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Milk — Determination of total milk-clotting activity of bovine rennets

Lait — Détermination de l'activité totale de coagulation du lait dans la présure de bovins

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Foreword

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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 11815 | IDF 157 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.

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.

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

ISO 11815 | IDF 157 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 IDF-ISO Action Team on *Enzymes in cheese making*, of the Standing Committee on *Minor compounds and characterization of physical properties*, under the aegis of its project leader Mr A. van Boven (NL).

This edition of ISO 11815|IDF 157 cancels and replaces IDF 157A: 1997, which has been technically revised.

Introduction

Bovine rennets (calf and adult) contain, in various amounts, both chymosin and bovine pepsin as main milk-clotting enzymes. Each of these enzymes has its own characteristics as far as milk-clotting activity and cheese-making properties are concerned. The most obvious difference between these enzymes is the stronger pH dependence of the milk-clotting activity of pepsin. For economic reasons, therefore, it is very important to know the total milk-clotting activity of a certain rennet type and to have that characterized relative to an internationally recognized reference standard with known composition and milk-clotting activity.

The method is commonly known as the relative milk-clotting activity test (acronym: REMCAT).

A qualitative determination of the six most common milk-clotting enzymes in a sample is performed according to IDF 110B. In the case of mixtures of milk-clotting enzymes other than bovine chymosin and pepsin, no correct determination of the total milk-clotting activity for the sample can be obtained.

Milk — Determination of total milk-clotting activity of bovine rennets

1 Scope

This International Standard describes a method for the determination of the total milk-clotting activity of bovine rennet containing only chymosin and bovine pepsin as the active coagulating enzymes on a standard milk substrate at pH 6,5.

To produce accurate results with this method, test samples of unknown origin are to be checked for the absence of main milk-clotting enzymes of non-bovine origin by using an appropriate method (e.g. IDF 110B).

This International Standard can also be applied to determine the total milk-clotting activity of fermentation-produced chymosin.

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 648, *Laboratory glassware — One-mark pipettes*

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

IDF 110B:1997 *Calf rennet and adult bovine rennet — Determination of chymosin and bovine pepsin contents (Chromatographic method)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
total milk-clotting activity

amount of activity set relative to the first batch of international calf rennet reference standard powder and the first batch of adult bovine rennet reference standard powder, respectively

NOTE 1 For the first batch of both the calf and the adult bovine rennet reference standard powder, the activity was defined at 1 000 International Milk-Clotting Units per gram (IMCU/g). Future preparations of reference standards will be set relative to the previous reference standards.

NOTE 2 The total milk clotting activity of the calf and adult bovine rennet reference standard powders is approximately 1 000 IMCU/g, but their exact activity is stated on the certificate of analysis.

NOTE 3 The total proteolytic (milk-clotting) activity of these calf (or adult bovine) rennet reference standard powders is checked every second year by an alternative method, for example on a synthetic hexapeptide substrate by NIZO[1)] .

4 Principle

The time needed for a visible flocculation of a standard milk substrate prepared with a 0,5 g/l calcium chloride solution (pH ≈ 6,5) is determined. The clotting time of a rennet sample is compared under identical chemical and physical conditions to that of a reference standard with known milk-clotting activity and having the same enzyme composition as the sample, which was determined using IDF 110B.

5 Reagents

Use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent purity.

5.1 Buffer solution, pH 5,5.

Add, using a pipette (6.1), 10,0 ml of 1 mol/l acetic acid (CH_3COOH) to 10,0 g of sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$) and mix. Dilute with water to 1 000 ml. If necessary, adjust the pH to 5,5 with acetic acid or sodium acetate trihydrate.

5.2 Calcium chloride stock solution, $c(CaCl_2)$ = 500 g/l.

Calcium chloride solutions with the required accurate concentration of 500 g/l calcium chloride and the actual density stated are commercially available[2)]. Store the solution as specified by the manufacturer.

Prior to use, bring the calcium chloride stock solution to room temperature (18 °C to 22 °C). Check the concentration of the solution by titration with EDTA (ethylenediaminetetraacetic acid) every year.

5.3 Calcium chloride working solution, $c(CaCl_2)$ = 0,5 g/l.

Use the density of the stock solution (5.2) to calculate the mass needed to obtain a final amount of 0,5 g/l calcium chloride in the working solution.

The mass of the solution should be equivalent to the addition of 2,00 ml of the stock solution with the exact concentration required, $c(CaCl_2)$ = 500 g/l; in that case, the solution mass is ≈ 2,70 g.

Weigh, to the nearest 0,01 g, about 2,70 g of calcium chloride stock solution (5.2) of exactly known concentration at room temperature (18 °C to 22 °C) in a 2 000 ml one-mark volumetric flask. Dilute to the mark with water and mix. The calcium chloride solution shall be freshly prepared on the day of use.

Weighing of the calcium chloride stock solution (5.2) is recommended in order to prepare correctly the calcium chloride working solution as the viscous solution is difficult to pipette.

Alternatively, an intermediate calcium chloride solution of 50 g/l may be prepared and further diluted before use.

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1) Netherlands Institute for Dairy Research (NIZO), PO Box 20, 6710 BA Ede, The Netherlands. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

2) Ordering address: Chr. Hansen A/S, 1-27 Jernholmen, 2650 Hvidovre, Denmark (Fax: +45 36 86 77 76). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

5.4 Low-heat, low-fat, spray-dried milk powders, of good renneting and bacteriological quality.

NOTE Low-heat, low-fat, spray-dried milk powders meeting the requirements are commercially available [2),3)].

5.5 Calf rennet reference standard powder [4)], in pouches of approx. 2,7 g powder, containing > 98 % chymosin and < 2 % bovine pepsin in terms of enzyme activity as determined according to IDF 110B.

The exact total milk-clotting activity is indicated on the certificate of analysis and should be about 1 000 IMCU/g.

The calf rennet reference standard powder is a primary reference standard; a secondary liquid standard may be made and used if it has been determined that the same result is obtained.

Store the calf rennet reference standard powder in the dark at −18 °C, protected against moisture. For short periods, for example during transport, it may be kept at ambient temperatures.

5.6 Adult bovine rennet reference standard powder [4)], in pouches of approx. 2,7 g powder, containing < 2 % chymosin and > 98 % bovine pepsin in terms of enzyme activity as determined according to IDF 110B.

The exact total milk-clotting activity is indicated on the certificate of analysis and should be about 1 000 IMCU/g.

The adult bovine rennet reference standard powder is a primary reference standard; a secondary liquid standard may be made and used if it has been determined that the same result is obtained.

Store the adult bovine reference standard powder in the dark at −18 °C, protected against moisture. For short periods, for example during transport, it may be kept at ambient temperatures.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Micropipette or any other pipette, capable of delivering 0,5 ml in less than 1 s with a repeatability of 0,2 % or better.

6.2 One-mark pipettes, in accordance with ISO 648, to deliver appropriate amounts.

Alternatively, a diluter (e.g. a Hamilton diluter) with the same high precision may be used for diluting the rennets. For measuring the substrate, a syringe or a dispenser delivering the appropriate amount with a repeatability of 0,4 % may also be used.

6.3 One-mark volumetric flasks, as specified in ISO 1042, of the required capacities.

6.4 Thermometer, calibrated, graduated between 20 °C and 45 °C, with a precision of ± 0,1 °C.

6.5 pH meter, capable of measuring the pH in 0,01 units.

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

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3) Institut national de la Recherche agronomique, France, which is dependent on the Station expérimentale laitière, BP 94, 39800 Poligny, France. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

4) AMAFE. Ordering address: Chr. Hansen A/S, 1-27 Jernholmen, 2650 Hvidovre, Denmark (Fax: +45 36 86 77 76). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

6.7 Stopwatch, capable of reading in seconds.

6.8 Flasks or test tubes, for milk-clotting testing, with a suitable capacity (see 6.9.1 and 9.5.1).

6.9 Water bath, capable of maintaining a temperature of 32 °C ± 1 °C, but also capable of maintaining the temperature constant to within ± 0,2 °C throughout the bath. The water bath should have following attachments.

6.9.1 Electric motor, provided with a rotating spindle to which the flask or test tube (6.8) can be attached, capable of rotating at a suitable angle of about 30° with the water surface of the water bath.

NOTE The rotation speed is not important for this method; a speed of 2 r/min to 4 r/min is suitable.

6.9.2 Electric lamp, placed in such a position as to illuminate the flask or test tube (6.8) effectively.

A screen with a dark background, placed in the water bath, may be used to improve the determination of the milk-clotting flocculation point in the flask or test tube.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. Recommended sampling methods are given: for liquid rennet (8.1) in Clause 9 of ISO 707|IDF 50:1997[1]; and for powdered rennet (8.2) in Clause 13 of ISO 707|IDF 50:1997[1].

Store the test samples in the dark at a temperature of between 0 °C and 5 °C.

8 Preparation of test sample

8.1 Liquid rennet

Mix the test sample by swirling while avoiding foam formation. Bring the sample to room temperature (18 °C to 22 °C) prior to starting the preparation of the rennet test solution (9.4).

Liquid rennet is viscous. When pipetting the sample, use the correct technique. Alternatively, accurate and precise dilutions, especially for high strength rennets, can be made. This is done by weighing the liquid samples on an analytical balance and by calculating its volume, in millilitres, by dividing its mass by the density of the rennet used.

8.2 Powdered rennet

Mix the test sample thoroughly to obtain a homogeneous powder. Bring the sample to room temperature (18 °C to 22 °C) before preparing the rennet test solution (9.4).

NOTE 1 Note that powdered products can separate rapidly.

NOTE 2 Consider the amount of sample to be taken out. Often sample amounts of 3 g and 5 g are sufficient, but when testing inhomogeneous samples or when very accurate results are desired, then larger sample sizes such as 10 g are needed for analysis.

9 Procedure

Measure the test samples and reference standards under identical chemical and physical conditions.

Note that differences in conditions that can affect the clotting, such as pH or salt and buffer level in the diluted rennet, will produce slightly deviant results.

9.1 Preparation of substrate

Measure 1 000 ml of calcium chloride working solution (5.3) into a 1 000 ml volumetric flask (6.3). Weigh, to the nearest 0,1 g, 110 g of the low-heat, low-fat, spray-dried milk powder (5.4) into a 2 000 ml beaker. Add about 100 ml of calcium chloride working solution (5.3) to the powder in the beaker. Stir manually to obtain a homogeneous mixture. Add the remaining 900 ml of calcium chloride working solution to the contents of the beaker, allowing the 1 000 ml volumetric flask to drain. Stir the substrate thus obtained with a magnetic stirrer for 30 min, while taking care to avoid foam formation.

Leave the substrate at room temperature for 30 min. The substrate may be kept at room temperature for no longer than 4 h, or refrigerated during the day of preparation.

The pH of the prepared substrate will be approximately 6,50 and should not be adjusted.

9.2 Preparation of rennet reference standard solutions

9.2.1 Calf rennet reference standard solution

Bring the pouch with the calf rennet reference standard powder (5.5) to room temperature (18 °C to 22 °C) before opening it, to avoid moisture getting into the powder.

Open the pouch and weigh, to the nearest 1 mg, 2,500 g of calf rennet reference standard powder into a 50 ml volumetric flask (6.3). Add 15 ml to 20 ml of buffer solution (5.1) and mix by swirling, while avoiding foam formation, to dissolve the powder. Dilute to the 50 ml mark with buffer solution (5.1) and mix well again.

9.2.2 Adult bovine rennet reference standard solution

Repeat the procedure in 9.2.1, but replace the calf rennet reference powder (5.5) with the adult bovine rennet reference standard powder (5.6).

9.3 Preparation of the calf and adult bovine rennet reference working solutions

9.3.1 Calf rennet reference working solution

In order to obtain a proper clotting time, pipette (6.2) 3 ml of the calf rennet reference standard solution (9.2.1) into another 50 ml volumetric flask (6.3). Dilute to the 50 ml mark with buffer solution (5.1) and mix well.

The dilution factor finally obtained is 333,33 times. The clotting time for the calf rennet reference working solution should be in the range 350 s to 550 s.

Keep the calf rennet reference working solution at room temperature during the day of its preparation. It may be stored at between 0 °C and 5 °C for 2 days.

9.3.2 Adult bovine rennet reference working solution

Repeat the procedure in 9.3.1, but replacing the calf rennet reference standard solution (9.2.1) with the adult bovine rennet reference standard solution (9.2.2).

The dilution factor finally obtained is 333,33 times. The clotting time for the adult bovine rennet reference working solution should be in the range 350 s to 550 s.

Keep the adult bovine rennet reference working solution at room temperature during the day of its preparation. It may be stored at between 0 °C and 5 °C for 2 days.

9.4 Preparation of rennet test solution

Take an appropriate test portion (3 ml to 5 ml of liquid, or 3 g to 5 g of powder) from the prepared test sample (8.1 or 8.2). Dilute the test portion with the buffer (5.1) until a test solution is obtained with a clotting time that is similar to the calf or adult bovine rennet reference working solution (9.3.1 or 9.3.2) with a tolerance of ± 40 s. Record the final dilution factor of the test solution for use in the calculation (10.1).

9.5 Clotting

9.5.1 Add, using a pipette (6.2), 25 ml ± 0,1 ml of substrate (9.1) to a dry flask or test tube (6.8). Pre-heat the substrate, while rotating the flask or test tube in the water bath (6.9) set at 32 °C for at least 12 min, but no longer than 20 min.

Then quickly add, using the micropipette (6.1), 0,5 ml of calf rennet reference working solution (9.3.1) to the substrate while activating the stopwatch (6.7) at the same time, mix by swirling, while avoiding foam formation, and immediately attach the flask or test tube to the rotating spindle.

Read the clotting time from the stopwatch when the first flocculation is observed in the substrate film on the wall of the flask or test tube.

Always place test samples as close as possible to the reference samples in the bath, in order to obtain, as far as possible, identical conditions. The method being relative analysis, maintaining the same clotting temperature for the test samples and the reference samples is of vital importance. To fulfil the aforementioned requirement, check the temperature of the water bath by measuring the temperature of milk samples at different positions in the bath. If the maximum allowed variation of ± 0,2 °C (see 6.9) cannot be reached, then the design of the water bath or its water-circulating system has to be improved.

9.5.2 Repeat the procedure in 9.5.1 without delay, but replacing the calf rennet reference working solution with the rennet test solution (9.4), and then with the adult bovine rennet reference working solution (9.3.2).

9.5.3 Repeat the procedure in 9.5.1 and 9.5.2 without delay to obtain duplicate values. Calculate the means of the clotting times for the calf and adult bovine rennet reference working solutions, respectively, and then for the rennet test solution.

9.5.4 Instead of 25 ml of substrate and 0,5 ml of calf or adult bovine rennet reference working (or test) solution in 9.5.1 or 9.5.2, 10 ml substrate plus 0,2 ml of working (or test) solution, or 50 ml of substrate plus 1,0 ml of working (or test) solution may be used. In either case, the ratio between the amount of substrate used and working solution should be 50:1.

10 Calculation and expression of results

10.1 Calculation

First, calculate the milk-clotting activity of the test sample relative to both the activity of the calf reference standard powder (5.5), $a_{c,ref}$, and the activity of the adult bovine rennet reference standard powder (5.6), $a_{a,ref}$ (see 10.1.1).

Then perform the final calculation of the total milk-clotting activity by interpolation (see 10.1.2) expressed in International Milk Clotting Units (IMCU) per gram or per millilitre.

10.1.1 Calculation of the milk-clotting activity relative to calf rennet reference standard powder

Calculate the milk-clotting activity of the test sample relative to both reference standards, a_{tc} and a_{ta}, by using the following equations:

$$a_{tc} = \frac{t_{c,ref} \times m_{c,ref} \times V_1 \times d \times a_{c,ref}}{t_t \times V_2 \times V_3} \quad (1)$$

$$a_{ta} = \frac{t_{a,ref} \times m_{a,ref} \times V_1 \times d \times a_{a,ref}}{t_t \times V_2 \times V_3} \quad (2)$$

where

a_{tc} is the total milk-clotting activity of the test sample relative to the calf rennet reference standard;

a_{ta} is the total milk-clotting activity of the test sample relative to the adult bovine rennet reference standard;

$t_{c,ref}$ is the mean clotting time, in seconds, obtained with the calf rennet reference working solution (9.5.1 and 9.5.3);

$t_{a,ref}$ is the mean clotting time obtained with the adult bovine rennet reference working solution (9.5.2 and 9.5.3), in seconds;

$m_{c,ref}$ is the mass, in grams, of the calf rennet reference standard weighed in 9.2.1;

$m_{a,ref}$ is the mass, in grams, of the adult bovine rennet reference standard weighed in 9.2.2;

V_1 is the volume, in millilitres, taken in either the calf (9.3.1) or the adult bovine (9.3.2) rennet reference standard solution (V_1 = 3 ml);

d is the noted final value of the dilution factor obtained with the test solution (9.4);

$a_{c,ref}$ is the milk-clotting activity (strength), in IMCU per gram, indicated on the container, of the calf rennet reference standard powder (5.5);

$a_{a,ref}$ is the milk-clotting activity (strength), in IMCU per gram, indicated on the container, of the adult bovine rennet reference standard powder (5.6);

t_t is the mean clotting time, in seconds, obtained with the rennet test solution (9.5.2 and 9.5.3);

V_2 is the final volume, in millilitres, of either the calf (9.2.1) or the adult bovine (9.2.2) rennet reference standard solution (V_2 = 50 ml);

V_3 is the final volume, in millilitres, in either the calf (9.3.1) or the adult bovine (9.3.2) rennet reference working solution (V_3 = 50 ml).

Simplified Equations (3) and (4) result from introducing the following values: $m_{c,ref}$ = $m_{a,ref}$ = 2,500 g; V_1 = 3 ml; V_2 = 50 ml; V_3 = 50 ml:

$$a_{tc} = \frac{t_{c,ref} \times 0{,}003 \times d \times a_{c,ref}}{t_t} \quad (3)$$

$$a_{ta} = \frac{t_{a,ref} \times 0{,}003 \times d \times a_{a,ref}}{t_t} \quad (4)$$

10.1.2 Final calculation of the total milk-clotting activity by interpolation

Calculate the total milk-clotting activity of the test sample, a_t, in IMCU/ml when using liquid rennet (8.1) and in IMCU/g when using powder rennet (8.2), relative to an interpolated rennet reference solution with the same composition as the test sample, by using the formula:

$$a_t = \left(\frac{c_C \times a_{tc}}{100} \right) + \left(\frac{c_P \times a_{ta}}{100} \right)$$

where:

c_C is the numerical value, as a percentage, of the chymosin content in the test sample (8.1 or 8.2), determined by the method specified in IDF 110B;

c_P is the numerical value, as a percentage, of the bovine pepsin content in the test sample (8.1 or 8.2), determined by the method specified in IDF 110B.

10.2 Expression of results

Express the test results in International Milk-Clotting Units (IMCU) per gram or per millilitre to the nearest whole number.

11 Precision

11.1 Interlaboratory test

Details of the interlaboratory test on the precision of the method have been published[3,4]. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

The values for repeatability and reproducibility are derived from the standard deviations (SD) which are estimates of the true standard deviation of the method. If, in the long run, significantly less than 95 % of the cases are within the values given in 11.2 and 11.3, it is recommended that execution of the analysis be improved.

Due to some differences in solubility and a certain degree of inhomogenity of rennet powders, the percentage value for the precision parameters, repeatability and reproducibility, mentioned below, may be somewhat higher when analysing rennet powders.

11.2 Repeatability

The coefficient of variation of repeatability, CV(r), which expresses the variability of independent analytical results obtained by the same operator, using the same apparatus under the same conditions on the same test sample and in a short interval of time, will in not more than 5 % of cases be greater than:

for liquid rennets: 1,8 % relative to the arithmetic mean of the test results.

If two determinations are obtained under these conditions, the absolute difference, r_{rel} %, between the two results, should not exceed:

for liquid rennets: 4,9 % relative to the arithmetic mean of the test results.

11.3 Reproducibility

The coefficient of variation of reproducibility, CV(R), which expresses the variability of independent analytical results by operators in different laboratories, using different apparatus under different conditions for the

analysis on the same test sample, will in not more than 5 % of cases be greater than:

for liquid rennets: 3,5 % relative to the arithmetic mean of the test results.

If two determinations are obtained under these conditions, the absolute difference, R_{rel} %, between the two results, should not exceed:

for liquid rennets: 9,7 % relative to the arithmetic mean of the test results.

NOTE The values for precision parameters are valid when considering a broad range of laboratories. Experience has shown that highly trained laboratories are able to perform the analysis with reproducibility between laboratories of 2 % relative.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample, including phase details (liquid or powder);

b) the sampling method used, if known;

c) reference to this International Standard;

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

e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Interlaboratory trial

A.1 General

An international collaborative test involving 13 laboratories from nine countries was carried out on three different batches of liquid rennets, each diluted to two different level of activity. The six samples thus obtained were divided into 12 blind duplicated samples. The test results were subjected to statistical analysis according to ISO 5725:1986[2], and the result published[3],[4].

A.2 Liquid rennet samples

The results below are from the second interlaboratory test carried out in 1990. The results in Table A.1 exclude those of laboratory 3 for samples 9/12 and laboratory 5 for samples 2/5 on reproducibility grounds[4].

Table A.1 — Results of interlaboratory trial

Sample	Chymosin : pepsin ratio	Mean IMCU/ml	CV(r) %	r	r_{rel} %	CV(R) %	R	R_{rel} %	Outliers
1/4	90 : 10	195,8	2,69	14,7	7,5	3,71	20,4	10,4	Grubbs
8/11	48 : 52	180,7	1,75	8,9	4,9	3,74	18,9	10,5	Grubbs
3/6	9 : 91	213,8	2,57	15,4	7,2	3,79	22,7	10,6	Grubbs
7/10	90 : 10	160,1	0,94	4,2	2,6	1,99	8,9	5,6	Grubbs
2/5	48 : 52	128,0	1,56	5,6	4,3	3,54	12,7	9,9	Grubbs
9/12	9 : 91	197,7	1,07	5,9	3,0	4,03	22,3	11,3	Grubbs
Mean			1,8		4,9	3,5		9,7	

Bibliography

[1] ISO 707|IDF 50:1997, *Milk and milk products — Guidance on sampling*

[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*[5)]

[3] Chapter 10: Rennets containing chymosin and bovine pepsin — Determination of the total milk-clotting activity. *Bull. IDF*, (285) 1993

[4] Chapter 11: Chymosin and pepsin in bovine rennets — Determination of the total milk-clotting activity at pH 6.5 — Relative milk-clotting activity test (REMCAT). *Bull. IDF*, (285) 1993

5) Now published in parts.

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