INTERNATIONAL STANDARD

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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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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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*

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.