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Microbiology of food and animal stuffs – Horizontal method for the detection of *Escherichia coli* O157 (ISO 16654:2001)

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Swedish Standards corresponding to documents referred to in this Standard are listed in "Catalogue of Swedish Standards", issued by SIS. The Catalogue lists, with reference number and year of Swedish approval, International and European Standards approved as Swedish Standards as well as other Swedish Standards.

Mikrobiologi i livsmedel och djurfoder – Analysmetod för bestämning av *Escherichia coli* O157 (ISO 16654:2001)

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EN ISO 16654

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English version

**Microbiology of food and animal feeding stuffs - Horizontal
method for the detection of Escherichia coli O157
(ISO 16654:2001)**

Microbiologie des aliments - Méthode horizontale pour la
recherche des Escherichia coli O157 (ISO 16654:2001)

Mikrobiologie von Lebensmitteln und Futtermitteln -
Horizontales Verfahren für den Nachweis von Escherichia
coli O157 (ISO 16654:2001)

This European Standard was approved by CEN on 1 May 2001.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



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Foreword

The text of the International Standard ISO 16654:2001 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2001, and conflicting national standards shall be withdrawn at the latest by November 2001.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

NOTE FROM CMC: The foreword is susceptible to be amended on reception of the German language version. The confirmed or amended foreword, and when appropriate, the normative annex ZA for the references to international publications with their relevant European publications will be circulated with the German version.

Endorsement notice

The text of the International Standard ISO 16654:2001 was approved by CEN as a European Standard without any modification.

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 $41,5\text{ °C} \pm 1\text{ °C}$ 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 | 1 000 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 $7,4 \pm 0,2$ 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 .