INTERNATIONAL STANDARD

ISO 4833-2

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

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

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

Partie 2: Comptage des colonies à 30 °C par la technique d'ensemencement en surface

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Foreword

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

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This first edition, together with ISO 4833-1, 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*

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

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

1 Scope

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

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

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

This part of ISO 4833 is applicable to:

1) products containing heat-sensitive organisms that are likely to form a significant proportion of the total flora (e.g. psychrotrophic organisms in chilled and frozen foods, dried foods, other foods that may contain heat-sensitive organisms);

2) products containing obligately aerobic bacteria that are likely to form a significant proportion of the total flora (e.g. *Pseudomonas* spp.);

3) products that contain small particles that can prove difficult to distinguish from colonies in a pour plate;

4) products whose intense colour prevents the recognition of colonies in a pour plate;

5) products for which distinction between different types of colony is required as part of the assessment of food quality.

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In addition to the manual spread plating technique, this part of ISO 4833 also specifies the use of a spiral plater, a rapid method of performing surface colony counts (Annex A).

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 these products are not detected effectively.

For some matrices, the method described in this part of ISO 4833 can give different results to those obtained using the method described in ISO 4833-1.

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*

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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 test sample, or a specified quantity of an initial suspension in the case of other products, is surface plated on a solid agar culture medium contained in Petri dishes.

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 of sample or the number of microorganisms per millilitre of sample is calculated from the number of colonies obtained on the plates containing fewer than 300 colonies.

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5 Culture media and diluents

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5.1 General

Follow ISO 11133 for the 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 digest of animal tissues	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.

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