SRI LANKA STANDARD 393 PART 5 :2013
ISO 6887-5: 2010

CODE OF PRACTICE FOR PREPARATION OF TEST SAMPLES, INITIAL SUSPENSION AND DECIMAL DILUTIONS FOR MICROBIOLOGICAL EXAMINATION OF FOOD AND ANIMAL FEEDING STUFFS

PART 5 –SPECIFIC RULES FOR THE PREPARATION OF MILK AND MILK PRODUCTS (FIRST REVISION)

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

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Sri Lanka.

Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This standard does not purport to include all the necessary provisions of a contract.

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CODE OF PRACTICE FOR PREPARATION OF TEST SAMPLES, INITIAL SUSPENSION AND DECIMAL DILUTIONS FOR MICROBIOLOGICAL EXAMINATION OF FOOD AND ANIMAL FEEDING STUFFS
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(First Revision)

NATIONAL FOREWORD

This Sri Lanka standard was approved by the Sectoral Committee on Agricultural and Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2013-01-22.

This standard was first published in 1976 with a view of providing a general guidance with regard to the practice to be followed, precautions to be observed in the sampling of different types of foods and in the handling of the samples for microbiological analysis. However, keeping in view the experience gained during the years and various International Standards brought out by the International Organization for Standardization (ISO) on the subject of microbiology of food and animal feeding stuffs, it was decided to revise it with a view to updating the existing rules and by incorporating those not covered earlier.

For different products of food and animal feeding stuffs, it is necessary to take special precautions and in order to accommodate these precautions within the scope of one standard, this revised standard is published in several parts.

This part of the standard is identical with ISO 6887-5 : 2010, Microbiology of food and animal feeding stuffs –Preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 5 –Specific rules for the preparation of milk and milk products published by the International Organization for Standardization (ISO).

Terminology and Conventions:

The text of the International Standard has been accepted as suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this standard should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards it is the current practice to use the full point on the base line as the decimal marker.

c) Wherever page numbers are quoted, they are ISO page numbers.

Cross References

International Standard	Corresponding Sri Lanka Standard
ISO 707|IDF 50, Milk and milk products- Guidance on sampling.	SLS 1404 Methods of sampling for milk and milk products.
ISO 6887-1: 1999, Microbiology of food and animal feeding stuffs –Preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 1 – General rules for the preparation of the initial suspension and decimal dilutions	SLS 393 : Part 1, code of practice for Preparation of test samples, initial suspension and decimal dilutions for microbiological examination of food and animal feeding stuffs. Part 1 – General rules for the preparation of the initial suspension and decimal dilutions
ISO 7218, Microbiology of food and animal feeding stuffs- General rules for microbiological examinations.	SLS 516 Part 14, Microbiology of food and animal feeding stuffs- General requirements and guidance for microbiological examinations.
ISO/TS 11133-2, Microbiology of food and animal feeding stuffs-Guidelines on preparation and production of culture media, Part 2 -Practical guidelines on performance testing of culture media.	No corresponding Sri Lanka Standard.

SLS 393-5: 2013

INTERNATIONAL STANDARD

ISO 6887-5

First edition
2010-08-15

Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 5: Specific rules for the preparation of milk and milk products

Microbiologie des aliments — Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique —

Partie 5: Règles spécifiques pour la préparation du lait et des produits laitiers

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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 2.

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 6887-5 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition cancels and replaces ISO 8261|IDF 122:2001, which has been technically revised.

ISO 8261 | IDF 122:2001 was elaborated by ISO/TC 34/SC 5, *Milk and milk products*, and, with its agreement, has been renumbered as ISO 6887-5 and technically revised by ISO/TC 34/SC 9 with the following modifications:

a) the introduction of buffered peptone water as a diluent for general use;

b) the systematic pre-heating of the diluent has been kept only for those cases where it resolves problems of homogeneity of the suspension;

c) the reactivation step has been removed.

ISO 6887 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*:

— *Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

— *Part 2: Specific rules for the preparation of meat and meat products*

— *Part 3: Specific rules for the preparation of fish and fishery products*

— *Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

— *Part 5: Specific rules for the preparation of milk and milk products*

The following part is under preparation:

— *Part 6: Specific rules for the preparation of samples taken at the primary production stage*

INTERNATIONAL STANDARD **ISO 6887-5:2010(E)**

Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 5:
Specific rules for the preparation of milk and milk products

WARNING — The use of this International Standard may involve hazardous materials, operations, and equipment. It is the responsibility of the user to establish appropriate health and safety practices and to determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 6887 specifies rules for the preparation of samples of milk and milk products and their suspension for microbiological examination when the samples require a different preparation from the general methods specified in ISO 6887-1. ISO 6887-1 defines the general rules for the preparation of the initial suspension and decimal dilutions for microbiological examination.

This part of ISO 6887 excludes preparation of samples for both enumeration and detection test methods where preparation details are specified in the relevant International Standards.

This part of ISO 6887 is applicable to:

a) milk and liquid milk products;

b) dried milk products;

c) cheese;

d) casein and caseinates;

e) butter;

f) ice-cream;

g) custard, desserts and sweet cream;

h) fermented milk and sour cream;

i) milk-based infant foods.

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 707 | IDF 50, *Milk and milk products — Guidance on sampling*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO/TS 11133-2, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

3 Terms and definitions

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

3.1
laboratory sample
sample prepared for sending to the laboratory and intended for inspection or testing

NOTE Adapted from ISO 7002:1986[1], A.19.

3.2
test portion
〈microbiology〉 measured volume or measured mass of representative sample taken from the laboratory sample for use in the preparation of the initial suspension

3.3
initial suspension
primary dilution
suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed, if necessary, using a blender and observing appropriate precautions, with a nine-fold quantity of dilution fluid (diluent), allowing large particles, if present, to settle

NOTE 1 For appropriate precautions, see 8.1.

NOTE 2 For details of diluents, see Clause 5.

3.4
further decimal dilutions
suspensions, solutions or emulsions obtained by mixing a specific volume of the primary dilution (3.3) with a nine-fold volume of diluent, and by repeating this operation with every dilution thus prepared, until a decimal dilution series, suitable for the inoculation of culture media, is obtained

4 Principle

An initial suspension (3.3) is prepared to obtain as uniform a distribution as possible of the microorganisms contained in the test sample.

If necessary, further decimal dilutions (3.4) are prepared in order to reduce the number of microorganisms per volume to allow, after incubation, observation of any growth (in the case of liquid media) or colonies (in the case of agar plates), as stated in each relevant International Standard.

In order to restrict, if required, the range of enumeration to a given interval, or if high numbers of microorganisms are foreseen, it is possible to inoculate only the necessary decimal dilutions (at least two successive dilutions) needed to achieve enumeration according to the calculation specified in ISO 7218.

5 Diluents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade, and only sterile distilled or deionized water.

5.1 Basic materials.

See ISO 6887-1.

5.2 Diluents for general use.

5.2.1 Peptone-salt solution.

5.2.1.1 Composition.

Enzymatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

5.2.1.2 Preparation.

Dissolve the components in the water, heating slightly on a hotplate (6.6) if necessary. Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.2.2 Quarter-strength Ringer's solution.

5.2.2.1 Composition.

Sodium chloride (NaCl)	2,25 g
Potassium chloride (KCl)	0,105 g
Calcium chloride ($CaCl_2$), anhydrous	0,06 g[a]
Sodium hydrogencarbonate ($NaHCO_3$)	0,05 g
Water	1 000 ml

[a] Alternatively, use 0,12 g of $CaCl_2 \cdot 6H_2O$.

5.2.2.2 Preparation.

Dissolve the salts in the water. Adjust the pH, if necessary, so that after sterilization it is 6,9 ± 0,2 at 25 °C.

5.2.3 Peptone solution.

5.2.3.1 Composition.

Enzymatic digest of casein	1,0 g
Water	1 000 ml

5.2.3.2 Preparation.

Dissolve the peptone in the water. Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.2.4 Phosphate buffer solution.

5.2.4.1 Composition.

Potassium dihydrogenphosphate (KH_2PO_4)	42,5 g
Water	1 000 ml

5.2.4.2 Preparation.

Dissolve the salt in 500 ml of water. Adjust the pH, if necessary, so that after sterilization it is 7,2 ± 0,2 at 25 °C. Dilute to 1 000 ml with the remaining water.

Store the stock solution under refrigerated conditions.

Add 1 ml of this stock solution to 1 000 ml of water for use as diluent.

5.2.5 Buffered peptone water.

5.2.5.1 Composition.

Enzymatic digest of animal tissues	10,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogenphosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9,0 g[a]
Potassium dihydrogenphosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

[a] Alternatively, use 3,56 g of anhydrous disodium hydrogenphosphate (Na_2HPO_4).

5.2.5.2 Preparation.

Dissolve the components in the water by heating slightly, if necessary, on a hotplate (6.6). Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.2.5.3 Application.

This diluent is recommended in particular for detection of *Salmonella* spp. or enumeration of *Listeria monocytogenes*, but can also be used for the preparation of initial suspensions for other determinations.

5.3 Diluents for special purposes.

These diluents shall only be used for the preparation of initial suspensions.

5.3.1 Sodium citrate solution.

5.3.1.1 Composition.

Trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	20,0 g
Water	1 000 ml

5.3.1.2 Preparation.

Dissolve the salt in water by heating, if necessary, on a hotplate (6.6) at a temperature between 45 °C and 50 °C. Adjust the pH, if necessary, so that after sterilization it is 7,5 ± 0,2 at 25 °C.

5.3.1.3 Application.

This solution is used for cheese and (roller-) dried milk, and some caseinates.

5.3.2 Dipotassium hydrogenphosphate solution.

5.3.2.1 Composition.

Dipotassium hydrogenphosphate (K_2HPO_4)	20,0 g
Water	1 000 ml

5.3.2.2 Preparation.

Dissolve the salt in the water by heating, if necessary, on a hotplate (6.6) at a temperature between 45 °C and 50 °C. For acid whey powder, adjust the pH so that for the primary dilution after sterilization it is 8,4 ± 0,2 at 25 °C. For cheese, roller-dried milk, fermented milk, caseinates, and sour cream, adjust the pH so that after sterilization it is 7,5 ± 0,2 at 25 °C.

5.3.2.3 Application.

This solution is used for cheese, (roller-) dried milk, fermented milk, some caseinates, dried acid whey, and sour cream.

5.3.3 Dipotassium hydrogenphosphate solution with antifoam agent.

5.3.3.1 Dipotassium hydrogenphosphate solution.

5.3.3.1.1 Composition.

Dipotassium hydrogenphosphate (K_2HPO_4)	20,0 g
Water	1 000 ml

5.3.3.1.2 Preparation.

Dissolve dipotassium hydrogenphosphate in water by heating, if necessary, on a hotplate (6.6) at a temperature between 45 °C and 50 °C.

5.3.3.2 Antifoam stock solution.

5.3.3.2.1 Composition.

Polyethylene glycol 2000	1 g
Water	100 ml

5.3.3.2.2 Preparation.

Dissolve the polyethylene glycol 2000 in the water by mixing.

5.3.3.3 Preparation.

Add 1 ml of the antifoam stock solution (5.3.3.2) to 1 l of the K_2HPO_4 solution (5.3.3.1). Adjust the pH so that for the primary dilution of both acid and lactic casein, after sterilization, it is 8,4 ± 0,2 at 25 °C, and for rennet casein, after sterilization, it is 7,5 ± 0,2 at 25 °C.

5.3.3.4 Application.

This solution is used for acid casein, lactic casein and rennet caseins.

5.3.4 Tripolyphosphate solution.

5.3.4.1 Composition.

Sodium tripolyphosphate ($Na_5O_{10}P_3$)	20,0 g
Water	1 000 ml

5.3.4.2 Preparation.

Dissolve the salt in the water by heating slightly on a hotplate (6.6), if necessary. Dispense the tripolyphosphate solution in bottles in portions of 90 ml and sterilize them. The medium may be stored at a temperature of 5 °C ± 3 °C for a maximum of 1 month.

5.3.4.3 Application.

This solution is used as alternative diluent for rennet caseins which are difficult to dissolve.

5.3.5 Diluent for general use with α-amylase solution.

5.3.5.1 Preparation.

Add 12,5 mg of α-amylase (EC 3.2.1.1, see Reference [3]) with a specific activity of approximately 400 units[1)] per milligram to 225 ml of the diluent for general use (see 5.2). This diluent is used for a 25 g test portion. Use amounts in the same proportion for preparation of other test portions (e.g. for a 10 g test portion, add 5 mg of α-amylase to 90 ml of the diluent for general use).

5.3.5.2 Application.

This solution is used for foods containing starch.

5.3.6 Buffered peptone water with bromocresol purple.

5.3.6.1 Composition.

Buffered peptone water (see 5.2.5)	1 000 ml
Bromocresol purple (4 % alcohol solution, e.g. ethanol solution)	0,1 ml

5.3.6.2 Preparation.

Add 0,1 ml of bromocresol purple solution to 1 000 ml of buffered peptone water (5.2.5).

5.3.6.3 Application.

This solution may be used in certain acidic products so that adjustment of the pH can be carried out without the use of a sterile pH probe (see 8.3).

Bromocresol purple is yellow at acidic pH, changing to purple at pH above 6,8.

1) This unit (often called the International Unit or Standard Unit) is defined as the amount of enzyme which catalyses the transformation of 1 µmol of substrate per minute under standard conditions.

5.4 Distribution and sterilization of the diluent.

See ISO 6887-1.

5.5 Performance testing for quality control.

Carry out quality control for all diluents included in this part of ISO 6887 as specified for peptone-salt solution in ISO/TS 11133-2.

Incubation:	45 min at 20 °C to 25 °C
Strain:	*Escherichia coli* WDCM 00013[a,2)] or WDCM 00012[a,2)]
	Staphylococcus aureus WDCM 00034[2)]
Medium of control:	TSA at 37 °C ± 1 °C for 24 h ± 2 h
Method of control:	Quantitative
Criteria:	± 50 % of the enumeration at t_0
[a] Strains to be used by the user laboratory (minimum).	

6 Apparatus

Usual microbiological laboratory equipment for general use (see ISO 7218 and ISO 6887-1) and, in particular, the following.

6.1 Peristaltic or **rotary blender**.

6.2 Vortex mixer.

6.3 Glass beads, of diameter about 6 mm.

6.4 Water baths, capable of maintaining temperatures of 37 °C ± 1 °C and 45 °C ± 1 °C.

6.5 Spatulas or **glass rods**.

6.6 Hotplate or other apparatus, capable of gentle heating (not gas burners), and capable of operating at the required temperature.

7 Preparation of samples

7.1 Frozen products

Products stored frozen should be brought to a consistency that allows sampling; i.e. by storing at 18 °C to 27 °C (laboratory temperature) for a maximum of 3 h, or at 3 °C ± 2 °C for a maximum of 24 h. Samples should be tested as quickly as possible after this. See ISO 6887-1.

If the product is still frozen when portioning, some diluent (Clause 5) at laboratory temperature may be used to facilitate defrosting.

2) For more information on culture collection strain numbers and contact details, refer to the reference strain catalogue available (viewed 2010-07-19) on http://www.wfcc.nig.ac.jp/WDCM_Reference_Strain_Catalogue.

7.2 Hard and dry products

To mix hard products in a peristaltic blender (6.1), contain the sample and diluent in two or more sterile bags to prevent puncturing and possible sample spillage.

For hard or dry products, do not homogenize in a rotary homogenizer for more than 2,5 min at a time.

For dry and hard or heterogeneous products, it may be necessary to mince or to grind the laboratory sample. In this case, to avoid an excessive rise in temperature, do not mince or grind for more than 1 min.

7.3 Liquid and non-viscous products

Shake the test sample by hand (see 9.1) or mechanically to ensure uniform distribution of microorganisms before analysis.

7.4 Heterogeneous products

For heterogeneous products (which contain pieces of different foods), sampling should be carried out by taking aliquots of each component representative of their proportions in the initial product.

It is also possible to homogenize the whole laboratory sample to allow sampling of a more homogeneous test sample.

It may be necessary to mince or to grind the laboratory sample. In this case, to avoid an excessive rise in temperature, do not mince or grind for more than 1 min.

8 General procedures

8.1 General

All preparations and manipulations should be carried out using good aseptic techniques and with sterile equipment to prevent microbial contamination of samples from all external sources. See ISO 7218.

Indicate in the report which procedure is used for the analysis if it is different from the procedure specified in this part of ISO 6887.

8.2 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 part of ISO 6887. A recommended sampling method is given in ISO 707 | IDF 50.

If there is no specific International Standard dealing with the product concerned, it is recommended that the parties concerned come to an agreement on the subject.

8.3 General case for acidic products

When using a suspension solution of acidic products, ensure that the pH is brought back to neutral. The use of diluent with an added pH indicator (5.3.6) can avoid the need to use and sterilize pH probes; add sodium hydroxide (NaOH) until the indicator starts to change colour.

For use with buffered diluents, the addition of NaOH is often necessary to increase the buffering capacity of the alkaline component. The concentration of added NaOH depends on the product acidity. The most suitable concentration (e.g. 0,1 mol/l or 1 mol/l) is the concentration which is still close to a ratio of 1 in 9 with the diluent.

8.4 High-fat foods (fat content > 20 % mass fraction)

The use of a diluent with between 1 g/l and 10 g/l of added sorbitan monooleate [polysorbate 80[3)]], approximately according to fat levels (e.g. at a fat content of 40 %, add 4 g/l) may improve emulsification during suspension.

9 Specific procedures

9.1 Milk and liquid milk products

Mix the test sample thoroughly so that the microorganisms are distributed as evenly as possible by rapidly inverting the sample container 25 times. Avoid foaming or allow any foam to disperse. The interval between mixing and removing the test portion shall not exceed 3 min.

Remove at least 1 ml of test sample with a sterile pipette and add a nine-fold volume of diluent for general use (5.2). Shake this primary dilution [e.g. 25 times with a movement of about 300 mm for 7 s manually, or using a vortex mixer (6.2) for 5 s to 10 s] to obtain a 10^{-1} dilution.

Prepare further dilutions in accordance with Clause 10.

9.2 Dried milk, dried sweet whey, dried acid whey, dried buttermilk, and lactose

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it.

If the test sample is in the original unopened container and this is too full to permit thorough mixing, transfer it to a larger container, then mix. Open the container, remove the test portion required with a spatula and proceed as indicated below. Immediately close the container again.

Weigh 10 g of the test sample into a sterile glass vessel (e.g. a beaker) and then add the powder to the dilution bottle containing a diluent for general use (5.2). For dried acid whey, use dipotassium hydrogenphosphate solution (5.3.2) at pH 8,4 ± 0,2 or, if necessary, use for roller-dried milk sodium citrate solution (5.3.1) or dipotassium hydrogenphosphate solution (5.3.2) at pH 7,5 ± 0,2.

Alternatively, weigh 10 g of the test sample directly into the bottle with the required diluent.

NOTE For better reconstitution and in particular with roller-dried milk, glass beads (6.3) can be helpful. If used, they should be added to the bottle before sterilization.

To dissolve the test sample, swirl slowly to wet the powder then shake the bottle e.g. 25 times, with a movement of about 300 mm, for about 7 s. A peristaltic blender (6.1) may be used as an alternative to shaking.

Allow to stand for 5 min, shaking occasionally.

The diluent may be pre-warmed to 45 °C if a homogeneous suspension cannot be obtained even after blending. Mention such an additional procedure in the test report.

Prepare further dilutions in accordance with Clause 10.

9.3 Cheese and processed cheese

Weigh 10 g of test sample in a dish and transfer it to the container of a rotary blender or of a peristaltic blender (6.1). Alternatively, weigh 10 g of test sample directly into the container.

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3) Tween 80 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Add 90 ml of diluent for general use (5.2) or, as diluent for cheese, 90 ml of sodium citrate solution (5.3.1) or dipotassium hydrogenphosphate solution (5.3.2) at pH 7,5 ± 0,2.

Blend until the cheese is thoroughly dispersed.

Allow any foam to disperse.

The diluent may be pre-warmed to 45 °C if a homogeneous suspension cannot be obtained even after blending. Mention such an additional procedure in the test report.

Prepare further dilutions in accordance with Clause 10.

9.4 Acid casein, lactic casein, rennet casein, and caseinate

9.4.1 General case

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it.

Weigh 10 g of the test sample into a sterile plastic bag for a peristaltic blender (6.1). Add 90 ml of the appropriate diluent at room temperature, as follows:

a) for acid and lactic casein — dilute with dipotassium hydrogenphosphate solution with antifoam agent (5.3.3) at pH 8,4 ± 0,2;

b) for caseinate — dilute with citrate solution (5.3.1) or dipotassium hydrogenphosphate solution (5.3.2) at pH 7,5 ± 0,2 or peptone-salt solution (5.2.1);

c) for rennet casein — dilute with dipotassium hydrogenphosphate solution with antifoam agent (5.3.3) at pH 7,5 ± 0,2.

Mix well manually and allow to stand at room temperature for 15 min. Blend if necessary for 2 min in the peristaltic blender (6.1) by using two sterile bags for granular products. Allow to stand for 5 min.

Prepare further dilutions in accordance with Clause 10.

9.4.2 Special case: rennet casein

Rennet casein can be difficult to dissolve. An alternative procedure to that described in 9.4.1 may be used.

Using dipotassium hydrogenphosphate solution with antifoam agent (5.3.3) as diluent for rennet caseins may not be efficient to dissolve the grains. These casein grains hamper the enumeration of microorganisms at 30 °C. Therefore, the following alternative procedure is recommended.

If necessary, grind the dry casein before taking the test portion. Transfer approximately 20 g of the test sample into a suitable container. Grind it using an apparatus with knives able to rotate at approximately 20 000 r/min, equipped with a device that prevents the sample from heating during grinding[4)].

Weigh 5 g of the thus-prepared test sample in a sterile bottle of 250 ml. Add glass beads (6.3) for mixing and 95 ml of the sodium tripolyphosphate solution (5.3.4) preheated to 37 °C. Mix by leaving the bottle on a mixing device for 15 min. Then place it in the water bath (6.4) set at 37 °C for 15 min while mixing from time to time.

Prepare further dilutions in accordance with Clause 10.

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4) The VirTis apparatus is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

9.5 Butter

If it is necessary to exclude the surface of a butter sample from investigation, consult ISO 707|IDF 50.

Weigh 10 g of test sample into a sample container. Place the container in the water bath (6.4) set at 45 °C. Keep it in the water bath until the whole test portion has just melted. Add 90 ml of diluent for general use (5.2) warmed to 45 °C and mix. This operation is more easily carried out in a peristaltic blender (6.1).

Alternatively, use only the aqueous phase for dilution, as follows.

Take a test portion of 50 g containing a volume-to-mass ratio of water of W %. Add (50 – [50 × W/100]) ml of diluent for general use (5.2) pre-warmed in the water bath (6.4) at 45 °C. In these conditions, 1 ml of the aqueous phase corresponds to 1 g of butter.

EXAMPLE For 50 g butter containing a volume-to-mass ratio of water of about 16 %, the aqueous phase represents 8 ml of liquid. Add (50 – [50 × 16/100]) = 42 ml of diluent for general use (5.2) pre-warmed in the water bath (6.4) at 45 °C.

Place a container in the water bath (6.4) set at 45 °C until the butter melts. Remove from the water bath, shake well, and allow phases to separate for no longer than 15 min. If necessary, remove the fat phase with a spatula or a glass rod (6.5).

If necessary, to separate the phases, transfer the melted test portion to a sterile centrifuge tube (or melt the test portion directly in the tube) and centrifuge at a rotational frequency allowing phases to separate. It may be necessary to remove the fatty (upper) phase aseptically with a sterile tube connected to a vacuum pump. Pipette from the bottom layer.

Prepare further dilutions in accordance with Clause 10.

9.6 Ice-cream

Weigh 10 g of test sample into a flask or into a sterile plastic bag for a peristaltic blender (6.1). Add 90 ml of diluent at room temperature and blend. The product melts during blending.

Prepare further dilutions in accordance with Clause 10.

9.7 Custard, desserts and sweet cream (pH > 5)

Weigh 10 g of test sample into a flask containing glass beads (6.3). Add 90 ml of diluent for general use (5.2) at room temperature and shake to disperse. Alternatively, a peristaltic blender (6.1) may be used following the manufacturer's instructions. In this case, the bag should not contain any glass beads.

Prepare further dilutions in accordance with Clause 10.

9.8 Fermented milk and sour cream (pH < 5)

Weigh 10 g of test sample into a flask containing glass beads (6.3). Add as diluent 90 ml of buffered peptone water (5.2.5) or dipotassium hydrogenphosphate solution (5.3.2) at pH 7,5 ± 0,2 at room temperature and shake manually. Alternatively, a peristaltic blender (6.1) may be used following the manufacturer's instructions. In this case, the bag should not contain any glass beads.

Prepare further dilutions in accordance with Clause 10.

9.9 Milk-based infant foods

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it. If the test sample is in an original unopened container which is too full to permit thorough mixing, transfer it to a larger container, then mix. Open the container. Remove the required test portion with a spatula (6.5) and proceed as indicated below. Immediately close the container again.

Weigh 10 g of test sample into a suitable sterile glass vessel (e.g. a beaker). Then add the powder to the dilution bottle containing a diluent for general use (5.2) or, for samples with high starch content, a diluent for special purposes (5.3.5).

Alternatively, weigh 10 g of test sample directly into the bottle with the required diluent.

The diluent may be pre-warmed to 45 °C if a homogeneous suspension cannot be obtained even after blending. Mention such an additional procedure in the test report.

For better reconstitution, using glass beads (6.3) might be helpful. If used, add them to the bottle before sterilization.

In order to dissolve the sample, swirl slowly to wet the powder, then manually shake the bottle, e.g. 25 times, with a movement of about 300 mm, for about 7 s. Alternatively, a peristaltic blender (6.1) may be used. Allow to stand for 5 min, shaking occasionally. Prepare further dilutions in accordance with Clause 10.

Samples with high starch content may cause problems because of the high viscosity of the primary dilution.

Use a diluent for general use (5.2) with α-amylase solution (5.3.5) to reduce the viscosity of the initial solution, or use twice the quantity of the diluent. Take this further dilution into consideration in the subsequent examinations.

10 Further decimal dilutions

See ISO 6887-1.

When transferring from a viscous primary dilution such as acid or rennet casein (9.4), rinse the pipette with diluent by aspirating several times, using the diluent in the tube used for making the decimal dilution.

IMPORTANT — If the aforementioned step is done without rinsing of the pipette when transferring a viscous primary dilution, an incorrect volume of primary dilution is transferred.

When 10 ml plus 90 ml, or 11 ml plus 99 ml, have been taken, shake manually as described in 9.1.

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[1] ISO 7002:1986, *Agricultural food products — Layout for a standard method of sampling from a lot*

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