

STN	Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu β-glukuronidázopozitívnych Escherichia coli. Časť 3: Dôkaz a metóda najpravdepodobnejšieho počtu použitím 5-bromo-4-chloro-3-indolyl-β-D-glukuronidu (ISO 16649-3: 2015).	STN EN ISO 16649-3
		56 0120

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- β -D-glucuronide (ISO 16649-3:2015)

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 08/15

Obsahuje: EN ISO 16649-3:2015, ISO 16649-3:2015

Oznámením tejto normy sa ruší
STN P ISO/TS 16649-3 (56 0120) z marca 2007

121412

Úrad pre normalizáciu, metrológiu a skúšobníctvo SR, 2015
Podľa zákona č. 264/1999 Z. z. v znení neskorších predpisov sa môžu slovenské technické normy rozmnožovať a rozširovať iba so súhlasom Úradu pre normalizáciu, metrológiu a skúšobníctvo SR.

ICS 07.100.30

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- β -D-glucuronide (ISO 16649-3:2015)

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 β -D-glucuronate (ISO 16649-3:2015)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zur Zählung von β -Glucuronidase-positiven *Escherichia coli* - Teil 3: Nachweis und Bestimmung der wahrscheinlichsten Keimzahl unter Verwendung von 5-Brom-4-Chlor-3-Indol- β -D-Glucuronid (ISO 16649-3:2015)

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

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a 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 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.

CEN members are the national standards bodies of 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 United Kingdom.



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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword.....3

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 has been approved by CEN as EN ISO 16649-3:2015 without any modification.

**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- β -D-glucuronide**

*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 β -D-glucuronate*





COPYRIGHT PROTECTED DOCUMENT

© ISO 2015, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle	2
4.1 Detection method.....	2
4.2 Enumeration method.....	3
4.2.1 Inoculation of three or five tubes of double strength liquid selective enrichment medium [5.3.1.1 a)] with an equal volume of the test sample if the initial product is liquid, or with an equal volume of the initial suspension in the case of other products.....	3
5 Dilution fluids and culture media	3
5.1 General.....	3
5.2 Dilution fluids.....	3
5.3 Culture media.....	3
5.3.1 Minerals modified glutamate medium (selective enrichment medium).....	4
5.3.2 Tryptone bile glucuronide agar (second selective enrichment medium).....	5
5.3.3 Performance testing for the quality assurance of the culture media.....	5
6 Apparatus and glassware	6
7 Sampling	6
8 Preparation of test sample	7
9 Procedure	7
9.1 Detection method.....	7
9.1.1 Test portion, initial suspension, and dilutions.....	7
9.1.2 Incubation of selective enrichment medium.....	7
9.1.3 Subculturing.....	7
9.1.4 Secondary incubation.....	7
9.1.5 Examination of the plates.....	7
9.1.6 Interpretation.....	7
9.2 Enumeration method.....	7
9.2.1 Test portion, initial suspension, and dilutions.....	7
9.2.2 Inoculation of the selective enrichment medium.....	8
9.2.3 Incubation.....	8
9.2.4 Subculturing.....	8
9.2.5 Second incubation.....	8
9.2.6 Examination of the plates.....	8
9.2.7 Interpretation.....	8
10 Expression of results	9
10.1 Detection method.....	9
10.2 Enumeration method.....	9
11 Precision	9
12 Test report	9
Bibliography	10

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).

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 (see 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.

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*

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- β -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*

ISO 16649-3:2015(E)

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products*

ISO 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

ISO 6887-6, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 6: Specific rules for the preparation of samples taken at the primary production stage*

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*

koniec náhľadu – text ďalej pokračuje v platenej verzii STN