SLOVENSKI STANDARD
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Mikrobiologija v prehranski verigi - Odkrivanje prisotnosti, izolacija in karakterizacija Escherichia coli (STEC), ki proizvaja Shiga toksin - 1. del: Horizontalna metoda za odkrivanje prisotnosti in izolacijo Escherichia coli (STEC), ki proizvaja Shiga toksin (ISO/DIS 13136-1:2024)

Microbiology of the food chain - Detection, isolation and characterization of Shiga toxin-producing Escherichia coli (STEC) - Part 1: Horizontal method for the detection and isolation of Shiga toxin-producing Escherichia coli (STEC) (ISO/DIS 13136-1:2024)

Mikrobiologie der Lebensmittelkette - Nachweis, Isolierung und Charakterisierung von Shiga-Toxin bildenden Escherichia coli (STEC) - Teil 1: Horizontales Verfahren zum Nachweis und zur Isolierung von Shiga-Toxin bildenden Escherichia coli (STEC) (ISO/DIS 13136-1:2024)

Microbiologie de la chaîne alimentaire - Détection, isolement et caractérisation des Escherichia coli producteurs de Shigatoxines (STEC) - Partie 1: Méthode horizontale pour la détection et l'isolement des Escherichia coli producteurs de Shigatoxines (STEC (ISO/DIS 13136-1:2024)

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Microbiology of the food chain — Detection, isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) —

Part 1: Horizontal method for the detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC)

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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.

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).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition of ISO 13136-1, together with ISO 13136-2, cancels and replaces the first edition Technical Specification (ISO/TS 13136:2012), which has been technically revised.

The main changes compared to the previous edition are as follows:

— structure of the standard (Full Standard rather than Technical Specification, split in Part 1 and Part 2)

— Change in the enrichment conditions (including medium and temperature)

— Removal of the determination of the presence of the gene *eae* and the genes associated to serogroups O157, O111, O26, O103 and O145 from the screening of the enrichment cultures and in the STEC isolates (included in Part 2 as characterization of the STEC isolates)

A list of all parts in the ISO 13136 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens causing human disease ranging from uncomplicated diarrhoea to severe illness, such as haemorrhagic colitis and haemolytic uremic syndrome (HUS). STEC are distinguished from other *E. coli* by the production of Shiga toxin (Stx), synonymous with Verocytotoxin (VT). The Stx family is divided antigenically into two major types, Stx1 and Stx2. STEC strains may possess Stx1- and/or Stx2-encoding genes. The serotypes of STEC causing human disease are highly diverse and continuously evolving, and so all STEC isolates are considered potential human pathogens.[1] Therefore, STEC strains regardless of the serogroup represent the target of this International Standard. The following nomenclature has been adopted in this standard method:

stx: Shiga toxin genes (synonymous with *vtx*);

Stx: Shiga toxin (synonymous with VT);

STEC: Shiga toxin-producing *Escherichia coli* (synonymous with VTEC: Verocytotoxin-producing *Escherichia coli*).

The main technical changes listed in the Foreword, introduced in this document compared to ISO/TS 13136:2012, are considered as major (see ISO 17468[2]).

These technical changes have a major impact on the performance characteristics of the method.

When this document is reviewed in the future, 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.

Identification of patent holders: the following text shall be included if patent rights have been identified. [The process is ongoing]

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Microbiology of the food chain — Detection, isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) —

Part 1: Horizontal method for the detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC)

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting STEC are only undertaken in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all the safety aspects associated with its use. It is the responsibility of the user to establish appropriate safety and health practices in compliance with national and international regulations concerning the handling and containment of biological agents.

1 Scope

This standard describes the detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC). The procedure includes the detection by real-time PCR of *stx1* and *stx2*, the major virulence genes of STEC (Reference [1]), in enrichment cultures. Isolation of STEC from the enrichment culture is attempted if one or both *stx* genes are detected.

This document is applicable to

— products intended for human consumption,

— products intended for animal feeding,

— environmental samples in the area of food and feed production, handling, and

— samples from the primary production stage.

2 Normative references

The following documents are referred to in the text in such a way that some or all their content constitutes requirements of this document. The latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension, and decimal dilutions for microbiological examination*

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*

ISO 22118, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection and quantification of food-borne pathogens — Performance characteristics*

ISO 22174, *Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

ISO 22119, *Microbiology of food and animal feeding stuffs — Real-time polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
Shiga toxin-producing *Escherichia coli* (STEC)
E. coli strain possessing one or multiple Shiga toxin (Stx)-encoding genes (*stx*)

3.2
Stx1 and Stx2
Shiga Toxin type 1 and Shiga Toxin type 2, respectively

3.3
stx1* and *stx2
Genes encoding Stx1 and Stx2, respectively

4 Principle

4.1 General

The method specified comprises the following sequential steps see the flowchart in Annex A:

a) microbial enrichment;

b) nucleic acid extraction;

c) detection of *stx* virulence genes (described in Annex D — this can also be done as a multiplex PCR, together with Internal Amplification Control, see 9.4.3);

d) isolation of STEC strains from enrichment culture positive for *stx* by determining the presence of Stx-encoding genes in isolated colonies to confirm the presence of STEC—(see 9.5 and Annex C)

4.2 Microbial enrichment

The number of viable STEC cells available to be detected in the test portion is increased by static incubation of the sample in buffered peptone water (BPW) (a non-selective liquid nutrient medium) at 41,5 °C for 21 h ± 3 h.

If injured cells are suspected to be present in the sample (e.g. frozen, UV or pressure treated food stuff, flours, dried food) incubate the BPW culture at 37 °C for 6 h ± 1 h to increase the number of viable STEC cells and then at 41,5 °C for further 18 h ± 3 h.

4.3 Nucleic acid extraction

Deoxyribonucleic acid (DNA) is extracted from enrichment cultures for analysis by real-time PCR assay for the detection of the genes encoding Shiga toxins.

4.4 Target genes

The primary virulence genes of STEC are the *stx* genes, encoding the Shiga toxins. The *stx* genes encode a family of toxins, which includes two main types: Stx1 and Stx2. Large variability in *stx* sequences has been described, and they can be divided in three subtypes of *stx1* (*stx1a*, *stx1c* and *stx1d*) and at least seven of *stx2* (*stx2a* to *stx2g)* (Reference [3]). The PCR primers and probes described in this standard detect all the previously mentioned *stx* subtypes, with the exception of *stx2f*. The gene sequence encoding Stx2f differs significantly from that of other subtypes and is not targeted by the *stx* primers and probes indicated in this standard. According to currently available data, *stx2f* has rarely been reported in foodstuff (References [4] and [5]), nevertheless it has been recently associated with with human disease (Reference [6]:). If there is a specific need for the detection of Stx2f-producing *E. coli* in food, such as prevalence studies, epidemiological investigations, or regulatory requests, a protocol issued by the European Union Reference Laboratory (EURL) for *E. coli* for the detection of *stx2f* by real-time PCR can be used. This procedure, EU-RL VTEC_ Method_10, Reference [7], is available at the following link: https://www.iss.it/documents/20126/0/EURL-VTEC_Method_10_Rev+0.pdf/a4eb6e2b-fd13-c52a-112a-255c005b4872?t=1644309297880, and is included in an informative Annex in this standard (Annex G). The EURL for *E. coli* protocol is used to determine the presence or absence of *stx2f* in enrichment cultures that give negative result for the presence of *stx* genes in the screening step described in the present standard.

In addition, new Stx subtypes are continuously identified, including Stx1e, Stx2h, Stx2i, Stx2j, Stx2k, Stx2l, Stx2m, and Stx2o (References [8-13]). An *ad-hoc* inclusivity assay has shown that these sets of primers described in this standard, including those described in Annex G, enable the efficient detection all the *stx*-subtypes except *stx1e*, *stx2h*, *stx2j*, *stx2n*, *stx2o* (courtesy of Alexander Gill and Sarah Clark, Health Canada, 2023).

The detection of the target genes, *stx1* and *stx2*, is performed either on DNA extracted from the enrichment culture as a screening step, or from isolated colonies when attempting to identify STEC among colonies recovered from *stx*-positive enrichment cultures.

4.5 Detection and isolation

Real-time PCR is applied to detect *stx1* and *stx2* genes in the enrichment culture, which is performed according to the PCR platform used and to manufacturers' instructions for the real-time PCR kit in use.

The isolation of the STEC strains from the enrichment culture is required to confirm that the positive PCR signals are generated from genes present in live bacterial cells.

If *stx* genes are detected in the enrichment culture, the isolation of STEC shall be attempted as a mandatory step. STEC isolation shall be attempted by plating the enrichment culture onto solid media, and isolated colonies are tested for the presence of *stx1* and *stx2*, either by the real-time PCR described in this standard (Section 9.4), or by conventional PCR (Annex E).

5 Culture media and reagents

Follow current laboratory practices in accordance with ISO 7218. The composition of culture media and reagents and their preparation are specified in Annex B. For performance testing of culture media, see table B.1 and follow the procedures in accordance with ISO 11133.

6 Equipment and consumables

Disposable plasticware is an acceptable alternative to reusable glassware if it has suitable specifications. Usual microbiological laboratory equipment (see ISO 7218) and the following:

6.1 Water bath or heating block capable of being maintained at temperatures up to 100 °C.

6.2 Incubator capable of operating at 41,5 °C ± 1 °C and at 37 °C ± 1 °C.