Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (ISO 16649-3:2015, Corrected version 2016-12-15)

EESTI STANDARDIKESKUS
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EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 16649-3:2015 sisaldab Euroopa standardi EN ISO 16649-3:2015 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 16649-3:2015 consists of the English text of the European standard EN ISO 16649-3:2015.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 20.05.2015.	Date of Availability of the European standard is 20.05.2015.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 07.100.30

EUROPEAN STANDARD

NORME EUROPÉENNE

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EN ISO 16649-3

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English Version

Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (ISO 16649-3:2015, Corrected version 2016-12-15)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour le dénombrement des Escherichia coli bêta-glucuronidase positive - Partie 3: Recherche et technique du nombre le plus probable utilisant le bromo-5-chloro-4-indolyl-3 ß-D-glucuronate (ISO 16649-3:2015, Version corrigée 2016-12-15)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zur Zählung von ß-Glucuronidase-positiven Escherichia coli - Teil 3: Nachweis und Bestimmung der wahrscheinlichsten Keimzahl unter Verwendung von 5-Brom-4-Chlor-3-Indol-ß-D-Glucuronid (ISO 16649-3:2015, korrigierte Fassung 2016-12-15)

This European Standard was approved by CEN on 16 April 2015.

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This European Standard exists 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: Avenue Marnix 17, B-1000 Brussels

European foreword

This document (EN ISO 16649-3:2015) 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 November 2015, and conflicting national standards shall be withdrawn at the latest by November 2015.

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.

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 16649-3:2015, Corrected version 2016-12-15 has been approved by CEN as EN ISO 16649-3:2015 without any modification.

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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 (see 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.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

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

This first edition cancels and replaces ISO/TS 16649-3:2005, which has been technically revised.

ISO 16649 consists of the following parts, under the general title *Microbiology of the food chain — Horizontal method for the enumeration of β-glucuronidase positive* Escherichia coli:

— *Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide*

— *Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide*

— *Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide*

This corrected version of ISO 16649-3:2015 incorporates the following corrections:

— in 4.1.4, first line, "(22 ± 2) h" has been replaced with "(21 ± 3) h";

— in 4.2.5, first line, "(22 ± 2) h" has been replaced with "(21 ± 3) h";

— Table 1 has been replaced;

— Table 2 has been replaced;

— in 9.1.4, first line, "(22 ± 2) h" has been replaced with "(21 ± 3) h";

— in 9.2.5, first line, "(22 ± 2) h" has been replaced with "(21 ± 3) h";

— in 9.2.5, second line, "three" has been replaced with "six".

Introduction

Because of the large variety of food and feed products, this horizontal method might not be appropriate in every detail for certain products. In this case, different methods which are specific to these products might be used if absolutely necessary, for justified technical reasons. Nevertheless, every attempt will be made to apply this horizontal method as far as possible.

When this part of ISO 16649 is next reviewed, account will be taken of all information 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 groups of products, International Standards and/or national standards might 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 16649 so that eventually, the only remaining departures will be those necessary for well-established technical reasons.

Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* —

Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide

WARNING — Strains of *Escherichia coli* that do not grow at 44 °C and, in particular, those that are β-glucuronidase negative, such as *Escherichia coli* O157 and some other strains of pathogenic *E. coli*, will not be detected by the method described in this part of ISO 16649.

1 Scope

This part of ISO 16649 specifies a horizontal method for the detection and enumeration of β-glucuronidase positive *Escherichia coli*, by means of the liquid-medium culture technique and calculation of the most probable number (MPN) after incubation at (37 ± 1) °C, then at (44 ± 1) °C. This part of ISO 16649 is applicable to the following:

— products intended for human consumption and the feeding of animals;

— environmental samples in the area of food production and food handling.

The method is suitable for the enumeration of cells of *E. coli* that might have been subjected to stress arising from dehydration, freezing, and exposure to a saline (such as marine) environment or damage by disinfectants such as chlorine-containing products.

A limitation of the applicability of this part of ISO 16649 is imposed by the susceptibility of the method to a large degree of variability. The method is intended to be applied and the results interpreted in the light of the information given in Clause 11.

This method has not been fully evaluated for all matrices (e.g. for milk and milk products). ISO 7251 is intended to be used for milk and milk products.

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 6887-1, *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*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*