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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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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.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 6887-1:1999), which has been technically revised.

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

Introduction

Because of the large variety of food and animal feed products, this horizontal method might not be appropriate in every detail for certain products. In this case, different methods which are specific to these products can be used if absolutely necessary for justified technical reasons.

When this document 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 document so that eventually, the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

This document 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 document.

Any special diluents or practices required for particular products or microorganisms in specific standard methods take priority over the general rules listed in the ISO 6887 series. These can include the following:

— 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 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 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 document is applicable to the general case and other parts apply to specific groups of products as mentioned in the foreword. Some aspects might also be applicable to molecular methods where matrices can be associated with inhibition of the PCR steps and consequently affect the test result.

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

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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 http://www.iso.org/obp

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

[SOURCE: ISO 7002:1986, A.19]

3.2
composite sample
mixed sample of a number of the same items of food, animal feed, animals or environment, from which a test portion is taken for examination in the laboratory

Note 1 to entry: See illustration of a composite sample in Annex A.

3.3
pooled sample
mixed sample of a number of the same items 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 1 to entry: See illustration of a pooled sample in Annex A.

3.4
test sample
sample prepared from the *laboratory sample* (3.1) according to the procedure specified in the method of test and from which *test portions* (3.5) are taken

Note 1 to entry: Preparation of the laboratory sample before the test portion is taken is infrequently used in microbiological examinations.

[SOURCE: ISO 7002:1986, A.47]

3.5
test portion
measured (volume or mass) representative sample taken from the *laboratory sample* (3.1) for use in the preparation of the *initial suspension* (3.6)

Note 1 to entry: Sometimes preparation of the *laboratory sample* (3.1) 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 to entry: Nine-fold quantities of diluent are normally used to produce a decimal dilution series, but other ratios may be required for specific purposes, such as to enumerate low numbers.

3.7
further dilution
suspension or solution 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 to entry: Ten-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 the same items of food, animal feed, animals or environment, where the complete mixture is the test portion examined

Note 1 to entry: 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 the same items of food, animal feed, animals or environment, from which specified volumes are combined for further examination

Note 1 to entry: See illustration of pooled (pre-)enriched test portions in Annex A.

3.10
specific standard
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 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).

For more detailed rules on preparation and performance testing of culture media, see ISO 11133.

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

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 ± 0,2 at 25 °C.

5.2.2 Buffered peptone water

5.2.2.1 Composition

Peptone[a]	10,0 g
Sodium chloride	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)[b] ‡	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4) ‡	1,5 g
Water	1 000 ml

[a] For example, enzymatic digest of casein.

[b] If disodium hydrogen phosphate with a different water content is used, amend the mass of the ingredient accordingly. For example, in case of anhydrous disodium hydrogen phosphate (Na_2HPO_4), use 3,57 g.

‡ Buffer ingredients, see 5.2.3.

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 ± 0,2 at 25 °C.

5.2.3 Double-strength buffered peptone water

This diluent may be necessary for high acid samples (see 8.6) and is prepared by dissolving double the quantities of a complete dehydrated medium in 1 000 ml of water and processing in the same manner.

If the diluent is prepared from individual ingredients, only double the quantities of the two buffer ingredients (marked ‡) are required.

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.

The tolerance allowable on final diluent volumes, after sterilization, shall not exceed ±2 %.

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 °C ± 3 °C for 15 min (see ISO 7218).

After autoclaving, check that the volumes from a proportion of the batch of diluent prepared are within the permitted tolerance of ±2 %. This may be achieved either destructively by emptying the contents

of the vessels into a tared container after autoclaving or non-destructively by marking and weighing vessels positioned through the autoclave both before and after autoclaving. For small batches of less than 100 units, check at least one unit; for larger batches, check 3 % to 5 % by either method.

To ensure diluent volumes meet the permitted tolerance, autoclaving bulk volumes and dispensing the required amounts into sterile vessels aseptically may also be used.

5.5 Performance testing for diluents

Test all diluents before use, according to Table 1, by the method given in ISO 11133.

The productivity target for diluents requires that the number of colonies counted after the specified incubation time at laboratory ambient temperature (18 °C to 27 °C) shall be within ±30 % of the number counted initially.

Table 1 — Test microorganisms and productivity criterion for diluents

Media	Incubation	Test microorganisms	WDCM number[a]	Reference medium	Control method	Criteria
Peptone salt diluent Buffered peptone water (single and double strength) Peptone solution Phosphate buffered diluent	45 min to 1 h at laboratory ambient temperature (18 °C to 27 °C)	*Escherichia coli*[c] *Staphylococcus aureus*	00012 or 00013 00034[b]	TSA	Quantitative	±30 % of original count

[a] Refer to the reference strain catalogue available at http://www.wfcc.info for information on culture collection strain numbers and contact details.

[b] Strain to be used as a minimum.

[c] Free choice of strain; one of the strains must be used as a minimum.

6 Apparatus

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) and wet sterilization (autoclave), see ISO 7218.

6.2 Homogenizer.

6.2.1 Rotary homogenizer (blender).

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

6.2.2 Peristaltic homogenizer.

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

6.3 Mechanical stirrer, see ISO 7218.

6.4 Flasks, test tubes or screw-cap bottles, of appropriate capacities.

6.5 Total-delivery graduated pipettes, of nominal capacities 1 ml and 10 ml, graduated in 0,1 ml and 0,5 ml divisions, respectively. Mechanical pipettors of suitable accuracy may also be used (see ISO 7218).

6.6 pH-meter, capable of being read to the nearest 0,01 pH unit at 25 °C, enabling measurements to be made which are accurate to ±0,1 pH unit (see ISO 7218).

6.7 Balances and gravimetric diluters, capable of weighing to 1 % of the mass (see ISO 7218).

6.8 Sterile sample trays, of appropriate dimensions.

6.9 Sterile scissors, forceps or tongs, straight scalpels or **knives**, and **spatulas**.

6.10 Special opening equipment, such as bottle and can openers.

6.11 Equipment for collecting test portions from frozen laboratory samples.

6.11.1 Variable speed electric drill, with maximum speed in use of 900 r/min, or **hand drill**.

6.11.2 Sterile wood bit for electric drill, of 14 mm or 16 mm diameter.

6.11.3 Sterile wood chisel, of 20 mm width, and **hammer** or **plastic mallet**.

6.11.4 Other apparatus that does not cause overheating or contamination of the sample.

6.11.5 Equipment for cauterization of sample surfaces, e.g. portable gas blowtorch.

6.11.6 Template for surface sampling, metallic frame of appropriate dimensions to delineate the surface to be sampled, sterilized by flaming after immersion in 70 % (volume fraction) alcohol.

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

6.13 Sterile wide-necked bowls, containers or **plastic bags**, of 500 ml capacity.

6.14 Sterile glass beads or **balls**, to disperse samples such as swabs.

7 Sampling

Carry out sampling in accordance with the specific standard appropriate to the product concerned or see ISO/TS 17728. If specific sampling instructions are not available, it is recommended that agreement be reached on this subject by the parties concerned.

Some guidance is given in other parts of ISO 6887 on sampling specific to certain products (see, for example, ISO 6887-3).

8 Preparation of samples

8.1 General

Requirements for the different general categories of products subjected to microbiological examination are given in this clause. For specific requirements on certain product types, see the appropriate part of ISO 6887 for further information.

In other cases, see the specific standard appropriate to the product concerned. If such a specific standard is not available, it is recommended that agreement be reached on this subject by the parties concerned.

