International Standard 7667

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Microbiology — Standard layout for methods of microbiological examination

Microbiologie — Plan normalisé pour les méthodes d'examen microbiologique

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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 developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been authorized 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 7667 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in November 1982.

It has been approved by the member bodies of the following countries:

Australia	Iran	Portugal
Austria	Iraq	Romania
Brazil	Israel	South Africa, Rep. of
Canada	Kenya	Spain
Chile	Korea, Dem. P. Rep. of	Thailand
Czechoslovakia	Malaysia	Turkey
Egypt, Arab Rep. of	Mexico	United Kingdom
Ethiopia	Netherlands	USA
France	New Zealand	USSR
Germany, F. R.	Peru	Venezuela
Hungary	Philippines	Yugoslavia
India	Poland	

No member body expressed disapproval of the document.

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Microbiology — Standard layout for methods of microbiological examination

Introduction

This standard layout has been prepared taking into account published International Standards dealing with methods for the microbiological examination of agricultural food products, and the experience gained in the preparation of these International Standards.

It should always be remembered, in making use of this layout, that it is for guidance only. It should be adapted to suit any special requirements.

The adoption of a standard form of layout and drafting ensures

— that no important point is overlooked in the preparation of the standard;

— that the various items of information to be included in the standard are always given in the same order;

— that any desired clause may be found rapidly, whatever the origin or scope of the standard.

(This is important particularly if a partial translation of a text is being studied or two texts are being compared.)

In drafting methods of microbiological examination, the subjects should be dealt with in the order shown in the standard layout but any clause or subclause which may be unnecessary in a particular case should be omitted and others, if required, should be added in the most appropriate places.

Scope and field of application

This International Standard establishes a standard layout for methods of microbiological examination. It is intended primarily for the drafting of methods for the microbiological examination of agricultural food products, but it is equally applicable for all other products for which microbiological examinations may be required.

Standard layout

Clause number

Title

0 Introduction

1 Scope and field of application

2 References

3 Definitions

4 Principle and reactions

5 Diluents, culture media, reagents and other products

6 Apparatus and glassware

7 Sampling

8 Preparation of test sample

9 Procedure

NOTE — In this clause, the relevant subclauses shall be given in the following order:

- Test portion, initial suspension and dilutions
- Liquid-medium method
  - Inoculation and incubation
  - Interpretation
  - Confirmation
- Solid-medium method
  - Inoculation and incubation
  - Interpretation
  - Confirmation

10 Expression of results

Liquid-medium method

Solid-medium method

11 Precision

12 Test report

Annexes

Rules for drafting individual elements and clauses

NOTE — The following rules supplement those laid down in parts 2 and 3 of the Directives for the technical work of ISO.

0.1 Title

The title of the International Standard shall state briefly and without ambiguity:

a) in the case of a specific standard [1], the type of product to which the method is applicable;

b) the category(ies) of micro-organisms concerned and whether the method is for their detection or enumeration;

c) if considered useful, the temperature(s) of incubation and, in the case of counting, the technique used.

0.2 Introduction

The introduction (clause 0), if any, may give information about the method, the reason prompting elaboration of the International Standard, and background information.

0.3 Safety warnings

If there is a danger, for example of toxicity or contamination, requiring special precautions, these shall be stated at the beginning of the introduction in the form of a **Warning**; such warnings are printed in bold characters in International Standards.

NOTE — It may be necessary in certain cases to draw attention to the need to comply with relevant national regulations.

Warnings may also be included at appropriate places in the text where it is necessary to emphasize a particular danger.

If necessary, further information on safety measures to be taken may be given in an annex.

1 Scope and field of application

This clause shall state succinctly the aim of the International Standard and specify the products to which it applies.

It shall contain, in particular, additional, but useful, information which could not be included in the title (for example, not only *"Clostridium perfringens"*, but also "vegetative forms and spores").

It shall give all the information required to enable the user to determine rapidly whether the method is applicable to the products he is to analyse, without restriction or under certain conditions.

It shall indicate, in particular, the type of products for which the method is applicable and the limits between which it can be applied without modification. These limits shall take into account the presence of other micro-organisms in the product, and also any restrictive properties of the product or medium.

NOTE — It is sometimes necessary to specify several methods of detection or enumeration in one International Standard, for example, for enumeration, the MPN method for low contents and the colony count technique for high contents. In this case, a clear distinction should be made between the fields of application of each of the methods specified. In other cases, where the methods are specified in different International Standards, it may be useful to make reference to them in a note.

2 References

This clause shall contain a complete list of all the documents necessary for the use of the International Standard.

Example: For general guidance for the preparation of dilutions:

ISO 6887, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

NOTE — This list is not intended for documents serving only as reference material during the preparation of the International Standard; such documents may be quoted in the clause or sub-clause concerned, or a list of these documents may be given in a bibliography (see clause 14).

3 Definitions

If required to facilitate the understanding of the text, definitions of the basic terms used in the International Standard shall be given in this clause.

The micro-organisms concerned shall be defined according to the methods used and the confirmatory procedures described.

4 Principle and reactions

This clause shall indicate the basic stages of the method, and also the basic ideas, the properties used and, where necessary, the reasons behind the choice of certain procedures.

If necessary, basic chemical or biochemical reactions may be described.

NOTE — This clause does not constitute a summary of the International Standard and cross-references are not necessary.

5 Diluents, culture media, reagents and other products

5.1 Title

The title of this clause shall mention the different categories of products used.

Example: For *Salmonella*: Culture media, reagents and sera.

1) For the definition of this term, see ISO 6887, *Microbiology — General guidance for the preparation of dilutions for microbiological analysis.*

5.2 Numbering

Each of the diluents, culture media, reagents and other products shall be designated by a number corresponding to the subclause in which it is described; by repeating this subclause number, in parentheses, in the clause "Procedure", following mention of the diluent, culture medium, reagent, or other product, and thus referring back to the subclause concerned, repetition of all the features of the product in question is avoided, thus reducing the wording.

If a medium or reagent is used more than once in the procedure, its full composition shall be given in the clause corresponding to the part of the procedure in which it is used for the first time. In clauses where subsequent use is specified, a reference to the previous full description will suffice.

5.3 Listing

5.3.1 In general, the clause shall begin with a subclause "Basic material", numbered 5.1, and worded as follows:

> "In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluents and culture media, dehydrated basic components or complete dehydrated media be used. Similarly, commercially prepared reagents may also be used. The manufacturer's instructions shall be rigorously followed.
>
> The chemical products used for the preparation of the culture media and the reagents shall be of recognized analytical quality.
>
> The water used shall be distilled or deionized water, free from substances that might inhibit the growth of micro-organisms under the test conditions.
>
> Measurements of pH shall be made using a pH meter, measurements being referred to a temperature of 25 °C.
>
> If the prepared culture media are not used immediately, they shall, unless otherwise stated, be stored in the dark at approximately 4 °C, for no longer than 1 month, in conditions which do not produce any change in their composition."

5.3.2 This standard introduction shall be followed by subclauses (5.2., 5.3, etc.) describing all the diluents, culture media, reagents and other products used during the test, together with their concentrations and, if necessary, their degrees of purity, according to the specific use, in the order in which they are referred to in clause 9 "Procedure".

This means, for example, that if a diluent is used, it shall be mentioned first. If a stage of the procedure (for example an identification reaction) requires the use of more than one medium, or of both media and reagents, these shall appear under the same heading sub-divided into two or more sections (in this case, do not make a general division in the International Standard for one subclause "Media" and another "Reagents").

5.4 Composition

The constituents of the culture media shall be listed with their proportions in tabular form, preferably in the following order: sources of protein, carbohydrates, salts, selective substances, other constituents, agar (or other setting agent), water. Chemical names approved by the International Union of Pure and Applied Chemistry shall be used with any common name in parentheses.

If a culture medium comprises a basic medium to which sterile or sterilized constituents are added separately, it shall be described in the following order:

a) basic medium (with list of basic constituents only);

b) solutions to be added;

c) complete medium.

The concentrations of the complete medium (or basic medium) shall be expressed in grams per litre, and those of the solutions to be added [b)] should, preferably, be expressed in grams per 100 ml. Different volumes may be prepared, depending on the examination to be carried out, provided that the concentrations are as specified.

In the description of the complete medium [c)], the quantities (volumes) of the basic medium and of the solutions to be added in order to obtain the complete medium at the required concentrations shall be given.

The list of the constituents of the medium (or basic medium or of a solution), shall be immediately followed with details regarding its preparation (see the note); for example

— the pH after sterilization;

— conditions of sterilization;

— where necessary containers to be used for storage (U-tubes, Petri dishes);

— precautions to be taken for conservation (duration, temperature, absence of light, etc.).

If a constituent is required to satisfy a specification (for example, brilliant green at the minimum concentration for the control of spreading by *Proteus*), a description shall be given of the corresponding procedure.

Methods for keeping reference strains and for their culture (for example in the case of a method for phage typing) shall also be described in this clause together with the composition of the corresponding culture medium.

NOTE — International Standards giving general guidance recommend that dehydrated basic constituents or complete dehydrated media are preferably used.

However, the preparation of diluents and media should be given when commercially available complete media are not available.

In addition, when a large number of media and reagents is required (for example, as in the case of ISO 6579[1]), it is possible, in order to reduce the wording, to give only a list of the products in this clause and to place their description and preparation in an annex.

1) ISO 6579, *Microbiology — General guidance on methods for the detection of* Salmonella.

6 Apparatus and glassware

This clause shall be introduced with the following phrase:

"Usual microbiological laboratory equipment and"

The clause shall then list the glassware and special apparatus used, with the exception of usual laboratory equipment. Mention may, however, be made of the latter in these terms or by means of the expression "standard laboratory apparatus".

If necessary, it shall make reference to apparatus forming the subjects of International Standards prepared by ISO/TC 48, *Laboratory glassware and related apparatus*, or standardized by other ISO Technical Committees.[1] If the apparatus or glassware is subjected to any special or unusual preliminary treatment, this operation shall also be mentioned in this clause.

Example: Sterilization of glassware (operation not specified for chemical analysis).

The characteristics (for example dimensions, accuracy of measurement) of apparatus which is not in normal use shall be given in this clause, especially those with significant effects on the execution or accuracy of the method.

Each item of apparatus and glassware shall be described in a separately numbered subclause; by repeating this subclause number, in parentheses, in the clause "Procedure" and thus referring back to the subclause concerned, repetition of all the features of the apparatus or glassware concerned is avoided, thus reducing the wording.

Diagrams showing special types of apparatus and the specified assembly are recommended. These diagrams shall be prepared in accordance with the International Standards prepared by ISO/TC 10, *Technical drawings,* in as far as they are applicable.

7 Sampling

Sampling is, in principle, independent of the examination itself and it is generally sufficient to refer to an International Standard giving general guidance on sampling, or to the International Standard specifying sampling, or to the corresponding clause of the product standard. If there is no such text, this clause may specify a plan and sampling procedure, taking into account the recommendations drawn up for the terminology and application of statistics in this field by ISO/TC 69, *Applications of statistical methods,* and the information given in ISO 7002.[2]

If necessary, this clause should also give useful instructions concerning the mass or the volume of the laboratory sample, or the number of units to be sampled, the characteristics of the container to be used for storage and the conditions for storage and transport to be applied.

8 Preparation of the test sample

8.1 In the case of a specific Standard[3] for a product or group of products, this clause shall give all the information necessary for the preparation of the test sample from which the test portions will be taken (for example chopping and mixing of meat in a mincer).

The information given shall, in all cases, specify the operations to be carried out.

This clause shall also give full information concerning the features of the sample thus prepared and, if necessary, of the containers to be used for storing it; it shall also specify the conditions for storage.

If there is, for a group of products, a particular document dealing with the preparation of the test sample, this document may be referred to (for example, "Meats and meat-based products — Preparation of a laboratory sample for microbiological examination").

8.2 In the case of an International Standard giving general guidance, this clause shall include the following text:

"Refer to the specific Standard. If there is no specific Standard, it is recommended that the parties concerned come to an agreement on this subject."

9 Procedure

This clause may be divided into as many subclauses as there are separate operations or series of operations to be carried out.

Each of these operations shall be accurately described in the chronological order of execution using the imperative mood in the English text and the infinitive in the French text.

It is recommended that subclauses containing several operations be subdivided, each subdivision corresponding to a given operation, including all the essential preliminary operations.

If the method to be described has already appeared in another International Standard, state: "use the method described in ISO ..." adding, if necessary, an instruction regarding the modifications which may be required.

If it is necessary to carry out a check of the apparatus (for example, to check the temperature of a water bath, or the anaerobiosis of a jar) or to carry out controls or other checks on the procedure by using selected strains of micro-organisms or any other biochemical systems, all the details necessary for carrying out this check shall be given in this clause.

If modifications (for example, the neutralization of an acid sample or resuscitation of damaged micro-organisms) are required to the procedure specified in this clause, they shall be given in an annex with a cross-reference at the appropriate place in the procedure.

1) Such references are not included in clause 2 "References", however, because it is not essential to consult them in order to carry out the specified procedure.

2) ISO 7002, *Agricultural food products — Layout for a standard method of sampling from a lot*. (At present at the stage of draft.)

3) This term is defined in ISO 6887.

9.1 Test portion, initial suspension and dilutions

9.1.1 In the case of a specific Standard for a product or group of products, this subclause shall refer to ISO 6887, supplementing the information given therein and, if necessary, amending it to suit the products concerned.

As a guide, this information may refer to the following points :

— the method of taking the test portion from the test sample (see clause 8), or, if it is identical, from the laboratory sample (see clause 7);

— the procedure for weighing or measuring the test portion, the accuracy with which this shall be carried out, and any other useful information;

— the method(s) for the preparation of the initial suspension and dilutions, the quantity of diluent used for the initial suspension, frequency of rotation at which a blender is operated, inoculation and mixing in the tubes with the diluent, any heating of the initial suspension and dilutions;

— the duration of the operations;

— the repetition of the operations for the preparation of the initial suspension and dilutions.

NOTE — If a special technique is required, this may be described in an annex.

9.1.2 in the case of an International Standard giving general guidance, this subclause shall include the following text:

"Refer to ISO 6887 and to the specific Standard. If there is no specific Standard, it is recommended that the parties concerned come to an agreement on this subject."

9.2 Liquid-medium method

This subclause shall describe the methods to be used when the presence or absence of a given micro-organism in an inoculated liquid-medium is to be demonstrated (enrichment).

This detection may be carried out using a single dilution (detection of presence or absence) or a series of dilutions (calculation of MPN).

The subclause shall be subdivided as follows.

9.2.1 Inoculation and incubation

This subclause shall give details concerning the inoculation of the pre-enrichment medium and/or the enrichment medium, with the initial suspension and/or dilutions, together with the temperature, duration and other conditions of incubation (for example anaerobiosis). The number of inoculations per dilution shall be specified, as well as the number of dilutions used.

If the inoculated tubes are to be heated, details of this operation shall be described.

9.2.2 Interpretation

This subclause shall give information relating to the interpretation of the reactions observed in the liquid media.

In addition, it shall give information concerning subsequent stages to be carried out if the reactions observed do not give conclusive proof as to the presence of the micro-organisms to be detected.

It shall describe the initial stages for confirmation, for example inoculation in a selective liquid medium or on a selective solid medium.

9.2.3 Confirmation

This subclause shall describe the subsequent stages intended to confirm the presence of the micro-organisms to be detected, in the flasks, bottles or tubes of enrichment medium. These stages may include the selection and purification of colonies, the inoculation of these colonies onto other, selective or non-selective, media, serological examination, phage typing, etc.

Details of all the biochemical and serological reactions required for the identification of the cultures shall be given.

NOTE — If possible, the order of the media and reactions should be the same as in clause 5 "Diluents, culture media, reagents and other products".

If necessary, a table shall be given summarizing the reactions of the micro-organism(s) to the tests performed.

Example:

Fermentation of glucose	+
Fermentation of lactose	−
Decarboxylation of lysine	+

9.3 Solid-medium method

This subclause shall describe the method of enumeration by colony counting on a solid medium.

It shall be subdivided as follows.

9.3.1 Inoculation and incubation

This subclause shall describe the inoculation, using the initial suspension and dilutions, of a solid, selective or non-selective, medium, which is already present or which is to be added later to Petri dishes, bottles and tubes. It shall also give the temperature, time and conditions of incubation.

Supplementary information concerning the preparation of agar plates, including pre-drying before inoculation, mixing with the inoculum, or layering with agar after inoculation, shall also be given.

9.3.2 Interpretation

This subclause shall give information concerning the nature of the colonies to be counted, the counting itself, and the measures to be taken when counting proves difficult (for example in the case of excessive growth). In addition, it shall specify an upper and a lower limit for the number of colonies to be counted.

If some of the colonies thus enumerated are to be confirmed, instructions shall be given concerning the selection and purification of the colonies. The initial stages of confirmation shall also be given in this subclause.

9.3.3 Confirmation

This subclause shall describe the subsequent stages intended to confirm that a representative part of the colonies enumerated shows the properties of the micro-organisms to be detected.

It shall list all the biochemical reactions and the microscopic examinations necessary to identify suspect colonies.

NOTE — If possible, the order of the media and reactions shall be the same as in clause 5 "Diluents, culture media, reagents and other products".

10 Expression of results

10.1 Liquid-medium method

If a single dilution or the initial suspension is used for inoculation, this subclause shall describe concisely the method of reporting the presence or absence of micro-organisms, taking into account the quantity of product examined and the number of cultures confirmed.

If more than one dilution has been used, calculation of the most probable number (MPN) of the micro-organisms to be detected shall be described on the basis of the number of tubes confirmed, using a most probable number (MPN) table with statistical limits given in an annex.

It may be desirable to add some information regarding the choice of one or more media, to specify if serological typing or phage typing has been carried out, etc.

It shall also indicate how the average is to be taken if the test has been repeated.

10.2 Solid-medium method

This subclause shall indicate how to calculate the number of micro-organisms present in the product from the number of colonies if a non-selective medium is used or from the percentage of confirmed colonies in the case of a selective medium.

It shall also indicate how the average is to be taken if the test has been repeated.

11 Precision

This clause shall give values for the repeatability and reproducibility of the method if such data are available. If not, any other information relevant to the precision or accuracy of the method may be included.

12 Test report

This clause shall require the analyst to give all the information required for the identification of the sample, the reference of the method used, the results obtained and the form in which they are expressed, to report any particular details noted during the test, and to report all optional operations which may have had an influence on the results.

The following standard wording is suggested :

> "The test report shall show the method used and the results obtained, indicating clearly the method of expression used. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.
>
> The test report shall include all the information necessary for the complete identification of the sample."

13 Annexes

13.1 The annexes may be either

a) integral parts of the International Standard, for example calculation tables for the MPN, preparation and/or specification of reagents, which have been placed after the main text for practical reasons, or

b) parts giving supplementary information, for example a diagram of the procedure or more comprehensive information regarding the safety measures to be taken, or

c) modifications for special cases.

Whether the annex belongs to category a), b) or c) shall be clearly shown by unambiguous wording; if necessary, a full explanation shall be given in the introduction.

13.2 If there is more than one annex, they shall be designated by the capital letters of the alphabet, beginning with A. The letters "I", "O" and "X" shall not be used in this connection. The word "Annex", followed by the letter designating its serial order, shall be placed above the title. Numbers given to the divisions and subdivisions of an annex shall be preceded by the letter assigned to that annex.

A single annex is not designated by a letter. However, numbers given to the divisions and subdivisions of a single annex shall be preceded by the letter A to distinguish them for reference purposes from numbers used elsewhere in the International Standard.

13.3 Each annex shall have a title.

14 Bibliographic references

If it is desired to give bibliographic references, these shall constitute a final element entitled "Bibliography".