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Microbiology of the food chain — Horizontal method for the enumeration of microorganisms —

Part 1: Colony count at 30 °C by the pour plate technique

Microbiologie de la chaîne alimentaire — Méthode horizontale pour le dénombrement des micro-organismes —

Partie 1: Comptage des colonies à 30 °C par la technique d'ensemencement en profondeur

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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, www.iso.org/directives.

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

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

This first edition, together with ISO 4833-2, cancels and replaces ISO 4833:2003.

ISO 4833 consists of the following parts, under the general title *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms*:

— *Part 1: Colony count at 30 °C by the pour plate technique*

— *Part 2: Colony count at 30 °C by the surface plating technique*

Microbiology of the food chain — Horizontal method for the enumeration of microorganisms —

Part 1:
Colony count at 30 °C by the pour plate technique

1 Scope

This part of ISO 4833 specifies a horizontal method for enumeration of microorganisms that are able to grow and form colonies in a solid medium after aerobic incubation at 30 °C. The method is applicable to:

a) products intended for human consumption and for animal feed;

b) environmental samples in the area of food and feed production and handling.

This part of ISO 4833 is applicable to:

1) products that require a reliable count when a low limit of detection is specified (below 10^2/g or 10^2/ml for liquid samples or below 10^3/g for solid samples);

2) products expected to contain spreading colonies that obscure colonies of other organisms, e.g. milk and milk products likely to contain spreading *Bacillus* spp.

The applicability of this part of ISO 4833 to the examination of certain fermented food and animal feeds is limited and other media or incubation conditions can be more appropriate. However, this method can be applied to such products even though it is possible that the predominant microorganisms in those products are not detected effectively.

For some matrices, the method specified in this part of ISO 4833 can give different results to those obtained using the method specified in ISO 4833-2.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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.

3.1
microorganism
entity of microscopic size, encompassing bacteria, fungi, protozoa and viruses

[SOURCE: ISO/TS 11139:2006,[3] 2.26]

Note 1 to entry: For the purposes of this part of ISO 4833, microorganisms are bacteria, yeasts and moulds that are able to produce colonies under the conditions specified in this part of ISO 4833.

4 Principle

A specified quantity of the liquid test sample, or a specified quantity of an initial suspension in the case of other products, is dispensed into an empty Petri dish and mixed with a specified molten agar culture medium to form a poured plate.

Other plates are prepared under the same conditions using decimal dilutions of the test sample or of the initial suspension.

The plates are incubated under aerobic conditions at 30 °C for 72 h.

The number of microorganisms per gram or per millilitre of the test sample is calculated from the number of colonies obtained in the plates containing fewer than 300 colonies.

5 Culture media and diluents

5.1 General

Follow ISO 11133 for preparation, production and performance testing of culture media.

5.2 Diluents

Use the diluent(s) specified in ISO 6887 for the product concerned or the specific International Standard dealing with the product under examination.

5.3 **Agar medium**: plate count agar (PCA)

5.3.1 Composition

Enzymatic digestion of casein	5,0 g
Yeast extract	2,5 g
Glucose, anhydrous ($C_6H_{12}O_6$)	1,0 g
Agar[a]	9 g to 18 g
Water	1 000 ml

[a] Depending on the gel strength of the agar.

When dairy products are examined, add skimmed milk powder at 1,0 g/l of the culture medium. The skimmed milk powder shall be free from inhibitory substances.

5.3.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary. Mix thoroughly and leave to stand for several minutes.