INTERNATIONAL STANDARD

ISO 16649-2

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Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* —

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

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

Partie 2: Technique de comptage des colonies à 44 °C au moyen de 5-bromo-4-chloro-3-indolyl β-D-glucuronate

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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 3.

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 part of ISO 16649 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 16649-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 16649 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — 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: Most probable number technique*

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 should 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 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 part of ISO 16649 so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

This International Standard describes two horizontal methods (ISO 16649-1 and ISO 16649-2) for the enumeration of β-glucuronidase-positive *Escherichia coli*.

The user may choose either ISO 16649-1 or ISO 16649-2. Either part is for general application. However, ISO 16649-1 should be used for foodstuffs which may contain severely stressed cells.

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* —

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

1 Scope

This part of ISO 16649 specifies a horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* in products intended for human consumption or for the feeding of animals. It uses a colony-count technique at 44 °C on a solid medium containing a chromogenic ingredient for detection of the enzyme β-glucuronidase.

WARNING — Strains of *Escherichia coli* which do not grow at 44 °C and, in particular, those that are β-glucuronidase negative, such as *Escherichia coli* O157, will not be detected.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 16649. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 16649 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Terms and definitions

For the purposes of this part of ISO 16649, the following terms and definitions apply.

3.1
β-glucuronidase-positive *Escherichia coli*
bacteria which at 44 °C form typical blue colony on tryptone-bile-glucuronide medium (TBX) under the conditions specified in this part of ISO 16649

3.2
enumeration of β-glucuronidase-positive *Escherichia coli*
determination of the number of colony-forming units (CFU) of β-glucuronidase-positive *Escherichia coli*, per millilitre or per gram of sample, when test and calculations are carried out in accordance with this part of ISO 16649

4 Principle

4.1 Duplicate plates of tryptone-bile-glucuronic medium (TBX) are inoculated with the specified quantity of the test sample or the initial suspension.

Under the same conditions, using decimal dilutions of the test sample or of the initial suspension, two plates per dilution are inoculated.

The dishes are incubated for 18 h to 24 h at 44 °C ± 1 °C then examined to detect the presence of colonies which, from their characteristics, are considered to be β-glucuronidase-positive *Escherichia coli*.

4.2 The number of colony-forming units (CFU) of β-glucuronidase-positive *Escherichia coli* per gram or per millilitre of sample is calculated (see clause 10).

5 Diluent and culture media

For current laboratory practice, see ISO 7218.

5.1 Diluent

See ISO 6887-1 or the specific International Standard dealing with the product to be examined.

5.2 Culture medium: Tryptone-bile-glucuronic medium (TBX)

5.2.1 Composition

Enzymatic digest of casein	20,0 g
Bile salts No. 3	1,5 g
5-Bromo-4-chloro-3-indolyl β-D-glucuronic acid (BCIG)	144 μmol [a]
Dimethyl sulfoxide (DMSO) [b]	3 ml
Agar	9 g to 18 g [c]
Water	1 000 ml

[a] e.g. 0,075 g of cyclohexylammonium salt.

[b] Dimethyl sulfoxide is harmful by inhalation and contact. The use of a fume cupboard when handling is advised. Because of this toxicity, a diluent recommended by the manufacturer may be used.

[c] Depending on the gel strength of the agar.

5.2.2 Preparation

Dissolve the BCIG in the dimethyl sulfoxide or in the diluent recommended by the manufacturer. Dissolve all components in the water and heat to boiling.

Adjust the pH, if necessary, so that after sterilization, it is 7,2 ± 0,2 at 25 °C.

Sterilize the medium in the autoclave set at 121 °C for 15 min. Immediately cool the medium in the water bath (6.3) at 44 °C to 47 °C.