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EAS 163 (2000) (English): Milk — Determination of freezing point — Thermistor

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EAST AFRICAN STANDARD

Milk — Determination of freezing point — Thermistor cryoscope method

EAST AFRICAN COMMUNITY

First Edition 2000

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to achieve this objective, the Partner States in the Community through their National Bureaux of Standards, have established an East African Standards Committee.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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INTERNATIONAL STANDARD

ISO 5764

IDF 108

Second edition
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Milk — Determination of freezing point — Thermistor cryoscope method (Reference method)

Lait — Détermination du point de congélation — Méthode au cryoscope à thermistance (Méthode de référence)

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Foreword

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

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 5764|IDF 108 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This joint edition of ISO 5764|IDF 108 cancels and replaces the first edition of ISO 5764:1987, which has been technically revised.

Annexes A to C of this International Standard are for information only.

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 and AOAC International 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 National Committees casting a vote.

International Standard ISO 5764|IDF 108 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Water*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr H.J.C.M. van den Bijgaart (NL).

This joint edition of ISO 5764|IDF 108 cancels and replaces the second edition of IDF 108:1991, which has been technically revised.

Introduction

The method described in this International Standard for the determination of the freezing point of milk uses a thermistor cryoscope, in which a thermostatically controlled device is cooled and a thermistor probe is used for the measurement of the freezing point.

This reference method requires the use of plateau-timed instruments. For routine measurements, other thermistor cryoscope methods, i.e. fixed time procedures, can be used. Guidelines for the application of other procedures are given in annex B.

Milk — Determination of freezing point — Thermistor cryoscope method (Reference method)

1 Scope

This International Standard specifies a reference method for the determination of the freezing point of raw, pasteurized, UHT-treated or sterilized whole milk, partially skimmed milk and skimmed milk by using a thermistor cryoscope.

The freezing point can be used for estimating the proportion of extraneous water in milk. Calculation of the amount of extraneous water is complicated by daily variation, seasonal variation, etc. and is not within the scope of this International Standard.

Results obtained from samples with a titratable acidity exceeding 20 ml of 0,1 mol/l sodium hydroxide solution per 10 g of non-fat solids will not be representative of the original milk.

NOTE Sterilization and vacuum pasteurization can affect the freezing point of milk (see reference [6]).

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6091, *Dried milk — Determination of titratable acidity (Reference method)*

3 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1
freezing point of milk
value obtained using the method specified in this International Standard

NOTE The freezing point is expressed in millidegrees Celsius (m°C).

4 Principle

A test sample of milk is super-cooled to an appropriate temperature and crystallization is induced by means sufficient to cause an instantaneous release of heat with an accompanying warming of the sample to a temperature

plateau. The plateau is reached when the temperature rise has not exceeded 0,5 m°C over the last 20 s. The thus-attained temperature corresponds to the freezing point of the milk sample.

The instrument is calibrated by adjusting it to give the correct readings for two sodium chloride standard solutions, using the same procedure as for test portions of milk.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Water, complying with grade 2 in accordance with ISO 3696, or water of equivalent quality distilled from borosilicate glass apparatus.

The water shall be boiled and cooled to 20 °C ± 2 °C shortly before use.

5.2 Sodium chloride (NaCl), finely ground, dried in the electric furnace (6.7) at 300 °C ± 25 °C for 5 h or alternatively dried in the drying oven (6.8) at 130 °C ± 2 °C for at least 24 h, then cooled to room temperature in a desiccator (6.9).

5.3 Sodium chloride standard solutions

Weigh, to the nearest 0,1 mg, the appropriate amount (see Table 1) of prepared dry sodium chloride (5.2) in a weighing bottle (6.5). Dissolve in water (5.1) and transfer quantitatively to a 1 000 ml one-mark volumetric flask (6.6). Dilute to the 1 000 ml mark with water (5.1) at 20 °C ± 2 °C and mix.

Alternatively and preferably, make up the sodium chloride standard solution on a gram per kilogram basis (see Table 1, second column) by weighing the required amount of prepared dry sodium chloride (5.2) to the nearest 0,1 mg and dissolving it in exactly 1 kg of water (5.1). Store standard solutions at about 5 °C in well-stoppered polyethylene bottles (6.10) of capacity not greater than 250 ml.

Before using a standard solution, gently invert and rotate the bottle several times to mix its contents thoroughly. At no time should the standard solution be agitated violently, as this can lead to incorporation of air. Pour samples of standard solutions from the bottles. Never use pipettes for this purpose. Do not use standard solutions from bottles less than one-quarter full, or more than 2 months old, or containing visible moulds.

Table 1 — Freezing point of sodium chloride standard solutions

NaCl solution at 20 °C g/l solution	**NaCl solution** g/kg water	**Freezing point** m°C
6,731	6,763	− 400,0
6,868	6,901	− 408,0
7,587	7,625	− 450,0
8,444	8,489	− 500,0
8,615	8,662	− 510,0
8,650	8,697	− 512,0
8,787	8,835	− 520,0
8,959	9,008	− 530,0
9,130	9,181	− 540,0
9,302	9,354	− 550,0
9,422	9,475	− 557,0
10,161	10,220	− 600,0

For this reference method, only unpreserved sodium chloride standard solutions should be used. For routine methods, sodium chloride standard solutions with a fungicidal or fungistatic agent may be used. For guidance, see annex B.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Cryoscope, consisting of a thermostatically controlled cooling device, a thermistor probe with associated circuit, a read-out device, a sample agitator and a crystallization device (see Figure 1).

6.1.1 Cooling device

Several types of thermostated cooling devices may be used. The following types are given as examples:

— immersion type: a cooling bath with a suitable buffer capacity;

— circulation type: a continuous stream of cooling liquid around the sample tube;

— cooling block type: a cooling block with a small amount of cooling liquid.

After the initiation of freezing, keep the temperature of the cooling liquid around the sample tube constant at $-$ 7,0 °C $\pm$ 0,5 °C.

NOTE A suitable cooling liquid is a 33 % (volume fraction) aqueous solution of propylene glycol.

6.1.2 Measuring device, associated circuitry and read-out device

The thermistor shall be of the glass-probe type with diameter of 1,60 mm $\pm$ 0,4 mm and an electrical resistance of between 3 Ω and 30 kΩ at 0 °C.

The type and dimensions of the shank material (including a possible filler) shall not allow a heat transfer into the sample greater than $2{,}5 \times 10^{-3}$ J/s, under operating conditions.

When the probe is in the measurement position, the thermistor bead shall lie on the axis of the sample and at equal distances from the inner walls and the inner bottom of the tube (see Figure 1).

The thermistor and the associated circuitry shall show a discrimination of 1 m°C or better over a range of $-$400 m°C to $-$600 m°C.

The linearity of the circuit shall be such that no error greater than 1 m°C is introduced at any point within the range of $-$400 m°C to $-$600 m°C when the instrument is correctly operated.

The read-out device shall provide a discrimination of 1 m°C or better over a range of at least 0 m°C to $-$1 000 m°C.

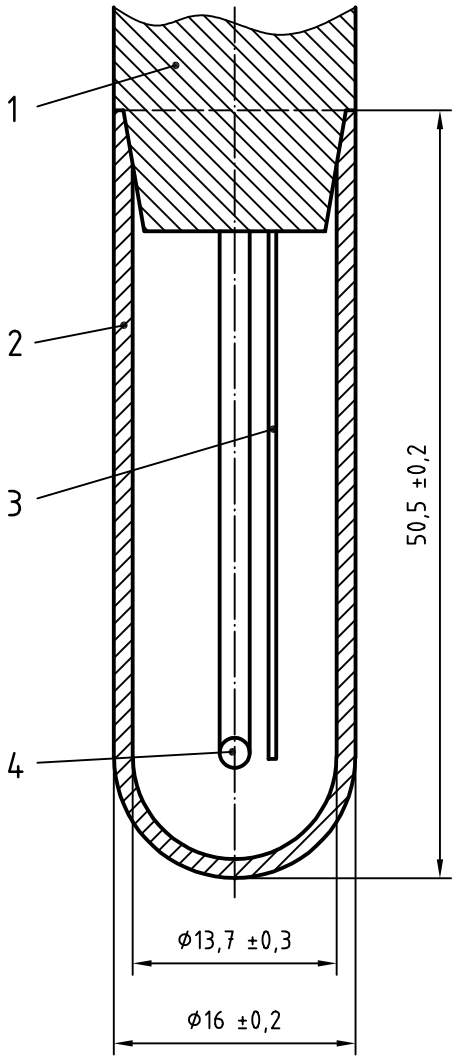
6.1.3 Stirring wire, inert to milk, used to stir the test portion during cooling.

The stirring wire shall be adjustable for amplitude and be mounted vertically in accordance with the manufacturer's instruction. The wire shall vibrate laterally with an amplitude of 2 mm to 3 mm to ensure that the temperature within the test portion remains uniform during cooling. At no time during its normal stirring operation shall the wire strike the glass probe or the wall of the tube.

6.1.4 Device for initiating freezing: any device that, when operated, instantaneously initiates freezing of the test portion when reaching $-$3,0 °C.

The stirring wire (6.1.3) may be used for this purpose. One method is to increase the amplitude of vibration for 1 s to 2 s such that the stirring wire strikes the wall of the sample tube (6.2).

Dimensions in millimetres



Key

1	Mandrel	3	Stirring wire
2	Sample tube	4	Thermistor bead

Figure 1 — Detail of thermistor cryoscope

6.2 Sample tubes, symmetrical, made of borosilicate glass, of length 50,5 mm ± 0,2 mm, external diameter 16,0 mm ± 0,2 mm, and internal diameter 13,7 mm ± 0,3 mm (see Figure 1).

The wall thickness throughout the tube shall not vary by more than 0,1 mm.

The tubes shall be equally shaped so that equal freezing points are obtained for equal volumes of the same solution. Check on equality before using the tubes.

6.3 Main power supply, capable of operating within the manufacturer's specifications.

6.4 Analytical balance, capable of weighing to the nearest 0,1 mg.

6.5 Weighing bottle

6.6 One-mark volumetric flasks, of capacity 1 000 ml, complying with the requirements of ISO 1042, class A.

6.7 Electric furnace, capable of being controlled at 300 °C ± 25 °C.

6.8 Drying oven, capable of being controlled at 130 °C ± 2 °C.

6.9 Desiccator

6.10 Polyethylene bottles, of maximum capacity 250 ml, with a suitable stopper.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

It is important that the laboratory receive a sample that is truly representative and has not been damaged or changed during transport or storage.

If necessary, the test samples may be stored at a temperature of between 0 °C and 6 °C.

It is preferable to test the samples immediately upon arrival at the laboratory.

8 Calibration of the thermistor cryoscope

Ensure that the cryoscope (6.1) is in working condition in accordance with the manufacturer's instructions. Check the position of the probe, the amplitude of vibration of the stirring wire and the temperature of the cooling device (6.1.1).

Select two sodium chloride standard solutions (see Table 1) which closely bracket the expected value of the freezing point of the milk to be tested. The difference in the freezing points between the two selected sodium chloride standard solutions shall not be less than 100 m°C. Take care that the temperatures of the selected sodium chloride standard solutions and that of the test sample are similar.

Pour 2,5 ml ± 0,1 ml of the sodium chloride standard solutions into clean, dry sample tubes (6.2) and calibrate the instrument as indicated by the manufacturer. Use sample tubes (6.2) of the same type as those being used during testing of the sample. Thereafter, the thermistor cryoscope is ready for use.

9 Preparation of test sample

9.1 Preparation

If necessary, remove any visible foreign bodies or solid butterfat from the test sample by filtering into a clean dry vessel. Mix the sample gently. Use a filter that is inert to milk and effective when used at laboratory temperature.

Test the samples at their storage temperature or after having reached the laboratory temperature before commencing the determination. However, the test samples and the sodium chloride standard solutions should have the same temperature when commencing the determination (see also clause 8).

9.2 Sample condition

If doubts exist about the sample condition, determine the titratable acidity of the test sample by the method specified in ISO 6091 as far as possible at the same time as determining the freezing point.

Results obtained from test samples with a titratable acidity exceeding 20 ml of 0,1 mol/l sodium hydroxide solution per 10 g of non-fat solids will not be representative of the original milk.

10 Procedure

10.1 Preliminary checks

Carry out preliminary instrument checks in accordance with the manufacturers' instructions.

10.2 Routine calibration check

Before each series of determinations, measure the freezing point of a sodium chloride standard solution (5.3) (e.g. a solution with a freezing point of −512 m°C) until the values obtained in two consecutive determinations do not differ by more than 1 m°C.

If the arithmetic mean of the two results differs from the freezing point of the sodium chloride standard solution used by more than 2 m°C, recalibrate the cryoscope as described in clause 8.

If the cryoscope is in continuous use, carry out a routine calibration check at least once every hour.

10.3 Determination

Gently invert and rotate the sample container several times to mix its contents, thereby avoiding the incorporation of air.

Use a pipette to transfer a test portion of 2,5 ml ± 0,1 ml of the prepared test sample (9.1) into a clean and dry sample tube (6.2). Ensure that the probe and the stirring wire (6.1.3) are clean and dry. If necessary, wipe carefully with a soft, clean and lint-less tissue.

Insert the sample tube into the calibrated cryoscope (6.1) according to the manufacturer's instructions. Start the instrument to cool the test portion and initiate freezing at −3,0 °C ± 0,1 °C.

The plateau is reached when the temperature rise over the last 20 s has not exceeded 0,5 m°C. For instruments with a resolution not better than 1 m°C, the plateau is reached as soon as the temperature has remained constant for 20 s. Record this temperature.

If, for any reason, freezing is initiated before reaching a temperature of −3,0 °C ± 0,1 °C, abandon the test. Repeat the determination with another test portion of 2,5 ml.

If this second test portion also freezes too early, warm another test portion of 2,5 ml ± 0,1 ml of the same sample to about 45 °C and maintain this temperature for 5 min in order to melt any crystalline fat. Cool the thus-prepared test portion to the testing temperature and test immediately.

NOTE The time between the initiation of freezing and the attainment of the plateau value, and the time during which the temperature remains constant, will differ from sample to sample and will be considerably shorter for water and sodium chloride standard solutions than for milk.

Remove the sample tube after each determination and rinse the thermistor probe and the stirring wire with water. Wipe the thermistor probe and the stirring wire with a soft, clean and lint-less tissue.

Carry out a second determination on another test portion of the test sample. If the two freezing points differ by more than the repeatability value (see 12.2), discard the results and carry out two consecutive determinations on fresh test portions.

11 Calculation and expression of results

11.1 Calculation

If the calibration is confirmed by the result of the routine calibration check (10.2), take as the result the mean of the two values obtained, rounded to the third significant figure. If the calibration is not confirmed, repeat the procedure (clause 10).

11.2 Expression of results

Express the test results of the freezing point to three significant figures. Round the mean results as follows: if the fourth significant figure is 5, change the third significant figure to the nearest even number.

Examples to round the mean results of the freezing point values (in millidegrees Celsius) are given below.

Duplicate results		Mean value
Result 1	Result 2	
−540	−542	−541
−541	−542	−542
−540	−541	−540

12 Precision

12.1 Interlaboratory test

The values for the repeatability and reproducibility limits were derived from the results of an interlaboratory test carried out in accordance with ISO 5725-1 and ISO 5725-2. Details of the interlaboratory test of the method are summarized in annex A.

The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

NOTE IDF 135 provides specific guidance for interlaboratory tests on methods of analysis and milk products. It is based on ISO 5725.

12.2 Repeatability

The absolute difference between two independent single test results, 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, will in not more than 5 % of cases be greater than 4 m°C.

12.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 6 m°C.

13 Test report

The test report shall specify:

— all information required for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incident which may have influenced the result(s);

— the titratable acidity, if determined;

— the test result(s) obtained, or, if the repeatability has been checked, the final quoted results obtained.

Annex A
(informative)

Results of the interlaboratory test

A.1 General

After a pre-study in October 1998, an international collaborative test involving nineteen laboratories and thirteen countries was carried out in September 1999. The test included 18 test samples, divided into 36 blind duplicates:

— 6 pairs of UHT skimmed milk samples at three different levels;

— 6 pairs of UHT whole milk samples at three different levels;

— 6 pairs of raw milk samples at three different levels.

The test was organized by the Netherlands Milk Control Station, Zutphen (NL) and the Inspectorate for Health Protection, Leeuwarden (NL). The test samples were prepared and distributed by Cecalait, Poligny (FR), which also performed the statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Tables A.1 to A.3.

A.2 Test results

Table A.1 — Results for UHT skimmilk

Sample	1	2	3	4	5	6	Grand mean[a]
No. of participating laboratories after eliminating outliers	17	16	17	18	18	17	
Mean value, m°C	–497,4	–498,8	–512,0	–518,1	–536,1	–539,4	–517,0
Repeatability standard deviation, s_r, m°C	1,2	1,0	1,2	1,1	1,2	1,0	1,1
Repeatability limit, r (2,8 s_r), m°C	3,3	2,9	3,2	3,0	3,3	2,9	3,1
Coefficient of variation of repeatability, %	0,23	0,21	0,22	0,21	0,22	0,19	0,21
Reproducibility standard deviation, s_R, m°C	1,2	1,3	1,2	1,6	1,4	2,0	1,5
Reproducibility limit, R (2,8 s_R), m°C	3,5	3,6	3,3	4,5	4,0	5,6	4,2
Coefficient of variation of reproducibility, %	0,25	0,26	0,23	0,31	0,26	0,37	0,29

[a] Grand mean values were calculated using only sample data with outliers removed. Grand sample mean value was calculated by averaging. All other statistical means were calculated from the square root of the average of the squared deviations.

Table A.2 — Results for UHT whole milk

Sample	1	2	3	4	5	6	Grand mean[a]
No. of participating laboratories after eliminating outliers	17	17	17	17	18	18	
Mean value, m°C	–491,6	–494,7	–512,6	–512,9	–534,8	–534,9	–513,6
Repeatability standard deviation, s_r, m°C	1,2	1,7	2,0	1,1	1,4	1,2	1,5
Repeatability limit, r (2,8 s_r), m°C	3,5	4,7	5,5	3,1	3,9	3,4	4,1
Coefficient of variation of repeatability, %	0,25	0,34	0,38	0,22	0,26	0,22	0,28
Reproducibility standard deviation, s_R, m°C	2,0	2,3	2,7	1,5	2,0	2,3	2,2
Reproducibility limit, R (2,8 s_R), m°C	5,6	6,4	7,7	4,2	5,6	6,4	6,1
Coefficient of variation of reproducibility, %	0,41	0,46	0,53	0,29	0,38	0,43	0,42

[a] Grand mean values were calculated using only sample data with outliers removed. Grand sample mean value was calculated by averaging. All other statistical means were calculated from the square root of the average of the squared deviations.

Table A.3 — Results for raw milk

Sample	1	2	3	4	5	6	Grand mean[a]
No. of participating laboratories after eliminating outliers	18	18	18	17	18	17	
Mean value, m°C	–496,2	–504,9	–518,3	–525,5	–540,0	–548,8	–522,3
Repeatability standard deviation, s_r, m°C	1,4	1,3	1,3	1,4	1,3	1,1	1,3
Repeatability limit, r (2,8 s_r), m°C	3,8	3,6	3,7	3,8	3,6	3,2	3,6
Coefficient of variation of repeatability, %	0,28	0,25	0,26	0,26	0,24	0,21	0,25
Reproducibility standard deviation, s_R, m°C	2,1	2,5	2,6	2,1	2,2	2,3	2,3
Reproducibility limit, R (2,8 s_R), m°C	5,9	7,0	7,4	6,0	6,2	6,5	6,5
Coefficient of variation of reproducibility, %	0,42	0,50	0,51	0,41	0,41	0,42	0,45

[a] Grand mean values were calculated using only sample data with outliers removed. Grand sample mean value was calculated by averaging. All other statistical means were calculated from the square root of the average of the squared deviations.

Annex B
(informative)

Guidelines for the application of routine thermistor cryoscope methods

B.1 Introduction

This International Standard describes the reference cryoscope method for the determination of the freezing point of milk. In practice, the application of other thermistor cryoscope methods may be attractive for reasons such as:

— achieving a shorter test duration by applying an earlier moment of measurement cut-off;

— keeping instruments in operation, for which a complete adaptation to the requirements of the reference method is not feasible.

Examples of methods and/or deviations in use are:

— measurement cut-off at 30 s, 50 s, 60 s or 90 s after the onset of freezing;

— measurement cut-off when the instrument read-out is constant to within ± 1 m°C or constant to within ± 1 m°C over 20 s;

— working with instrument parts with deviating characteristics;

— using other sample volumes;

— applying other freezing temperatures.

When applying routine methods, a correction should be made on routine test results in order to obtain an average agreement with the outcome of reference thermistor cryoscope measurements. The correction value should be determined for the relevant circumstances. This annex provides guidelines for establishing correction values.

B.2 Prerequisites

The applied routine method should fulfil requirements such as:

— using the same test principle, i.e. a thermistor cryoscope method;

— achieving a discrimination of 1 m°C or better over the range −400 m°C to −600 m°C;

— having a linearity with no error greater than 1 m°C within the range −400 m°C to −600 m°C;

— achieving measurement cut-off not earlier than 30 s after the onset of freezing;

— having a repeatability not greater than that specified in 12.2.

B.3 Determination of the correction value

B.3.1 General

The necessary correction shall be determined through comparative measurements with the reference method and the routine method, using a sufficient number of representative samples of the milk under consideration.

B.3.2 Sample taking and treatment

For this purpose, collect test samples which are representative of the target population, i.e. covering the normal range in compositional variation and the range of occurring freezing point values for the type of sample under consideration (e.g. raw, pasteurized and/or skimmed milk). Take and treat the test samples according to clauses 7 and 9.

B.3.3 Number of test samples

The minimum number of samples, n, shall be such that a critical difference of 0,4 m°C is not exceeded. Calculate n by using the following approximate equation:

$$n = \left(\frac{\sigma_{\mathrm{d}} \cdot u_{0,95}}{D} \right)^2$$

where

n is the minimum number of samples in the test;

σ_{d} is the standard deviation of differences between the methods for individual samples;

$u_{0,95}$ is the numerical value of the normal reduced distribution at its unilateral 95 % confidence interval level;

D is the defined value for the critical difference.

EXAMPLE For σ_d = 3,0 m°C, $u_{0,95}$ = 1,645 and D = 0,4 m°C, $n \geqslant 152$ samples.

B.3.4 Calibration and calibration check

Analyse the sodium chloride standard solutions (5.3) with known freezing point values under the same conditions as for the test samples. Take care that the conditions are the same for both the reference and the routine method. Also use the same sodium chloride standard solutions in both methods.

B.3.5 Measurement of test samples

With routine methods, sodium chloride standard solutions (5.3) with a fungicidal or a fungistatic agent may be used. However, an addition should not have an effect larger than $+1,0$ m°C as compared to an unpreserved solution with the same sodium chloride concentration. If used, the agent shall be of analytical grade quality and its use shall not be subject to limitations imposed by environmental regulations. When using a preservative, lower the sodium chloride concentration slightly so as to keep exactly the same freezing point as for the unpreserved sodium chloride standard solution (see Table 1). Establish a proper expiration date based on validation studies.

Determine the freezing point of the test samples in duplicate with the reference method described in this International Standard.

Determine the freezing point of the same set of test samples in duplicate with the routine method, thereby following clauses 9 and 10 of this International Standard. Ambient differences between successive measurements or between measurements made by the reference method and the routine method should be avoided; for example, ensure proper storage (at 0 °C to 6 °C) of samples in well-stoppered bottles.

B.3.6 Calculation of the correction value

Correct every individual result by an amount corresponding to the difference between the freezing point of the control sample used (see 5.3) and the value determined in the nearest calibration check. Then calculate the mean freezing point values for the duplicate measurements obtained using the reference method. Similarly, obtain mean values for the routine method measurements.

Calculate the standard deviation s of the individual differences between the mean results obtained by both methods. Delete outliers, i.e. samples with a difference larger than $3\,s$. Then calculate the average difference between the results obtained by the two methods. Round to the second significant figure. Use this value as the correction value.

If, after the deletion of outliers, s is still greater than 3,0 m°C, working with a correction value is considered to be too inaccurate and therefore unacceptable.

An example with a reduced sample set (n = 10) is shown in Table B.1.

Table B.1 — Example

Sample	Results with reference method (1)					Results with routine method (2)					Diff. (2) – (1)
			after correction					after correction			
	1	2	1	2	mean	1	2	1	2	mean	
512 control sample	–512,4	–513,0				–511,5	–512,7				
1	–517,5	–518,2	–516,8	–517,5	–517,15	–518,1	–519,2	–518,0	–519,1	–518,55	–1,40
2	–538,1	–536,9	–537,4	–536,2	–536,80	–537,3	–539,0	–537,2	–538,9	–538,05	–1,25
3	–521,8	–521,3	–521,1	–520,6	–520,85	–522,9	–521,8	–522,8	–521,7	–522,25	–1,40
4	–528,6	–529,9	–527,9	–529,2	–528,55	–530,3	–531,4	–530,2	–531,3	–530,75	–2,20
5	–522,3	–520,9	–521,6	–520,2	–520,90	–527,3	–529,2	–527,2	–529,1	–528,15	–7,25[a]
6	–515,7	–518,1	–514,9	–517,3	–516,10	–517,7	–518,9	–518,1	–519,3	–518,70	–2,60
7	–523,4	–522,1	–522,6	–521,3	–521,95	–525,2	–524,7	–525,6	–525,1	–525,35	–3,40
8	–512,2	–513,8	–511,4	–513,0	–512,20	–514,1	–513,4	–514,5	–513,8	–514,15	–1,95
9	–516,1	–514,2	–515,3	–513,4	–514,35	–517,5	–518,2	–517,9	–518,6	–518,25	–3,90
10	–521,6	–522,3	–520,8	–521,5	–521,15	–521,4	–523,4	–521,8	–523,8	–522,80	–1,65
512 control sample	–513,1	–512,5				–511,7	–511,5				
Average difference											–2,7
Value of s											1,73
Average difference after deleting outlier sample data											–2,2
Value of s after deleting outlier sample data											0,88

[a] Outlier

The average difference between the results when measuring with the routine method and the reference method in this example is –2,2 m°C. This is also the needed correction value when measuring with this routine method. In this case, it means a correction to a warmer temperature.

B.4 Validity

The calculated correction value is valid for the specific combination of type of test sample and routine method attributes, i.e. instrument properties, moment of measurement cut-off, test portion volume and freezing temperature.

Annex C
(informative)

Adjustment of the freezing point value used as the reference for genuine milk

To estimate whether or not a sample of milk contains extraneous water, it is necessary to compare the freezing point of that test sample with the freezing point of “genuine milk”, i.e. milk known to be free from extraneous water.

For many samples (e.g. processed milk made from a mixture of many different farm supplies) it is not always possible to obtain a reference value for genuine milk. In such cases, it is necessary to set an average target value for genuine milk. Some countries have arrived at a freezing point value for genuine milk after completing extensive surveys. Others have adopted a value based on many years of historic observations.

A shift in the freezing point temperature scale is likely to occur when changing from the method used so far to the reference method described in this International Standard. This change in the temperature scale is, in part, due to more vigorous standardization of instrument components and characteristics and in part due to redefining the “cut off” point on the freezing point curve of milk.

Clearly, this change in scale will give rise to a change in freezing point readings from those recorded previously. This will make it necessary to apply a small change to historical values used for genuine milk. If an adjustment were not made, a systematic error would apply to the assessment of extraneous water in milk.

It is recommended, therefore, to adjust “once only” the value applied to genuine milk to bring it into line with the new scale in this International Standard. Such an adjustment should be made by, or established under the supervision of, each national authority.

The adjustment may be made following the procedure given in annex B with the following additional precautions being taken.

a) Seek the guidance of a statistician in order to establish the optimum number of samples required in the comparison in order to keep any residual systematic error to an acceptably low level.

b) Use test samples in the trial representing as closely as possible the type of milk being tested routinely.

c) Test one set of samples in the trial by closely following this reference cryoscope standard method.

d) Test a duplicate set of samples by using the test method and the instrument characteristics used when the historic reference value for “genuine milk" was established.

e) Keep a record of this study for future reference.

Bibliography

[1] ISO 707, *Milk and milk products — Guidance on sampling*

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