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Second edition
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Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 4: Specific rules for the preparation of miscellaneous products

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

Partie 4: Règles spécifiques pour la préparation de produits variés

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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This second edition cancels and replaces the first edition (ISO 6887-4:2003), which has been technically revised.

It also incorporates the Amendment ISO 6887-4:2003/Amd.1:2011 and the Technical Corrigendum ISO 6887-4:2003/Cor.1:2004.

A list of all parts in the ISO 6887 series can be found on the ISO website.

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Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 4: Specific rules for the preparation of miscellaneous products

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

1 Scope

This document specifies rules for the preparation of samples and dilutions for the microbiological examination of specific food products not covered in other parts of ISO 6887, which deal with more general categories. This document covers a wide range of miscellaneous products, but does not include new products brought on to the market after publication.

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ISO 6887-1 defines the general rules for the preparation of the initial suspension and dilutions for microbiological examination.

This document excludes preparation of samples for both enumeration and detection test methods when preparation details are specified in the relevant International Standards.

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This document is applicable to the following products:

— acidic (low pH) products;

— hard and dry products;

— dehydrated, freeze-dried and other low a_W products (including those with inhibitory properties);

— flours, whole cereal grains, cereal by-products;

— animal feed, cattle cake, kibbles and pet chews;

— gelatine (powdered and leaf);

— margarines, spreads and non-dairy products with added water;

— eggs and egg products;

— bakery goods, pastries and cakes;

— fresh fruit and vegetables;

— fermented products and other products containing viable microorganisms;

— alcoholic and non-alcoholic beverages;

— alternative protein products.

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2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6887-1, *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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6887-1 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp/

4 Principle

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The general principles for sample preparation and subsequent steps are detailed in ISO 6887-1. This document describes specific measures for products not covered in other parts of ISO 6887.

An initial suspension is prepared to obtain as uniform a distribution as possible of the microorganisms contained in the test portion.

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For detection methods, the pre-enrichment or enrichment suspension is prepared in the same way, using the medium recommended in the method of examination concerned. For enumeration methods used for all food types covered in this document, general diluents are documented in the relevant clauses of ISO 6887-1 and specific diluents are included in Clause 5.

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

5 Diluents

5.1 Basic materials

Refer to ISO 6887-1.

5.2 Diluents for general use

5.2.1 Peptone salt solution

Refer to ISO 6887-1.

5.2.2 Buffered peptone water

Refer to ISO 6887-1.

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5.3 Diluents for special purposes

5.3.1 Double-strength buffered peptone water

5.3.1.1 Composition and preparation

Refer to ISO 6887-1.

5.3.1.2 Application

Use this diluent for highly acidic products of pH ≥ 3,5 to pH < 4,5 to obtain an initial suspension of pH 7,0 ± 0,5.

NOTE Buffered peptone water (5.2.2) is sufficient for products of pH 4,5 and above.

5.3.2 Phosphate buffered diluent

5.3.2.1 Composition

Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

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5.3.2.2 Preparation

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Dissolve the components in the water, by heating if necessary.

If necessary, adjust the pH so that, after sterilization, it is pH 7,0 ± 0,2 at 25 °C.

5.3.2.3 Application

Phosphate buffered solution is used as a diluent for gelatine (9.3).

5.4 Distribution and sterilization of the diluent

Refer to ISO 6887-1.

5.5 Performance testing of diluents

Refer to ISO 6887-1.

5.6 Enzyme solutions

5.6.1 Alpha-amylase solution

5.6.1.1 Composition

α-amylase	1,0 g
Water	100 ml

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5.6.1.2 Preparation

Dissolve the α-amylase in the water and sterilize the solution by passing through a 0,2 µm membrane filter. The enzyme solution can be stored for up to 1 month at 5 °C ± 3 °C or up to 3 months at ≤–20 °C.

Final composition of the α-amylase solution may need to be adjusted depending on the enzymatic activity of the commercial α-amylase used and the thickening properties of the test sample.

5.6.1.3 Application

This enzyme solution is added at the rate of 10 ml to 1 000 ml of diluent (1 % volume fraction) to improve solubility of swelling starch products, cereals and cereal-containing products (9.1.4.3).

5.6.2 Cellulase solution

5.6.2.1 Composition

Cellulase	1,0 g
Water	100 ml

5.6.2.2 Preparation

Dissolve the cellulase in the water and sterilize the solution by passing through a 0,2 µm membrane filter. The enzyme solution can be stored for up to two weeks at 5 °C ± 3 °C or up to 1 month at ≤–20 °C.

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NOTE Use of fresh solution will ensure maximum enzyme activity.

5.6.2.3 Application

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This enzyme solution is added at the rate of 10 ml to 1 000 ml of diluent (1 % volume fraction) to improve solubility of carboxymethyl cellulose, locust beans, carob, guar and cassia gums (9.1.4.3).

5.6.3 Papain solution

5.6.3.1 Composition

Papain	5,0 g
Water	100 ml

5.6.3.2 Preparation

Dissolve the papain in the water and sterilize the solution by passing through a 0,2 µm membrane filter. The enzyme solution can be stored for up to 1 month at 5 °C ± 3 °C.

NOTE Use of fresh solution will ensure maximum enzyme activity.

5.6.3.3 Application

This enzyme solution is added at the rate of 20 ml to 1 000 ml of diluent (2 % volume fraction) to improve solubility of gelatine (9.1.4.3 and 9.3).

6 Apparatus

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

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6.1 Homogenizers.

6.1.1 Rotary homogenizer (blender).

Refer to ISO 7218. If a large sample is to be homogenized, the equipment should include a sterile 1 l bowl.

6.1.2 Peristaltic homogenizer.

Refer to ISO 7218. With sterile plastic bags or filter bags to retain particulate material where necessary.

6.2 Domestic grater, sterile.

6.3 Hammer or **other heavy implement**, capable of crushing hard materials.

6.4 Water baths, capable of being maintained at 44 °C to 47 °C or as stated for specific purposes.

6.5 Sterile scissors, **knives**, **scalpels** and **forceps**.

6.6 Sterile spatulas, **spoons** or **scoops**.

6.7 Sterile corers, for taking samples at depth.

6.8 Stirrer, capable of operating with a horizontal motion.

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6.9 Sterile wide-necked flasks or **other containers**, of 500 ml capacity.

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6.10 Ultrasonic bath, with operating frequency of 35 MHz to 45 MHz.

7 Sampling and sample types

Carry out sampling in accordance with the specific standard appropriate to the product concerned or see ISO/TS 17728. Some guidance on sampling certain products is included in Clause 9 for clarity. If a specific standard is not available, it is recommended that agreement be reached on this subject by the parties concerned.

8 Preparation of samples

8.1 General

All preparations and manipulations shall be carried out using aseptic techniques and sterile equipment (see ISO 7218). General sample preparation procedures are given in ISO 6887-1, but additional detail for some categories is given in 8.2 to 8.4.

8.2 Acidic products

It is important to consider the end use of the product when testing acidic samples.

If the product is to be used as an ingredient in a final product of higher pH, then the pH of the initial suspension of the test portion shall be adjusted to pH 7,0 ± 0,5 with the diluents specified at 5.2.2 or 5.3.1 or others with equivalent buffering capacity.

For pH adjustment of moderately acidic samples (pH ≥ 3,5 to pH < 4,5), use double-strength buffered peptone water (5.3.1). See ISO 6887-1.

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