INTERNATIONAL STANDARD

ISO 6887-1

First edition
1999-02-15

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

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

Reference numbe
ISO 6887-1:199 (E)

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 3.

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.

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

This first edition of ISO 6887-1 cancels and replaces ISO 6887:1983.

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 for microbiological examination*

— *Part 2: Specific rules for the preparation of test samples and initial suspension*

Part 2 will probably be divided into several parts, for specific products such as meat, milk, fish and other products.

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

Printed in Switzerland

Introduction

Because of the large variety of food and 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 group 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 the initial suspension and of decimal dilutions for microbiological examination. Part 2 of ISO 6887 (under preparation) will specify specific rules for the preparation of the test sample and of the initial suspension, taking into account the variety of food and feed products 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 a dry product, a highly viscous product), or the presence of inhibitory substances (such as spices, salted fishes), or the acidity, etc.

It is recommended that, whilst waiting for the publication of part 2, any special diluents or practices specified for particular products in an appropriate specific standard be used in the preparation of the initial suspension. This may include:

— adjustment of the pH of a food suspension to neutrality;

— the use of buffered peptone water, and no other diluent, for products with high inhibitory effect, or products containing microorganisms that have been stressed (e.g. acidic pH);

— 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);

— the use of surface-active agents for high-fat foods.

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

1 Scope

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

This part of ISO 6887 is applicable to the general case, except for products mentioned in ISO 6887-2.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 6887. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreements based on this part of ISO 6887 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Definitions

For the purposes of this part of ISO 6887, the following definitions apply.

3.1
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 a nine-fold quantity of diluent, allowing large particles, if present, to settle

NOTE See clause 5 and notes 1 and 2 of 9.1.

3.2
further decimal dilutions
suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.1) with a ninefold 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

3.3
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 (3.1) in such a way as to obtain as uniform a distribution as possible of the microorganisms contained in the test portion.

Preparation, if necessary, of decimal dilutions (3.2) 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 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

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluent, dehydrated basic components or a dehydrated complete preparation should be used. The manufacturer's instructions shall be rigorously followed.

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

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

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, by heating if necessary.

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

5.2.2 Buffered peptone water

5.2.2.1 Composition

Enzymatic digest of animal tissues	10,0 g
Sodium chloride	5,0 g
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

5.2.2.2 Preparation

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

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

5.3 Diluents for special purposes

See the specific standard appropriate to the product concerned.

NOTE ISO 6887-2 (under preparation) will give specific rules.

5.4 Distribution and sterilization of the diluent

Dispense the diluent (5.2 or 5.3) in volumes as necessary for the preparation of the initial suspensions into flasks (6.4) of appropriate capacity.

Dispense the diluent (5.2 or 5.3) in volumes as necessary for the preparation of the decimal dilutions into test tubes (6.5) or flasks (6.4) in quantities such that, after sterilization, each tube or flask contains 9,0 ml. The uncertainty of measurement of this final volume, after sterilization, shall not exceed ± 2 %.

NOTE If it is intended to count 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; the size of the flasks (6.4) and test tubes (6.5) being specified accordingly.

Stopper the tubes or flasks.

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

6 Apparatus and glassware

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 Blending equipment

See ISO 7218.

6.3 Mechanical stirrer

See ISO 7218.

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

6.5 Test tubes, of appropriate capacities.

6.6 Total-delivery graduated pipettes, of nominal capacities 1 ml and 10 ml, graduated in 0,1 ml and 0,5 ml divisions respectively.

6.7 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.

6.8 Balance, capable of weighing to the nearest 0,01 g.

7 Sampling

Carry out sampling in accordance with 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.

8 Preparation of test sample

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.

9 Procedure

9.1 Test portion and initial suspension (primary dilution)

Into a sterile bowl or sterile plastic bag, weigh, to a measurement uncertainty of ± 5 %, a mass m g or measure, to a measurement uncertainty of ± 5 %, a volume of V ml (minimum 10 g or 10 ml, unless otherwise stated) representative of the test sample (see clause 8).

Add a quantity of diluent equal to $9 \times m$ g or $9 \times V$ ml. This quantity can be measured preferably by mass with a measurement uncertainty of ± 5 % or by volume with a measurement uncertainty of ± 5 %.

NOTE 1 It may be necessary, in certain cases, particularly for products giving an initial 1 + 9 suspension which is too viscous or too thick, to add more diluent. This should be taken into account for subsequent operations and/or in the expression of results.

NOTE 2 This primary dilution partly conditions the value of the lower limit of enumeration, which also depends on the technique used (for example, pour-plate technique with a 1 ml inoculum of a 1/10 suspension, for which the limit is 10 microorganisms per gram). If it is necessary, for some enumerations in certain products, to fall below this limit, it is possible to use a smaller volume of diluent[1)]. It should be noted that the inoculation of this initial suspension may result in difficulties due to inbalance in the inoculum/medium ratio (inhibition of the microbial growth by the increased concentration of the food components).

To avoid damage to the microorganisms by sudden changes in temperature, the temperature of the diluent during the operations given below shall be approximately the same as the ambient temperature, except for particular products (see specific standard).

Homogenize the mixture according to the recommendations of ISO 7218.

Allow large particles to settle, if necessary, for up to 15 min. Filtration systems giving equivalent results can be used.

[1)] In this case, the volume of diluent used should be reported in the test report.

In the case of enumeration of spores, a heat-treatment of the initial suspension, for example 10 min at 80 °C, shall be performed immediately after its preparation, followed by a quick cooling.

9.2 Further decimal dilutions

Transfer, by means of a pipette, 1 ml of the initial suspension with an uncertainty of measurement[2)] of ± 5 %, into a tube containing 9 ml of sterile diluent at the appropriate temperature.

NOTE If a larger volume is necessary, it is possible to add a determined volume (more than 1 ml) of the initial suspension, with an uncertainty of measurement of ± 5 %, into a tube containing the nine-fold volume of sterile diluent.

For optimal precision, do not introduce the pipette more than 1 cm into the initial suspension.

Avoid any contact between the pipette containing the inoculum and the sterile diluent.

Mix thoroughly, preferably by using a mechanical stirrer (6.3) for 5 s to 10 s, to obtain a 10^{-2} dilution.

If necessary repeat these operations using the 10^{-2} and further dilutions by using at each dilution a new sterile pipette to obtain 10^{-3}, 10^{-4}, etc., dilutions, until the appropriate number of microorganisms has been obtained (see clause 4).

9.3 Duration of the procedure

The time lapse between the end of the preparation of the initial suspension and the instant when the inoculum comes into contact with the culture medium shall not exceed 45 min while limiting to 30 min the lapsed time between the preparation of the initial suspension (9.1) and the beginning of preparation of the following decimal dilutions, unless otherwise specified in the specific International Standard.

NOTE If the ambient temperature of the laboratory is too high, these two maximum durations should be reduced.

2) The retained measuring uncertainty of ± 5 % takes into account the present limits of currently used pipettes.