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Microbiology of food and animal feeding stuffs — 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-beta-D-glucuronide

Microbiologie des aliments — Méthode horizontale pour le dénombrement des Escherichia coli bêta-glucuronidase positive —

Partie 3: Technique du nombre le plus probable utilisant le bromo-5-chloro-4-indolyl-3 beta-D-glucuronate

ICS 07.100.30

ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO-lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five-month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 16649-3 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second/third/... edition cancels and replaces the first/second/... edition (), [clause(s) / subclause(s) / table(s) / figure(s) / annex(es)] of which [has / have] been technically revised.

ISO 16649 consists of the following parts, under the general title *Microbiology of food and animal feed — Enumeration of β-glucuronidase positive Escherichia coli*:

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

— *Part [n]:*

— *Part [n+1]:*

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ISO 16649 consists of the following parts, under the general title *Microbiology of food and animal feed — 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 may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products may 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 International Standard is next reviewed, account will be taken of all information then 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 may 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 International Standard so that eventually the only remaining departures from will be those necessary for well-established technical reasons.

Microbiology of food and animal feed — Enumeration of β-glucuronidase positive *Escherichia coli* — Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide

1 Scope

This International Standard 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 °C, then at 44 °C. This International Standard is applicable to:

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

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

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

A limitation of the applicability of this International Standard is imposed by the susceptibility of the method to a large degree of variability. The method should be applied and the results interpreted in the light of the information given in 11.

This method has not been fully evaluated for all matrices, e.g. for milk and milk products. ISO 7251 should be used for milk and milk products.

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 International Standard.

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 products other than meat, dairy and seafood 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 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance of culture media in the laboratory*

ISO TS 11133-2, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on 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*
bacteria which, at 44 °C, form typical blue or blue-green colonies on tryptone bile glucuronide medium under the conditions specified in this part of ISO 16649

3.2
enumeration of β-glucuronidase positive *Escherichia coli*
determination of the most probable number of β-glucuronidase positive *Escherichia coli* per millilitre or gram of sample when the test is carried out in accordance with this part of ISO 16649.

4 Principle

4.1 Detection method

4.1.1 A liquid selective enrichment medium is inoculated with a specified quantity 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 °C for 24 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 [5.2.2].

4.1.4 Incubation of the tryptone bile glucuronide agar [5.2.2] at 44 °C for 20 – 24 h. Examination of the tryptone bile glucuronide agar [5.2.2] for the presence of blue or blue-green colonies, indicating the presence of β-glucuronidase positive *Escherichia coli*.

4.1.5 The result is expressed as the “presence” or “absence” of presumptive *Escherichia coli* in *x* g or *x* ml of product.

4.2 Enumeration method

4.2.1 Inoculation of three or five tubes [1)] of double-strength liquid selective enrichment medium [5.2.1.1 a] with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

4.2.2 Inoculation of three or five tubes of single-strength liquid enrichment medium [5.2.1.1 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- and single-strength medium at 37 ± 1 °C for 24 h. Examination of the tubes for acid production, indicating lactose fermentation.

4.2.4 For each tube of medium [5.2.1] showing acid production, subculture to tryptone bile glucuronide agar [5.2.2].

4.2.5 Incubation of the tryptone bile glucuronide agar [5.2.2] at 44 ± 1 °C for 20 – 24 h. Examination of the tryptone bile glucuronide agar [5.2.2] for the presence of blue or blue-green colonies, indicating the presence of β-glucuronidase positive *Escherichia coli.*

4.2.6 Determination of the most probable number of β-glucuronidase positive *Escherichia coli* (see ISO 7218), according to the number of tubes of medium [5.2.1] the subcultures of which have produced blue or blue-green colonies on tryptone bile glucuronide agar

5 Dilution fluids and culture media

For current laboratory practice, see ISO 7218.

5.1 Dilution fluids

See ISO 6887.

1) For live bivalve shellfish, or other special products, and/or where greater accuracy is needed, it is necessary to inoculate a series of five tubes