

SLOVENSKI STANDARD
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Mikrobiologija v prehranski verigi - Priprava vzorcev za preskušanje ter osnovne in decimalnih razredčin za mikrobiološko preiskavo - 1. del: Splošna pravila za pripravo osnovne in decimalnih razredčin (ISO/DIS 6887-1:2013)

Microbiology of the food chain - 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/DIS 6887-1:2013)

Mikrobiologie der Lebensmittelkette - Vorbereitung von Untersuchungsproben und Herstellung von Erstverdünnungen und von Dezimalverdünnungen für mikrobiologische Untersuchungen - Teil 1: Allgemeine Regeln für die Herstellung von Erstverdünnungen und Dezimalverdünnungen (ISO/DIS 6887-1:2013)

Microbiologie des aliments - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 1: Règles générales pour la préparation de la suspension mère et des dilutions décimales (ISO/DIS 6887-1:2013)

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Microbiology of the food chain — 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

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

Partie 1: Règles générales pour la préparation de la suspension mère et des dilutions décimales

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This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

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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 6887-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This document cancels and replaces ISO 6887-1:1999.

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

- *Part 1: General rules for the preparation of the initial suspension and 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 miscellaneous products*

[This part includes sample preparation for a variety of products not covered in the other parts, as follows: very acidic products; very hard products; cereals and cereal products; animal feeds; gelatine; margarines and non-dairy spreads; dehydrated and low a_w products; eggs and egg products; fermented products; bakery goods; and beverages.]

- *Part 5: Specific rules for the preparation of milk and milk products*
- *Part 6: Specific rules for the preparation of samples taken at the primary production stage.*

Introduction

Because of the large variety of food and animal feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods which are specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this part of ISO 6887 is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this part of ISO 6887 so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

This part of ISO 6887 defines the general rules for the preparation of samples, initial suspensions and subsequent dilutions for microbiological examination. The remaining parts of ISO 6887 give specific rules for the preparation of samples and initial suspensions, each covering the variety of food and feed products and environmental samples to which ISO 6887 applies.

For a number of products, it is necessary to take special precautions, especially when preparing the initial suspension, because of the physical state of the product (such as dry products, highly viscous products) or the presence of inhibitory substances (such as spices, high salt content) or the acidity, etc. These are covered in general terms in this part of ISO 6887.

Any special diluents or practices required for particular products or microorganisms in specific standard methods should still be used in preference to the general rules listed in this series of standards. These may include:

- specific rehydration procedures for foods of low water activity to minimize osmotic shock;
- the use of adequate temperatures to aid suspension of cocoa, gelatine, milk powder, etc.;
- resuscitation procedures for the improved recovery of stressed microorganisms resulting from food processing and storage;
- homogenization procedures and duration specific to certain products (e.g. cereals) and/or to certain determinations (e.g. yeasts and moulds).

Microbiology of the food chain — 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

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

1 Scope

This part of ISO 6887 defines general rules for the aerobic preparation of the initial suspension and of dilutions for microbiological examinations of products intended for human or animal consumption.

This part of ISO 6887 is applicable to the general case and other parts apply to specific groups of products as detailed in the Foreword. Some aspects may also be applicable to molecular methods where matrices may be associated with inhibition of the PCR steps and consequently affect the test result.

This part of ISO 6887 excludes preparation of samples for both enumeration and detection test methods where preparation instructions are detailed in specific International Standards.

2 Normative references

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

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

3 Terms and definitions

For the purposes of all parts of ISO 6887, the following definitions apply.

3.1

laboratory sample

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

[ISO 7002]

3.2

composite sample

mixed sample of a number of items of the same type of food, animal feed, animals or environment, from which a test portion is taken for examination in the laboratory.

NOTE See illustration of a composite sample in Annex A.

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3.3

pooled sample

mixed sample of a number of items of the same type of food, animal feed, animals or environment, where the complete mixture is the test portion and is taken as a whole for examination in the laboratory

NOTE See illustration of a pooled sample in Annex A.

3.4

test sample

sample prepared from the laboratory sample according to the procedure specified in the method of test and from which test portions are taken

[ISO 7002]

NOTE Preparation of the laboratory sample before the test portion is taken is infrequently used in microbiological examinations.

3.5

test portion

measured (volume or mass) representative sample taken from the laboratory sample for use in the preparation of the initial suspension

NOTE Sometimes preparation of the laboratory sample (3.4) is required before the test portion is taken but this is infrequently used in microbiological examinations.

3.6

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 with, normally, a nine-fold quantity of diluent, allowing large particles, if present, to settle

NOTE 1 Nine-fold dilutions are normally used to produce a decimal dilution series, but other ratios may be required for specific purposes.

3.7

further dilutions

suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.6) with an x-fold volume of diluent and by repeating this operation with further dilutions until a dilution series, suitable for the inoculation of culture media, is obtained

NOTE 1 Nine-fold dilutions are normally used to produce a decimal dilution series, but other ratios may be required for specific purposes.

3.8

pooled test portions

mixture of test portions from a number of items of the same type of food, animal feed, animals or environment, where the complete mixture is the test portion examined

NOTE See illustration of pooled test portions in Annex A.

3.9

pooled (pre-) enriched test portions

individually (pre-) enriched test portions from a number of items of the same type of food, animal feed, animals or environment, from which specified volumes are combined for further examination

NOTE See illustration of pooled (pre-) enriched test portions in Annex A.

3.10

specific standard

an International Standard or guidance document describing the examination of a specific product (or group of products) for the detection or enumeration of a specific microorganism (or group of microorganisms)

4 Principle

Preparation of the initial suspension (3.6) in such a way as to obtain as uniform a distribution as possible of the microorganisms contained in the test portion (3.5).

Preparation, if necessary, of further dilutions (3.7) in order to reduce the number of microorganisms per unit volume to allow, after incubation, observation of their growth or not (in the case of tubes or bottles) or colony counting (in the case of plates), as stated in each specific standard.

NOTE In order to restrict the range of enumeration to a given optimum 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 the enumeration according to the calculations described in ISO 7218.

5 Diluents

5.1 Basic materials

To improve the reproducibility of test results, it is recommended that either ready-made diluents or dehydrated basic components or a dehydrated complete preparation should be used. In all cases, the manufacturer's instructions shall be followed rigorously.

Chemical products shall be of recognized analytical quality and suitable for microbiological examinations.

The water used shall be distilled water or of equivalent quality (see ISO 7218 or ISO 11133 [2]).

For more detailed rules on preparation of culture media see ISO 11133 [2].

All diluents shall be tested for acceptable performance in accordance with ISO 11133 [2].

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	8,5 g
Water	1 000 ml

ISO/DIS 6887-1**5.2.1.2 Preparation**

Dissolve the components in the water in flasks, bottles or test tubes (6.4), by heating if necessary.

Adjust the pH if necessary so that, after sterilization, it is $7,0 \pm 0,2$ at 25 °C.

5.2.2 Buffered peptone water**5.2.2.1 Composition**

Peptone ¹	10,0 g
Sodium chloride	5,0 g
Disodium hydrogen phosphate dodecahydrate (Na ₂ HPO ₄ ·12H ₂ O)	9,0 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	1,5 g
Water	1 000 ml

¹: For example enzymatic digest of casein

5.2.2.2 Preparation

Dissolve the components in the water in flasks, bottles or test tubes (6.4), by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is $7,0 \pm 0,2$ at 25 °C.

5.2.3 Double-strength buffered peptone water

This diluent may be necessary for high acid samples (see 8.5) and is prepared by dissolving double the quantities of a complete dehydrated medium or the dry ingredients given at 5.2.2.1 in 1 000 ml of water and processing in the same manner. Alternatively only double the quantities of buffer ingredients may be used if the diluent is prepared from individual ingredients.

5.3 Diluents for special purposes

See the specific standard or part of ISO 6887 appropriate to the product concerned.

5.4 Distribution and sterilization of the diluent

Dispense the diluent in volumes as necessary for the preparation of the initial suspensions into vessels (6.4) of appropriate capacity.

Dispense further diluent in volumes as necessary for the preparation of the (decimal or other ratio) dilutions into vessels (6.4) of appropriate capacity in quantities such that, after sterilization, each vessel contains 9,0 ml \pm 0.2 ml. The tolerance allowable on this final volume, after sterilization, shall not exceed \pm 2 %.

NOTE In order to enumerate several groups of microorganisms using different culture media, it may be necessary to distribute all the diluents (or some of them) in quantities greater than 9,0 ml into vessels (6.4) of appropriate size.

Stopper the vessels loosely to allow for expansion on heating.

Sterilize in the autoclave at $121 \text{ °C} \pm 3 \text{ °C}$ for 15 min (see ISO 7218).