

# Australian Standard<sup>®</sup>

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## Food microbiology

### **Method 19.1: Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of $\beta$ -glucuronidase-positive *Escherichia coli*—Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl $\beta$ -D-glucuronide (ISO 16649-1:2001, MOD)**

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AS 5013.19.1—2012

#### PREFACE

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology to supersede AS/NZS 1766.2.12:2002, *Food microbiology, Method 2.12: Examination for specific organisms—Escherichia coli in bivalve molluscs—Rapid method*.

This Standard is an adoption with national modifications and has been reproduced from ISO 16649-1:2001, *Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of  $\beta$ -glucuronidase-positive Escherichia coli—Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl $\beta$ -D-glucuronide*.

The objectives of this revision are—

- (a) to transfer the procedure to the AS 5013 series; and
- (b) to adopt ISO 16649-1:2001, with national modifications, to supersede AS/NZS 1766.2.12:2002.

Australian technical variations to ISO 16649-1 have been made as follows:

- (i) New Clause 5.3, Cultures.
- (ii) Clause 9.3.
- (iii) New Clause 9.5, Summary of Procedure, including flow diagram on the procedure.

These variations, which are necessary for Australian conditions, are listed in Appendix ZZ, which is added at the end of the source text.

As this Standard is reproduced from an International Standard, the following applies:

- (A) In the source text ‘this part of ISO 16649’ should read ‘this Australian Standard’.
- (B) A full point substitutes for a comma when referring to a decimal marker.
- (C) Substitute ‘mL’ for ‘ml’ wherever it appears.

The laboratory should have a clearly defined quality control system to ensure the apparatus, culture media, reagents and technique are suitable for the test. The use of positive controls is part of this system.

References to International Standards should be replaced by references to Australian Standards, as follows:

<i>Reference to International Standard</i>		<i>Australian Standard</i>	
ISO		AS	
6887	Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination	5013	Food microbiology
6887-1	Part 1: General rules for the preparation of the initial suspension and decimal dilutions	5013.11.1	Method 11.1: Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—General rules for the preparation of the initial suspension and decimal dilutions
7218	Microbiology of food and animal feeding stuffs—General rules for microbiological examinations	5013.14.1	Method 14.1: Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations (ISO 7218:2007, MOD)

The term ‘normative’ has been used in this Standard to define the application of the annex to which it applies. A ‘normative’ annex is an integral part of a Standard.

## 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  $\beta$ -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.

NOTES

## 1 Scope

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

**WARNING** — Strains of *Escherichia coli* which do not grow at 44 °C and, in particular, those that are  $\beta$ -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

#### **$\beta$ -glucuronidase-positive *Escherichia coli***

bacteria which at 44 °C form typical blue colonies on tryptone-bile-glucuronide medium (TBX) under the conditions specified in this part of ISO 16649

### 3.2

#### **enumeration of $\beta$ -glucuronidase-positive *Escherichia coli***

determination of the number of colony-forming units (CFU) of  $\beta$ -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