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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

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

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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

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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).

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*

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*

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*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
β-glucuronidase positive *Escherichia coli*
strains of *E. coli* which, at 44 °C, form typical blue or blue green colonies on tryptone bile glucuronide medium (TBX) under the conditions specified in the procedure

3.2
enumeration of β-glucuronidase positive *Escherichia coli*
determination of the most probable number of β-glucuronidase positive *E. coli* per millilitre or gram of sample when the test is carried out in accordance with the specified procedure

4 Principle

4.1 Detection method

4.1.1 A liquid selective enrichment medium is inoculated with a specified quantity of test sample if the initial product is liquid or with a specified quantity of the initial suspension in the case of other products.

4.1.2 The tube is incubated at (37 ± 1) °C for (24 ± 2) h. The tube is examined for acid production, indicating lactose fermentation.

4.1.3 If the tube has given rise to acid production, it is subcultured onto tryptone bile glucuronide agar (5.3.2).

4.1.4 Incubation of the tryptone bile glucuronide agar (5.3.2) at (44 ± 1) °C for (21 ± 3) h. Examination of the tryptone bile glucuronide agar (5.3.2) for the presence of blue or blue green colonies, indicating the presence of β-glucuronidase positive *E. coli*.

4.1.5 The result is expressed as *E. coli* detected or not detected in *x* g or *x* ml of product.

4.2 Enumeration method

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.

For live bivalve molluscs or other products requiring greater precision, it is necessary to inoculate a series of five tubes.

4.2.2 Inoculation of three or five tubes of single strength liquid enrichment medium [5.3.1.1 b)] with a specified quantity of test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Then, under the same conditions, inoculation of the medium with decimal dilutions of the test sample or of the initial suspension.

4.2.3 Incubation of the tubes of double strength and single strength medium at (37 ± 1) °C for (24 ± 2) h. Examination of the tubes for acid production, indicating lactose fermentation.

4.2.4 For each tube of medium (5.3.1) showing acid production, subculture to tryptone bile glucuronide agar (TBX) (5.3.2).

4.2.5 Incubation of the tryptone bile glucuronide agar (5.3.2) at (44 ± 1) °C for (21 ± 3) h. Examination of the tryptone bile glucuronide agar (TBX) (5.3.2) for the presence of blue or blue green colonies, indicating the presence of β-glucuronidase positive *E. coli*.

4.2.6 Determination of the most probable number of β-glucuronidase positive *E. coli* (refer to ISO 7218) from the number of tubes of medium (5.3.1) that produced blue to blue green colonies after subculture to tryptone bile glucuronide agar (5.3.2), according to ISO 7218.

5 Dilution fluids and culture media

5.1 General

For current laboratory practice, use ISO 7218; for preparation and testing of culture media, refer to ISO 11133.

5.2 Dilution fluids

According to ISO 6887 (all parts).

5.3 Culture media

If commercially dehydrated media are used, prepare the media according to the manufacturer's instructions.