INTERNATIONAL STANDARD

ISO 16649-3

First edition
2015-05-15

Corrected version
2016-12-15

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

Reference number
ISO 16649-3:2015(E)

COPYRIGHT PROTECTED DOCUMENT

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

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.

5.3.1 Minerals modified glutamate medium (selective enrichment medium)

5.3.1.1 Composition

	a)	b)
	Double strength medium	**Single strength medium**
Sodium glutamate	12,7 g	6,35 g
Lactose	20,0 g	10,0 g
Sodium formate	0,5 g	0,25 g
L-cystine	0,04 g	0,02 g
L(-) aspartic acid	0,048 g	0,024 g
L(+) arginine	0,04 g	0,02 g
Thiamine	0,002 g	0,001 g
Nicotinic acid	0,002 g	0,001 g
Pantothenic acid	0,002 g	0,001 g
Magnesium sulfate septahydrate	0,2 g	0,1 g
Ammonium iron (III) citrate	0,02 g	0,01 g
Calcium chloride dihydrate	0,02 g	0,01 g
di-potassium hydrogen phosphate	1,8 g	0,9 g
Bromocresol purple	0,02 g	0,01 g
Ammonium chloride	5,0 g	2,5 g
Water	1 000 ml	1 000 ml

5.3.1.2 Preparation

Dissolve the ammonium chloride in the water. Add the remaining components or the dehydrated complete medium and dissolve by heating, if necessary.

To improve the stability of the dehydrated medium on storage, the sodium glutamate may be supplied separately.

Adjust the pH, if necessary, so that after sterilization, it is 6,7 ± 0,1 at (25 ± 1) °C.

Dispense the single strength medium in 10 ml volumes into tubes or bottle of dimensions at least 16 mm × 160 mm (6.7). Dispense the double strength medium in 10 ml volumes into tubes or bottles of dimensions at least 18 mm × 180 mm or 20 mm × 200 mm (6.7). If larger volumes of media are required, use tubes or bottles of appropriate capacity.

Sterilize the medium for 10 min in an autoclave (6.1) at 116 °C.

5.3.2 Tryptone bile glucoronide agar (second selective enrichment medium)

5.3.2.1 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 sulphoxide (DMSO)[b,c]	3 ml
Agar	9 g to 18 g[d]
Water	1 000 ml

[a] For example, 0,075 g of cyclohexylammonium salt.

[b] Dimethyl sulphoxide (DMSO) is harmful by inhalation and contact. The use of a protective cabinet and appropriate personal protective equipment when handling is advised.

[c] DMSO is only required if a stock solution of BCIG is made from first principles.

[d] Depending on the desired gel strength of the agar.

5.3.2.2 Preparation

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

Sterilize the medium in the autoclave (6.1) for 15 min at 121 °C.

5.3.2.3 Preparation of agar plates

Pour 18 ml to 20 ml of the molten medium into sterile Petri dishes (6.10) and allow to solidify.

The plates may be stored at (5 ± 3) °C in the dark and protected from desiccation for up to four weeks unless results of the laboratory shelf life validation indicate a longer shelf-life.

5.3.3 Performance testing for the quality assurance of the culture media

Refer to ISO 11133 for performance testing (see Table 1 and Table 2).

Table 1 — Performance testing of minerals modified glutamate medium

Function	Incubation	Control strain	WDCM numbers[a]	Method of control	Criteria	Characteristic reaction
Productivity	(37 ± 1) °C/ (24 ± 2) h	*Escherichia coli*[b]	00012 or 00013	Qualitative	Acid production	Colour change to yellow
Selectivity	(37 ± 1) °C/ (24 ± 2) h	*Enterococcus faecalis*[b]	00009 or 00087	Qualitative	Total inhibition	–

[a] Consult the reference strain catalogue available on http://www.wfcc.info/ for information on culture collection strain numbers and contact details.

[b] Strain free of choice; one of the strains has to be used as a minimum.

Table 2 — Performance testing of Tryptone bile glucuronide agar (TBX)

Function	Incubation	Control strain[a]	WDCM numbers[a]	Method of control	Criteria	Characteristic reaction
Productivity	(44 ± 1) °C/ (21 ± 3) h	*E. coli*[c]	00012[d] 00013[d] 00202[b]	Qualitative	Good growth	Blue to blue green colonies
Selectivity		*E. faecalis*[d]	00009 or 00087	Qualitative	Total inhibition	—
Specificity		*Citrobacter freundii* *Pseudomonas aeruginosa*	00006[d] 00025[d]	Qualitative	Good growth	White to green-beige colonies

[a] Consult the reference strain catalogue available on http://www.wfcc.info/ for information on culture collection strain numbers and contact details.

[b] Strain to be used as a minimum.

[c] WDCM 00013 is a strong β-glucuronidase producer and WDCM 00202 is a weak β-glucuronidase producer.

[d] Strain free of choice; one of the strains has to be used as a minimum.

6 Apparatus and glassware

Microbiological laboratory apparatus and the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave), as specified in ISO 7218.

6.2 Incubators, capable of operating at (37 ± 1) °C and (44 ± 1) °C.

6.3 Drying cabinet or ventilated oven, capable of being maintained between (25 ± 1) °C and (50 ± 1) °C, or a **laminar flow cabinet**.

6.4 Protective cabinet, for media preparation.

6.5 Refrigerator (for storage of prepared media), capable of operating at (5 ± 3) °C.

6.6 pH-meter, having a resolution of 0,01 pH units and accurate to within ± 0,1 pH units at 25 °C. The pH meter shall be equipped with either manual or automatic temperature equalization.

6.7 Test tubes or bottles, with dimensions of at least 16 mm × 125 mm (e.g. 16 mm × 160 mm, 18 mm × 180 mm or 20 mm × 200 mm).

6.8 Total-delivery pipettes, having nominal capacities of 1 ml and 10 ml, graduated in 0,1 ml divisions.

6.9 Sampling loops, made of platinum/iridium or nickel/chromium, approximately 3 mm in diameter or 10 µl sterile disposable sampling loops.

6.10 Petri dishes, approximately 90 mm in diameter.

7 Sampling

Use the appropriate part of ISO 6887 and the specific International Standard appropriate to the product concerned. If there is no specific International Standard dealing with sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

It is important that the laboratory receives a sample that is truly representative sample which has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned. If there is no specific International standard, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 Detection method

9.1.1 Test portion, initial suspension, and dilutions

Refer to the appropriate part of ISO 6887 and the specific International Standard appropriate to the product concerned.

9.1.2 Incubation of selective enrichment medium

Incubate single strength or double strength minerals modified glutamate broth (5.3.1.1) in the incubator (6.2) set at (37 ± 1) °C for (24 ± 2) h.

9.1.3 Subculturing

From each tube incubated according to 9.1.2 showing the presence of acid, indicated by the presence of any yellow colouration or change in colour or appearance when compared with the negative control, subculture with a loop (6.9) to a plate of tryptone bile glucuronide agar (TBX) (5.3.2) and streak to obtain isolated colonies.

9.1.4 Secondary incubation

Incubate the plates inoculated as in 9.1.3 for (21 ± 3) h in an incubator (6.2) set at (44 ± 1) °C. Plates should be stacked no more than six high and should be separated from each other and from the incubator walls by at least 25 mm. Higher stacks with less spacing may be acceptable in incubators fitted with air circulation systems; in this case, the temperature distribution should be verified.

9.1.5 Examination of the plates

After the specified period of incubation (9.1.4), examine the plates for the presence of colonies showing any shade of dark or light blue or blue green, indicating the presence of β-glucuronidase positive *E. coli*.

9.1.6 Interpretation

Consider the result as being positive for the presence of β-glucuronidase positive *E. coli* if the plate obtained in 9.1.5 showed the presence of blue or blue green colonies.

9.2 Enumeration method

9.2.1 Test portion, initial suspension, and dilutions

Refer to the appropriate part of ISO 6887 and the specific International Standard appropriate to the product concerned. A sufficient number of dilutions should be prepared to obtain a final dilution where all tubes yield a negative result.

9.2.2 Inoculation of the selective enrichment medium

9.2.2.1 General

As a general case, the following procedure prescribes series of three tubes for each dilution. For live bivalve shellfish or other special products, and/or whenever a greater precision in the results is needed, it is necessary to inoculate series of five tubes per dilution.

A sufficient number of dilutions should be prepared to obtain a final dilution for which all tubes yield a negative result on subculture.

9.2.2.2 Take three or five tubes of double strength minerals modified glutamate broth [5.3.1.1 a)]. Using a sterile pipette (6.8), transfer to each of these tubes 10 ml of the test sample if liquid, or 10 ml of the initial suspension in case of other products. Carefully mix the inoculum and the medium.

9.2.2.3 Take three or five tubes of single strength minerals modified glutamate broth [5.3.1.1 b)]. Using a fresh sterile pipette (6.8), transfer to each of these tubes 1 ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products. Carefully mix the inoculum and the medium.

9.2.2.4 Take a further three or five tubes of single strength minerals modified glutamate broth for each successive further dilution prepared from the test sample if liquid, or the initial suspension in the case of other products (i.e. 10^{-1}, 10^{-2}, 10^{-3}, etc). Using a fresh sterile pipette (6.8) for each dilution, transfer to each of these tubes 1 ml. Carefully mix the inoculum and the medium.

9.2.3 Incubation

Incubate the tubes of double strength selective medium inoculated in 9.2.2.2 and the tubes of single strength selective medium inoculated in 9.2.2.3 and 9.2.2.4 in the incubator (6.2) set at (37 ± 1) °C for (24 ± 2) h.

9.2.4 Subculturing

From each tube incubated according to 9.2.3 showing the presence of acid, indicated by the presence of any yellow colouration or change in colour or appearance when compared with the negative control, subculture with a loop (6.9) to a plate of tryptone bile glucuronide agar (TBX) (5.3.2) and streak to obtain isolated colonies.

9.2.5 Second incubation

Incubate the plates inoculated as in 9.1.3 for (21 ± 3) h in an incubator (6.2) set at (44 ± 1) °C. Plates should be stacked no more than six and should be separated from each other and from the incubator walls by at least 25 mm. Higher stacks with less spacing may be acceptable in incubators fitted with air circulation systems; in this case, the temperature distribution should be verified.

9.2.6 Examination of the plates

After the specified period of incubation (9.2.5), examine the plates for the presence of colonies showing any shade of blue or blue green, indicating the presence of β-glucuronidase positive *E. coli*.

9.2.7 Interpretation

Consider as positive each tube of double strength or single strength selective enrichment medium incubated according to 9.2.3 that has given rise, after subculturing (9.2.4) and incubation according to 9.2.5, to the presence of blue or blue green colonies on the plate of tryptone bile glucuronide agar (TBX) (5.3.2).

For each dilution, count the number of positive tubes of medium.

10 Expression of results

10.1 Detection method

In accordance with the results of the interpretation (see 9.1.6), indicate that β-glucuronidase positive *E. coli* is detected or not detected in the test portion.

10.2 Enumeration method

In accordance with the results of the interpretation (see 9.2.7), calculate the most probable number (MPN) per gram or millilitre from the number of positive tubes at each dilution according to ISO 7218.

11 Precision

It is well known that wide variations in results can occur with the MPN technique using series of three tubes per dilution. Results obtained with this method should therefore be used with caution. When using series of five tubes, it has been reported that precision obtained would be comparable to that of colony count methods. Confidence limits are given in ISO 7218.

EXAMPLE 1 Using three tubes per dilution, in 95 % of the cases, the confidence limits vary from 5,6 to 100 β-glucuronidase positive *E. coli* per gram for an MPN of 24 β–glucuronidase positive *E. coli* per gram.

EXAMPLE 2 Using five tubes per dilution, in 95 % of the cases, the confidence limits vary from 7,79 to 74 β-glucuronidase positive *E. coli* per gram for an MPN of 24 β–glucuronidase positive *E. coli* per gram.

12 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) sampling method used;

c) all operating details not specified in this part of ISO 16649, or regarded as optional, together with details of any issues likely to have influenced the test results;

d) test result(s) obtained.

Bibliography

[1] ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

[2] Blazko N. Evaluation of the β-glucuronidase substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide in a 24 hour direct plating method for *Escherichia coli.* J. Food Prot. 1988 p, **51** p. 402

[3] Damare J.M., Campbell D.F., Johnson R.W. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. J. Food Sci. 1985, **50** pp. 1736–1737

[4] Delisle G.L., & Ley A. Rapid detection of *Escherichia coli* in urine samples by a new chromogenic β -glucuronidase assay. J. Clin. Microbiol. 1989, **27** pp. 778–779

[5] Donovan T.J., Gallacher S., Andrews N.J., Greenwood M.H., Graham J., Russell J.E. ROBERTS D. and LEE R. Modification of the standard method used in the United Kingdom for counting *Escherichia coli* in live bivalve molluscs. Commun. Dis. Public Health. 1998, **1** pp. 188–196

[6] Killian m., bulow p. Rapid identification of Enterobacteriaceae. Use of a β -glucuronidase detecting agar (PGUA) for the identification of *Escherichia coli* in primary cultures of urine samples. *Acta pathologica Microbiologica Scandinavica* 1979, Sect. B. volume **87**, pp. 271- 276

[7] Killian m., bulow p. Rapid identification of Enterobacteriaceae. Detection of bacterial glycosidases. *Acta pathologica Microbiologica Scandinavica* 1976, Sect. B volume **84**, pp. 245-251

[8] Ley A.N., Bowers R.J., Wolfe S. Indoxyl- β -D-glucuronide, a novel chromogenic reagent for the specific detection and enumeration of *Escherichia coli* in environmental samples. Can. J. Microbiol. 1988, **34** pp. 690–693

[9] Manafi M., & Kneifel W. A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliforms and *E.coli* in water. Zentralbl. Hyg. 1989, **189** pp. 225–234

[10] Ogden I.D., & Watt A.J. An evaluation of fluorogenic and chromogenic assays for the direct enumeration of *Escherichia coli.* Lett. Appl. Microbiol. 1991, **13** pp. 212–215

[11] Restaino L., Frampton E.W., Lyon R.H. Use of chromogenic substrate 5-bromo-4-chloro-3-indolyl- β- D-glucuronide (X-GLUC) for enumeration of *Escherichia coli* in 24 hours from ground beef. J. Food Prot. 1990, **53** (6) pp. 508–510

[12] Watkins W.D., Rippey S.C., Clavet C.R., Kelly-Reitz D.J., Burkhart W. Novel compound for identifying *Escherichia coli.* Appl. Environ. Microbiol. 1988, **54** pp. 1874–1875

ICS 07.100.30

Price based on 10 pages