

**TOIDUAHELA MIKROBIOLOOGIA. MIKROORGANISMIDE
LOENDAMISE HORISONTAALNE MEETOD. OSA 2:
KOLOONIAATE LOENDAMINE PINDKÜLVI TEHNIKAT
KASUTADES TEMPERATUURIL 30 °C**

**Microbiology of the food chain - Horizontal method for
the enumeration of microorganisms - Part 2: Colony
count at 30 °C by the surface plating technique
(ISO 4833-2:2013 + ISO 4833-2:2013/Amd 1:2022)**

EESTI STANDARDI EESSÕNA**NATIONAL FOREWORD**

See Eesti standard EVS-EN ISO 4833-2:2013 +A1:2022 sisaldab Euroopa standardi EN ISO 4833-2:2013 ja selle muudatuse A1:2022 ja paranduse AC:2014 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 4833-2:2013 +A1:2022 consists of the English text of the European standard EN ISO 4833-2:2013 and its amendment A1:2022 and its corrigendum AC:2014.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas. Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 04.09.2013, muudatus A1 12.01.2022.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation. Date of Availability of the European standard is 04.09.2013, for A1 12.01.2022.
Muudatusega A1 lisatud või muudetud teksti algus ja lõpp on tekstis tähistatud sümbolitega $\boxed{A_1}$ $\langle A_1 \rangle$. Parandusega AC lisatud või muudetud teksti algus ja lõpp on tekstis tähistatud sümbolitega \boxed{AC} $\langle AC \rangle$. Standard on kättesaadav Eesti Standardimis- ja Akrediteerimiskeskusest.	The start and finish of text introduced or altered by amendment A1 is indicated in the text by tags $\boxed{A_1}$ $\langle A_1 \rangle$. The start and finish of text introduced or altered by corrigendum AC is indicated in the text by tags \boxed{AC} $\langle AC \rangle$. The standard is available from the Estonian Centre for Standardisation and Accreditation.

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EUROPEAN STANDARD

EN ISO 4833-2 + A1

NORME EUROPÉENNE

EUROPÄISCHE NORM

September 2013, January 2022

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Supersedes EN ISO 4833:2003

English Version

**Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by the surface plating technique
(ISO 4833-2:2013 + ISO 4833-2:2013/Amd 1:2022)**

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
(ISO 4833-2:2013 + ISO 4833-2:2013/Amd 1:2022)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zur Zählung von Mikroorganismen - Teil 2: Koloniezählung bei 30 °C mittels Oberflächenverfahren
(ISO 4833-2:2013 + ISO 4833-2:2013/Amd 1:2022)

This European Standard was approved by CEN on 26 July 2013. Amendment A1 was approved by CEN on 1 January 2022.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for inclusion of this European Standard and its amendment into the relevant national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard and its Amendment A1 exist in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Foreword

This document (EN ISO 4833-2:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2014, and conflicting national standards shall be withdrawn at the latest by March 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 4833:2003, together with EN ISO 4833-1:2013.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 4833-2:2013 has been approved by CEN as EN ISO 4833-2:2013 without any modification.

A1 Amendment A1 European foreword

This document (EN ISO 4833-2:2013/A1:2022) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 "Microbiology of the food chain" the secretariat of which is held by AFNOR.

This Amendment to the European Standard EN ISO 4833-2:2013 shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2022, and conflicting national standards shall be withdrawn at the latest by July 2022.

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Endorsement notice

The text of ISO 4833-2:2013/Amd 1:2022 has been approved by CEN as EN ISO 4833-2:2013/A1:2022 without any modification. **A1**

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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.

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, 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.

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

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*

A1 Amendment A1 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.

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For an explanation of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 4833 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html. **A1**

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

A1 This document 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 described in this document is applicable to:

- products intended for human consumption;
- products intended for feeding animals (including pets);
- environmental samples in the area of food and feed production and handling;
- all samples from the primary production stage.

This technique is suitable for, but not limited to, the enumeration of microorganisms in test samples with a minimum of 10 colonies counted on a plate. This corresponds to a level of contamination that is expected to be higher than 100 cfu/ml for liquid samples or higher than 1 000 cfu/g for solid samples.

This technique is especially suitable for:

- 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 can contain heat-sensitive organisms);
- products containing obligately aerobic bacteria that are likely to form a significant proportion of the total flora (e.g. *Pseudomonas* species.);
- products that contain small particles that can prove difficult to distinguish from colonies in a pour plate;
- products whose intense colour prevents the recognition of colonies in a pour plate;
- products for which a distinction between different types of colony is desired as part of the assessment of food quality.

In addition to the manual spread plating technique, this document also describes the use of a spiral plater, an automated method of performing surface colony counts (see Annex A).

This horizontal method was originally developed for the examination of samples belonging to the food chain. Because of the large variety of products in the food chain, it is possible that this horizontal method is not appropriate in every detail for all products. Nevertheless, it is expected that the required modifications are minimized so that they do not result in a significant deviation from this horizontal method.

Based on the information available at the time of publication of this document, the suitability of this method for the examination of certain fermented food and animal feeds is considered to be limited and other media or incubation conditions can be more appropriate. However, this method can still be applied to such products even though it is possible that the predominant microorganisms in those products are not detected effectively. A1

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 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.

5 Culture media and diluents

5.1 General

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