INTERNATIONAL STANDARD

ISO 6887-4

First edition
2003-08-01

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

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

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Microbiologie des aliments — 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 autres que les produits laitiers, les produits carnés et les produits de la pêche

Reference number
ISO 6887-4:2003(E)

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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 6887-4 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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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*:

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— *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, fish and fishery products*

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

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

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 prior the use.

1 Scope

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This part of ISO 6887 specifies rules for the preparation of samples and decimal dilutions for the microbiological examination of food products other than those covered in other parts of ISO 6887. ISO 6887-1 defines the general rules for the preparation of the initial suspension and decimal dilutions for microbiological examination.

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This part of ISO 6887 only describes methods of preparation that are applicable to several microorganisms simultaneously. It excludes the preparations that only apply to the detection and/or enumeration of a single microorganism where the methods of preparation are described in the relevant International Standard concerning that microorganism.

This part of ISO 6887 is applicable to the following products:

— general case for acidic products (see 8.2);

— foods with a high fat content, excluding margarine and spreads (see 8.3);

— flours, whole cereal grains, cereal by-products, animal feeds and cattle cake (see 9.1);

— very hard products, e.g. cassava (see 9.2);

— gelatine (see 9.3);

— margarine and spreads (see 9.4);

— dehydrated products and freeze-dried products (except dairy products and egg products) (see 9.5);

— egg and egg products (see 9.6);

— fermented products (products containing live microorganisms) (see 9.7);

— pastries and cakes (9.8).

NOTE Milk and milk products are dealt with in ISO 8261.

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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 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*

ISO 6887-2:2003, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

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

3 Terms and definitions

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

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

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

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

3.4
further decimal dilutions
suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.3) with a nine-fold volume of diluent and by repeating this operation with further dilutions, 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.

A pre-enrichment or enrichment suspension is prepared in the same way, using the medium recommended by the method of analysis concerned, except in special cases mentioned in each product section of this part of ISO 6887.

If necessary, decimal dilutions (3.4) 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.

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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 the enumeration according to the calculation described in ISO 7218.

5 Diluents

5.1 Basic materials

See ISO 6887-1.

5.2 Diluents for general use

5.2.1 Peptone salt solution

See ISO 6887-1:1999, 5.2.1.

5.2.2 Buffered peptone water

See ISO 6887-1:1999, 5.2.2.

5.3 Diluents for special purposes

5.3.1 Peptone salt solution with Bromocresol purple

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5.3.1.1 Composition

Peptone salt solution (5.2.1)	1 000 ml
Bromocresol purple (0,04 % alcohol solution, e.g. ethanol solution)	0,1 ml

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5.3.1.2 Preparation

Add 0,1 ml of Bromocresol purple to 1 000 ml of peptone salt solution (5.2.1).

5.3.1.3 Application

This solution may be used for the analysis of certain acidic products so that adjustment of the pH can be carried out without the use of a sterile pH probe (see 8.2).

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

5.3.2 Phosphate buffered solution

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

5.3.2.2 Preparation

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

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

Place 180 ml into each flask.

Sterilize for 15 min in the autoclave set at 121 °C.

5.3.2.3 Application

Phosphate buffered solution is used as a diluent for gelatine (see 9.3) and other samples.

5.4 Distribution and sterilization of the diluent

See ISO 6887-1:1999, 5.4.

6 Apparatus

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

6.1 Homogenizer

6.1.1 **Rotary homogenizer** (blender)

See ISO 7218. If a large test sample is used, the equipment should include a sterile 1 litre bowl.

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6.1.2 Peristaltic homogenizer

See ISO 7218.

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6.2 Domestic-type grater, sterile.

6.3 Hammer

6.4 Water baths, capable of being maintained at 45 ± 1 °C, or 40 ± 1 °C, or between 37 °C and 42 °C.

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

6.6 Sterile spatulas, spoons or **scoops**

6.7 Sterile corers (metallic probes), for taking samples at depth.

6.8 Stirring apparatus, with to-and-fro motion.

6.9 Wide-necked flasks, sterile, of capacity 500 ml.

7 Preparation of samples

7.1 Frozen products

Products stored frozen should be brought to a consistency that allows sampling; i.e. storing at 18 °C to 27 °C (laboratory temperature) for a maximum of 3 h, or 2 °C ± 2 °C for a maximum of 24 h. Samples should be tested as quickly as possible after that. See ISO 6887-1:1999, 9.3.

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

Powders should be well mixed in their containers before sampling.

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7.2 Hard and dry products

For hard or dry products, do not homogenize in a rotary homogenizer (6.1.1) 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

Before analysing, the test sample should be taken after having shaken the laboratory sample by hand (e.g. 25 times through an arc of 25 cm; see ISO 8261 for details) or by mechanical means in order to ensure that the microorganisms are uniformly distributed.

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 the sampling of an homogenized 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.

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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 analysis if it is different from the procedure described in this part of ISO 6887.

8.2 General case for acidic products

It is important when using a suspension solution of acidic products to ensure that the pH is brought back to neutral. The use of diluent with an added pH indicator (5.3.1) can avoid the need to use and sterilize pH probes: add sodium hydroxide (NaOH) to bring back the coloration of the suspension until the indicator starts to change.

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 is 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 + 9 with diluent.

8.3 High fat foods, excluding margarines and spreads (e.g. over 20 % of total mass is fat)

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

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