too large an empty volume can facilitate churning; too small an empty volume can cause problems with mixing.

5.2 Sampling

5.2.1 General

Sampling materials (i.e. sample bottles, beakers and sampling devices) should be clean and dry. Where automatic samplers are used, these should have been properly validated.

Test samples should preferably be cooled immediately after sampling to between 0 °C and 6 °C and be kept at that temperature until counting (see 5.4) rather than being preserved. Freezing should be avoided. If preservation is necessary, suitable means for chemical preservation of test samples are described in 5.3.

5.2.2 Bulk milk samples

Thorough mixing of the raw bulk milk to be sampled is essential. Somatic cells will concentrate in the upper and lower layers in the case of insufficient stirring.

5.2.3 Milk samples from individual animals

The release of somatic cells in the milk during milking is uneven. When the aim is to produce a representative counting result for a whole milking, it is essential that a representative sample of the whole milking be obtained. For diagnostic purposes, a sample of a partial milking may suffice.

5.3 Preservation

If chemical preservation is considered necessary, the test sample (5.2.1) should be preserved as soon as possible, but in any case within 24 h after sampling. In all cases, the test sample should be kept cool (0 °C to 6 °C) until the addition of the preservative.

Suitable preservatives are the following.

a) Boric acid: its final concentration in the test sample should not exceed 0,6 g/100 ml. Such preserved samples may be stored at between 6 °C and 12 °C for up to a further 24 h.

b) Sodium azide: its final concentration in the test sample should not exceed 0,024 g/100 ml. Such preserved samples may be stored at between 2 °C and 10 °C for up to a further 72 h.

c) Bronopol (2-bromo-2-nitro-1,3-propanediol): its final concentration in the test sample should not exceed 0,05 g/100 ml. Such preserved samples may be stored at between 2 °C and 12 °C for up to a further 6 d.

d) Potassium dichromate: its final concentration in the test sample should not exceed 0,2 g/100 ml. Such preserved samples may be stored at between 2 °C and 12 °C for up to a further 6 d.

Accompanying colour tracers found to be suitable are

— Patent Blue V with a final concentration in the test sample of up to 0,15 mg/100 ml,

— Yellow Orange S (E110) with a final concentration in the test sample of up to 1 mg/100 ml, and

— a mixture of Patent Blue V and Eosin B with a final concentration in the test sample of up to 0,03 mg and 0,45 mg/100 ml, respectively.

Other preservatives and colour tracers may be used provided that their effectiveness and conditions for use have been soundly validated.

In all cases, properly validate the absence of interference for the counting equipment concerned before application.

In cases where fluoro-opto-electronic cell counters are combined with milk analysers for the measurement of other test sample components, care should be taken that the applied preservatives and colour tracers do not affect the counting result.

5.4 Sample storage and transport

Unpreserved test samples should be stored at between 0 °C and 6 °C and should be counted within 96 h after the completion of sampling. Avoid freezing the test samples. Storage at higher temperatures and/or over longer time scales may result in non-representative counts. The measurement of samples after freezing and thawing may result in lower counts (by 10 % to 20 %). The age of the samples at freezing and the type of thawing process can influence the counting result.

5.5 Interfering substances

The use of substances that interfere in the counting should be avoided. Substances known to influence the instrument read-out are

a) preservatives and colour tracers at higher concentrations than specified in 5.3, and

b) Methylene blue at higher concentrations, i.e. $>$ 0,06 mg/100 ml.

5.6 Sample quality at analysis

Breakdown of somatic cells (lysis) will result in an increase of smaller cell fragments. The lower intensity of fluorescence after staining of these particles causes a shift in the pulse height distribution to the left. This will hamper proper differentiation from noise pulses and therefore result in a lower count.

NOTE In several types of instruments, features are available for evaluating the position and the shape of the pulse height distribution. See the relevant instructions and guidance from the instrument manufacturer.

After the processing of problematic samples, the flow path should be checked and possible cleaned. The proper functioning of the instrument should be tested before further use. Possible problematic test samples are

a) milk samples from severely infected udders, i.e. with clots,

b) milk samples with impurities,

c) milk samples with high numbers of erythrocytes,

d) colostrum,

e) late lactation milk, and

f) sour milk.

Where possible, analysis of problematic test samples should be avoided.

5.7 Chemicals used

All reagents used should be of recognized analytical and bacteriological quality. Water used should be demineralized (remaining conductivity < 10 µS/cm) or water of at least equivalent purity. Follow the manufacturer's instructions for the preparation of the working solutions, the maximum storage time and storage requirements.

Local conditions regarding the use and discharge of applied chemicals and effluents should be observed.

5.8 Instrument condition

5.8.1 General points of attention are

a) the functioning of the mixing device and the stirrer,

b) possible disturbances at sample intake and in the flow system due to blocking by impurities, clots or fouling in the mixing and incubation units, and

c) the condition and the functioning of the light source and the photo multiplier, the gain setting and the signal quality.

5.8.2 Specific points of attention with disc cytometric counters are

a) the positioning of the film on the rotating disc,

b) the cleanliness of the object plane and the functioning of the cleaning sponge; timely replacement of the sponge is essential, and

c) proper emptying of the collection vessel for the rinsing liquid.

5.8.3 A specific point of attention with flow cytometric counters is the variation in the behaviour of the sample string in the flow cell and the sheath liquid flow. Some instrument manufacturers offer special programme features for checking this, thereby indicating possible solutions in the case of deviations.

5.9 Working factor

The working factor is the number by which the actual number of somatic cells counted by an instrument is multiplied in order to arrive at the somatic cell count of the test sample. In theory, precision characteristics and accuracy should benefit from a lower working factor.

5.10 Testing volumes

A proper ratio between the volume of buffer/stain solution and that of the test sample is essential for correct counting.

6 Calibration

6.1 Reference materials

6.1.1 General

Reference materials should be produced under closely controlled conditions; i.e. working with a quality assurance system and fulfilling the requirements as listed in ISO Guide 34.

Reference materials may be

a) certified reference materials (CRMs) as produced by a recognized official organization,

b) secondary reference materials (SRMs) as prepared by an external supplier, or

c) in-house reference materials (IRMs) as prepared by the laboratory itself, whereby traceability is kept with CRMs, SRMs or via interlaboratory proficiency studies.

NOTE CRMs for somatic cell counting are not available. Examples of suitable procedures for the preparation of IRMs are listed below. IRMs with a composition as close as possible to natural milk are preferable.

6.1.2 Preparation of calibration samples

6.1.2.1 Preparation by addition of a bovine leucocyte suspension

a) Mix 1 000 ml of sterilized or UHT milk with low somatic cell count with 1 ml of polypropylene 2000 and 0,4 g of bronopol.

b) Add the required amount of a suitable leucocyte suspension to different portions of the mixture in order to obtain a suitable range of cell counts.

6.1.2.2 Preparation by microfiltration

a) Collect fresh bulk milk and add bronopol to a final mass fraction of 0,02 %.

b) Skim the milk in a cream separator to a mass fraction of fat of below 0,1 %.

c) Concentrate the skimmed milk 20-fold by applying tangential microfiltration over a membrane with a pore size of 0,8 µm, resulting in a portion with a high cell content (HCM) and a portion with a low cell content (LCM).

d) Mix cream, HCM and LCM in the required quantities so as to obtain 5 to 8 milk portions with a mass fraction of fat of 3,5 % and different levels of cell counts covering the range of interest.

6.1.2.3 Preparation by centrifugation

a) Collect fresh bulk milk and add bronopol to a final mass fraction of 0,02 %.

b) Filter the thus-prepared milk over a metal filter with a pore size of 0,5 mm.

c) Skim the filtered milk by centrifuging at a radial acceleration of 40 g for 10 min so as to obtain cream, skimmed milk and a pellet.

d) Mix the skimmed milk and pellet material in the required quantities so as to obtain 5 to 8 milk portions with different cell count levels covering the range of interest.

e) If necessary, add cream so as to obtain portions with a mass fraction of fat of 3,3 % $\pm$ 0,3 %.

f) Split the obtained portions into individual samples and heat these at 120 °C and 10^5 Pa for 3 min.

6.1.3 Assignment of reference values

Reference values should be determined by replicate analysis with the reference method described in ISO 13366-1 | IDF 148-1, preferably in at least two different laboratories.

Parallel replicate counting of calibration samples by instrumental methodology, accurately calibrated with a still valid former set of calibration samples, can play a valuable supportive role in suppressing fluctuations in counting levels over time.

Sound congruence should normally be found; that is a maximum difference between reference method results and instrumental countings for individual calibration samples of less than 10 %. In such cases, the obtained reference method values and instrumental countings may be combined, whereby the result of the reference method should be included with a weighting factor of at least 0,5 in the assigned reference value.

In cases where the results from counting in test samples are to be used for checking against official limits, approval from competent authorities should be sought for the procedure on the assignment of reference values.

6.1.4 Storage conditions and keeping time

The storage conditions and the keeping time of the prepared reference materials should be properly validated. A shelf life of one month is considered to be a minimum.

When chemical preservation is used, alignment should be sought with the type and concentration of the preservative applied to the test samples (see 5.3).

6.2 Calibration procedure

6.2.1 Calibration

Before calibration, it should be ascertained that the instrument is properly functioning and fulfilling the requirements with regard to blank checks (9.1), carry-over effect (9.2), volume ratio (9.3) and repeatability (9.6).

It is assumed that linear regression can be applied for calibration purposes.

Apply a calibration procedure according to ISO 8196-2 | IDF 128-2 using at least five calibration samples that cover the relevant range of somatic cell counts.

Indicative values for the relevant ranges of somatic cell counts of calibration samples are shown in Table 1.

Table 1 — Indicative values for relevant calibration ranges

Type of milk	Range cells/ml
Cow milk (herd bulk)	100 000 to 1 000 000
Cow milk (individual animals)	100 000 to 2 000 000
Goat milk	200 000 to 2 000 000
Sheep milk	100 000 to 2 000 000
Buffalo milk	100 000 to 2 000 000

The calibration should be checked at least once a month.

6.2.2 Check on linearity

The relationship between the instrument readings and the reference values should be linear within the relevant range of somatic cell counts. Deviations from linearity may stem from non-specific signals and coincidence effects.

First perform a linearity check visually using appropriate figures so as to obtain a clear picture of the shape of the relationship. Whenever deviation from linearity appears evident, use a quantitative parameter as a test to indicate whether the observed trend is acceptable or not.

To achieve this, use may be made of high-count milk diluted serially with low-count milk, resulting in a set of at least five samples covering the concentration range.

Measure the high-count milk and the low-count milk according to Clause 8 in at least quadruplicate and calculate the average result for each sample. Calculate values for the intermediate samples from the applied mixing ratio per sample, resulting in an expected value for each sample. Then measure all samples according to Clause 8 in at least quadruplicate and calculate the average result for each sample, which is equivalent to the measured value per sample.

Apply linear regression with the expected values per sample on the x-axis and the measured values per sample on the y-axis. Calculate the residuals $e_i = y_i - (\mathrm{b}x_i + \mathrm{a})$ from the regression. Plot the residuals e_i (y-axis) versus the expected values (x-axis) on a graph. A visual inspection of the data points will usually yield sufficient information about the linearity of the signal. Any outlying residual should lead to deleting the related result and renewing the calculation process before testing further.

When observed, the curving may be expressed by the ratio, r_C, by using the following equation:

$$r_\mathrm{C} = \frac{\left(e_\mathrm{max} - e_\mathrm{min}\right)}{\left(M_\mathrm{max} - M_\mathrm{min}\right)} \times 100$$

where

e_max is the numerical value of the maximum residual from the regression;

e_min is the numerical value of the minimum residual from the regression;

M_max is the numerical value of the upper measured value for the set of samples concerned;

M_min is the numerical value of the lower measured value for the set of samples concerned.

The ratio, r_C, should be less than 2 %. If this value is superseded, better performance may be obtained by making separate calibrations for distinct counting ranges.

NOTE Generally, it is possible to combine the required linearity check with the calibration.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage. Specific points of attention are listed in 5.2 to 5.6.

Sampling is not part of the method specified in this part of ISO 13366 | IDF 148. Guidance on sampling is given in ISO 707 | IDF 50.

8 Determination

Prior to analysis, the test sample (5.2.2 or 5.2.3) should be gradually heated to 40 °C ± 3 °C and should be mixed by inversion. Test samples may be kept at room temperature until tested, provided that the samples are tested within 30 min of having reached 40 °C ± 3 °C. Directly before testing, the samples should again be mixed thoroughly.

Follow the instructions of the instrument manufacturer for the measurement of test samples.

NOTE Extending the duration of counting, which is offered as an option with some instruments, coincides in fact with lowering the working factor (see 5.9). It can improve the repeatability and accuracy of the measurements.

9 Checks on performance in routine operation

9.1 Blank checks

Blank checks are meant to check the flow path through the instrument for contamination with impurities. At start-up, a blank check should be carried out in at least five-fold.

The mean should not exceed 3 000 cells/ml and all individual results should be below 8 000 cells/ml.

In routine testing, a blank check should be made after 100 samples as a maximum, or every 2 h, whichever comes first.

9.2 Carry-over effect

The test portion from one test sample may affect the result of the next sample. This carry-over effect should be checked at least monthly by measuring at least five sequences of a test sample with a somatic cell count of at least 750 000 per millilitre, followed by two blanks. Calculate the carry-over value, CO, expressed in percent, using the equation:

$$\mathrm{CO} = \frac{\left(\Sigma B_1 - \Sigma B_2\right)}{\left(\Sigma M - \Sigma B_2\right)} \times 100\ \%$$

where

B_1 is the numerical value of the reading for the first blank;

B_2 is the numerical value of the reading for the second blank;

M is the numerical value of the reading for the test sample.

The resulting carry-over effect should be less than 2 %.

Generally, the calculated carry-over effect can be automatically compensated for when testing samples routinely.

NOTE In some instruments, a carry-over effect can also occur between non-consecutive samples, for instance when a wheel with incubation cups is applied.

9.3 Ratio of reagent volume and test sample volume

A suitable ratio for the volumes of buffer/stain solution and the test milk sample is essential for correct counting. With rotating disc cytometers, this should be tested regularly. Collecting dispensed volumes of buffer/stain solution and test sample in preweighed bottles or tubes may be used for this test.

After weighing, the ratio can be calculated. The calculated ratio should be in agreement with the prescribed value of ± 5 % relative.

9.4 Pilot checks

9.4.1 General

The measurement of pilot samples with assigned pilot milk values is intended to check the short-term stability of the instrument. Use pilot milk(s) with average and preferably also high ($> 2 \times$ average) somatic cell counts in the relevant counting range.

9.4.2 Pilot milk

In order to obtain suitable pilot milk(s), the procedures for the preparation of reference materials may be applied (see 6.1). An alternative procedure is the selection of suitable test samples from routinely analysed batches and the subsequent preparation of combined milks with the addition of a suitable preservative (see 5.3). Store these samples at between 0 °C and 6 °C until use. Avoid freezing the pilot milk samples. It is to be noted that the period of use for unpreserved samples is generally limited to one to two days after preparation.

9.4.3 Assignment of pilot milk values

Analyse at least 10 pilot samples in duplicate on a calibrated instrument. Calculate from the results the repeatability limit r according to ISO 8196-2|IDF 128-2. Provided that the calculated value is lower than the target repeatability value as listed in 11.1, calculate the arithmetic mean of the obtained results and assign this result as the pilot milk value.

9.4.4 Use of pilot milk samples

Pilot checks should be made at the beginning of a working day and consecutively at least three times per hour while analysing samples routinely. Pilot milk samples should be representative of the milk under analysis and should be subject to the same procedure for sample pretreatment and analysis as the samples tested.

For a proper monitoring of the instrument stability, a control chart according to ISO 8196-2|IDF 128-2 may be used. Thereby, the assigned value to the pilot milk serves as the reference value. Appropriate action should be taken if one or more of the obtained values are outside the limits for an individual result or an arithmetic mean.

9.5 Additional instrument surveillance

Some instrument manufacturers offer artificial particle samples to assist in daily instrument surveillance.

9.6 Repeatability

A repeatability check should be made every working day at start-up according to the instructions of the manufacturer. Use may be made of pilot milk samples.

When routinely testing large numbers of samples with high capacity equipment, it is recommended to execute 10 replicate determinations in a pilot sample at start-up. Furthermore, it is recommended to test 20 different individual test samples twice in consecutive runs at regular intervals, e.g. about once per week.

The relative standard deviation of repeatability should be calculated according to ISO 8196-2|IDF 128-2. Appropriate action should be taken if an obtained value is larger than the value specified in 11.1.

9.7 Intralaboratory reproducibility

For instruments subject to the same calibration system, typically within one laboratory with several instruments, intralaboratory reproducibility (see 11.2) should be checked.

The term "intralaboratory reproducibility" relates to analysis performed in the same laboratory, using the same method on identical test material by possibly different operators using different instruments at different times (within at most a few hours).

Available individual pilot milk results from repeatability checks (see 9.6) may also be used to check intralaboratory reproducibility. Appropriate action should be taken if an obtained value is larger than the value specified in 11.2.

9.8 External comparisons

Participation in interlaboratory proficiency studies according to ISO Guide 43-1 at least twice per year is essential as part of a quality assurance scheme for fluoro-opto-electronic somatic cell counting. The number of participants should not be lower than 10. The relevant counting range should be covered by at least 10 samples, which are to be offered for duplicate analysis, two samples being taken from each sample bottle.

10 Specific remarks for the use with milk from different species

10.1 General

It is recommended to check the absence of a significant effect of elevated fat and protein concentrations, for instance by adding cream and ultrafiltration retentate.

If a significant influence is observed, an adjustment of the instrument and/or of the counting procedure should be made in accordance with the recommendations of the instrument manufacturer.

Possible necessary adaptations are as follows:

a) prediluting the test sample;

b) using a more concentrated stain;

c) adjusting the amount of buffer/stain solution;

d) changing the test sample temperature;

e) lengthening the pass time through the flow cell;

f) adjusting the mathematical processing of the obtained pulse height distribution.

10.2 Cow milk

For cow species delivering milk with high fat and protein content, possible interference with the somatic cell counting should be checked.

10.3 Goat milk

Somatic cells in goat milk are much smaller compared to those in cow milk. Additional cell material, such as cytoplasmatic particles, can introduce additional noise. This requires extra care to obtain a proper discrimination. However, depending on the instrument and if the total solids content is not too high, goat milk may be analysed under a cow milk calibration on fluoro-opto-electronic counters, provided that the relevant range of somatic cell counts is covered by the calibration set [4].

Somatic cell counts in goat milk and their variation with, for instance, the stage of lactation are generally higher compared to cow milk and are less strongly influenced by udder health status.

10.4 Sheep milk

In fluoro-opto-electronic counting processes, somatic cells in sheep milk have a similar appearance to those in cow milk. Depending on the instrument and if the total solids content is not too high, sheep milk may be analysed under a cow milk calibration on fluoro-opto-electronic counters, provided that the relevant range of somatic cell counts is covered by the calibration set [4].

Somatic cell counts in sheep milk might be at the same level as in cow milk but can also be higher.

10.5 Buffalo milk

In fluoro-opto-electronic counting processes, somatic cells in buffalo milk have a similar appearance to those in cow milk. Fat and protein content may be considerably higher, which requires checks for possible interference and necessary adjustments.

11 Precision

NOTE The indicative values for precision listed below were extracted from proficiency studies with cow milk. Values for milk from other species might be less favourable.

11.1 Repeatability

The absolute difference between two independent single test results (r), obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should in not more than 5 % of cases be greater than the values listed in Table 2.

Table 2 — Repeatability values

Cell count level cells/ml	s_r %	r cells/ml
150 000	6	25 000
300 000	5	42 000
450 000	4	50 000
750 000	3	63 000
1 500 000	3	126 000

11.2 Intralaboratory reproducibility

The absolute difference between two independent single test results (R_{intra}), obtained using the same method on identical test material in the same laboratory by possibly different operators using different instruments at different times (within at most a few hours), should in not more than 5 % of cases be greater than the values listed in Table 3.

Table 3 — Intralaboratory reproducibility values

Cell count level cells/ml	$s_{R\text{intra}}$ %	R_{intra} cells/ml
150 000	7	29 000
300 000	6	50 000
450 000	5	63 000
750 000	4	84 000
1 500 000	4	168 000

11.3 Reproducibility

The absolute difference between two independent single test results (R), obtained using the same method on identical test material in different laboratories within a short interval of time, should in not more than 5 % of cases be greater than the values listed in Table 4.

Table 4 — Reproducibility values

Cell count level cells/ml	s_R %	R cells/ml
150 000	9	38 000
300 000	8	67 000
450 000	7	88 000
750 000	6	126 000
1 500 000	6	252 000

12 Test report

The test report should specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the date of sampling;

d) the type of sample;

e) the test method used;

f) all operating details deviating from the guidelines in this part of ISO 13366|IDF 148, which may have influenced the test result(s);

g) the test result(s) obtained in thousands of cells per millilitre or, if the repeatability has been checked, the final quoted result obtained.

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