INTERNATIONAL STANDARD

**ISO 16654**

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# Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157

*Microbiologie des aliments — Méthode horizontale pour la recherche des* Escherichia coli *O157*



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# 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.

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

International Standard ISO 16654 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

Annex A forms a normative part of this International 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 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 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 this horizontal method will be those necessary for well-established technical reasons.

# Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157

**WARNING — *Escherichia coli* O157 can cause severe life-threatening illness and has a low infective dose. Laboratory-acquired infections have been reported.**

**In order to safeguard the health of laboratory personnel, it is essential that the whole of this method be carried out only by skilled personnel using good laboratory practices and preferably working in a containment facility. Relevant national Health and Safety Regulations relating to this organism must be adhered to.**

**Care must be taken in the disposal of all infectious materials.**

## 1 Scope

This International Standard specifies a horizontal method for the detection of *Escherichia coli* serogroup O157.

Subject to the limitations discussed in the introduction, this International Standard is applicable to products intended for human consumption or for animal feeding stuffs.



## 2 Normative references



The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard 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 Term and definition

For the purposes of this International Standard, the following term and definition applies.

**3.1**
***Escherichia coli* O157**
***E. coli* O157**
microorganisms which form typical colonies on the surface of the plating-out medium used in this International Standard, and which produce indole and agglutinate specifically with antiserum against the O157 antigen

NOTE 1 Sorbitol-positive *E. coli* O157 strains are not detected on CT-SMAC (5.2) media.

NOTE 2 Some indole-negative mutations have been found.

## 4 Principle

The detection of *Escherichia coli* O157 necessitates four successive stages (see annex A).

a) **Enrichment** of the test portion homogenized in modified tryptone soya broth containing novobiocin (mTSB + N) with incubation at 41,5 °C ± 1 °C for 6 h and subsequently for a further 12 h to 18h.

b) **Separation and concentration** of microorganisms by means of immunomagnetic particles coated with antibodies to *E. coli* O157.

c) **Isolation** by subculture of the immunomagnetic particles with adhering bacteria onto cefixime tellurite sorbitol MacConkey agar (CT-SMAC) and the user's choice of a second selective isolation agar.

d) **Confirmation** of sorbitol-negative colonies from CT-SMAC and colonies typical of *E. coli* O157 on the second isolation agar, by indole production and agglutination with *E. coli* O157 antiserum.

NOTE Further characterization, by for example pathogenic markers, of the positive isolates can be obtained by forwarding them to an appropriate reference laboratory.

## 5 Culture media, reagents and antisera

For current laboratory practices, see ISO 7218.

### 5.1 Enrichment medium: Modified tryptone soya broth with novobiocin (mTSB + N)



See reference [1].

#### 5.1.1 Modified tryptone soya broth (mTSB)



##### 5.1.1.1 Composition

| | |
|---|---|
| Enzymatic digest of casein | 17,0 g |
| Enzymatic digest of soya | 3,0 g |
| D(+)-glucose | 2,5 g |
| Bile salts No. 3 | 1,5 g |
| Sodium chloride | 5,0 g |
| Dipotassium hydrogen phosphate ($K_2HPO_4$) | 4,0 g |
| Water | 1000 ml |

##### 5.1.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary. Adjust the pH, using the pH-meter (6.6), if necessary, so that after sterilization it is 7,4 ± 0,2 at 25 °C.

Dispense the medium in appropriate amounts in flasks or bottles (6.7).

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

#### 5.1.2 Novobiocin solution

##### 5.1.2.1 Composition

| | |
|---|---|
| Novobiocin | 0,45 g |
| Water | 100 ml |

##### 5.1.2.2 Preparation

Dissolve the novobiocin in the water and sterilize by membrane filtration.

Prepare on the day of use.

##### 5.1.2.3 Preparation of the complete medium

Immediately before use, add 1 ml or 4 ml of novobiocin solution (5.1.2) to either 225 ml or 900 ml of cooled mTSB (5.1.1).

The final concentration of novobiocin is 20 mg per litre of mTSB.

### 5.2 First selective isolation medium: Cefixime tellurite sorbitol MacConkey agar (CT-SMAC)

See reference [2].

#### 5.2.1 Base medium



##### 5.2.1.1 Composition



| | |
|---|---|
| Enzymatic digest of casein | 17,0 g |
| Enzymatic digest of animal tissues | 3,0 g |
| Sorbitol | 10,0 g |
| Bile salts No. 3 | 1,5 g |
| Sodium chloride | 5,0 g |
| Neutral Red | 0,03 g |
| Crystal Violet | 0,001 g |
| Agar | 9 g to 18 g [a] |
| Water | 1000 ml |

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

##### 5.2.1.2 Preparation

Dissolve the basic components or the complete dehydrated base in the water by boiling. Adjust the pH (6.6), if necessary, so that after sterilization it is 7,1 ± 0,2 at 25 °C.

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

#### 5.2.2 Potassium tellurite solution

##### 5.2.2.1 Composition

| | |
|---|---|
| Potassium tellurite for bacteriological use | 0,25 g |
| Water | 100 ml |

#### 5.2.2.2 Preparation

Dissolve the potassium tellurite in the water and sterilize by membrane filtration.

This solution may be stored at room temperature for up to 1 month, but discard it if a white precipitate forms.

### 5.2.3 Cefixime solution

#### 5.2.3.1 Composition

| | |
|---|---|
| Cefixime | 5,0 mg |
| Water | 100,0 ml |

#### 5.2.3.2 Preparation

Dissolve the cefixime in the water and sterilize by membrane filtration.

NOTE Cefixime may need to be dissolved in ethanol.

This solution may be stored at 3 °C ± 2 °C for 1 week.

### 5.2.4 Complete medium

#### 5.2.4.1 Composition



| | |
|---|---|
| Base medium (5.2.1) | 1 000 ml |
| Potassium tellurite solution (5.2.2) | 1,0 ml |
| Cefixime solution (5.2.3) | 1,0 ml |



#### 5.2.4.2 Preparation

Either cool the freshly sterilized base medium (5.2.1) to between 44 °C and 47 °C (6.5), or melt it by steaming the previously sterilized and solidified base medium, then cool to between 44 °C and 47 °C.

Add 1 ml of the tellurite solution and 1 ml of the cefixime solution to 1000 ml of the base medium. Mix and pour about 15 ml amounts into sterile Petri dishes (6.15). Allow to solidify.

The final concentration of tellurite is 2,5 mg/l and cefixime 0,05 mg/l.

Immediately before use, dry the agar plates, preferably with the lids removed and with the agar surfaces facing downwards, in an oven set at a temperature between 25 °C and 50 °C (6.2), until the droplets have disappeared from the surface of the medium. Do not dry them any further. The agar plates may also be dried in a laminar-flow safety cabinet for 30 min with half-open lids, or overnight with the lids in place.

If prepared in advance, the undried plates may be stored in the dark in plastic bags or other moisture-retentive containers, in a refrigerator at 3 °C ± 2 °C for up to 2 weeks.

## 5.3 Second selective isolation medium

Use any other solid selective medium, at the choice of the laboratory, complementary to CT-SMAC agar and especially appropriate for the isolation of *Escherichia coli* O157.

Immediately before use, dry the agar plates, preferably with the lids removed and with the agar surfaces facing downwards, in an oven set at a temperature between 25 °C and 50 °C (6.2), until the droplets have disappeared

from the surface of the medium. Do not dry them any further. The agar plates may also be dried in a laminar-flow safety cabinet for 30 min with half-open lids, or overnight with the lids in place.

If prepared in advance, the undried plates may be stored in the dark in plastic bags or other moisture-retentive containers, in a refrigerator at 3 °C ± 2 °C for a time that causes no change to its performance.

### 5.4 Nutrient agar

#### 5.4.1 Composition

| | |
|---|---|
| Meat extract | 3,0 g |
| Peptone | 5,0 g |
| Agar | 9 g to 18 g [a] |
| Water | 1 000 ml |

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

#### 5.4.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Transfer the medium into flasks or bottles (6.7) of appropriate capacity.

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



#### 5.4.3 Preparation of nutrient agar plates



Transfer about 15 ml of the molten, cooled medium (5.4.2) at between 44 °C and 47 °C (6.5) to Petri dishes and allow to solidify.

Immediately before use, dry the agar plates, preferably with the lids removed and with the agar surfaces facing downwards, in an oven set at a temperature between 25 °C and 50 °C (6.2), until the droplets have disappeared from the surface of the medium. Do not dry them any further. The agar plates may also be dried in a laminar-flow safety cabinet for 30 min with half-open lids, or overnight with the lids in place.

If prepared in advance, the undried plates may be stored in the dark, in plastic bags or other moisture-retentive containers, in a refrigerator at 3 °C ± 2 °C for up to 2 weeks.

### 5.5 Tryptone/tryptophan medium

#### 5.5.1 Composition

| | |
|---|---|
| Tryptone | 10,0 g |
| Sodium chloride | 5,0 g |
| DL-Tryptophan | 1,0 g |
| Water | 1 000 ml |

#### 5.5.2 Preparation

Dissolve the components in the water by boiling if necessary. Adjust the pH (6.6) so that after sterilization it is 7,5 ± 0,2 at 25 °C.