SLOVENSKI STANDARD
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Mikrobiologija v prehranski verigi - Odkrivanje prisotnosti, izolacija in karakterizacija Escherichia coli (STEC), ki proizvaja Shiga toksin - 2. del: Horizontalna metoda za karakterizacijo izolatov 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 2: Horizontal method for the characterization of Shiga toxin-producing Escherichia coli (STEC) isolates (ISO/DIS 13136-2:2024)

Mikrobiologie der Lebensmittelkette - Nachweis, Isolierung und Charakterisierung von Shiga-Toxin bildenden Escherichia coli (STEC) - Teil 2: Horizontales Verfahren zur Charakterisierung von Shiga-Toxin bildenden Escherichia coli (STEC) Isolaten (ISO/DIS 13136-2:2024)

Microbiologie de la chaîne alimentaire - Détection, isolement et caractérisation des Escherichia coli producteurs de Shigatoxines (STEC) - Partie 2: Méthode horizontale pour la caractérisation d'isolats des Escherichia coli producteurs de Shigatoxines (STEC) (STEC) (ISO/DIS 13136-2:2024)

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

Part 2:
Horizontal method for the characterization of Shiga toxin-producing *Escherichia coli* (STEC) isolates

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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.

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 second edition, together with the ISO 13136 Part 1, cancels and replaces the first edition (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)

— Description of the characterization of STEC isolates, in terms of determining the presence of a set of virulence genes and serogroups, and subtyping of *stx* genes

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 (Stx)-producing *Escherichia coli* (STEC) are human pathogens causing severe disease, including haemorrhagic colitis (HC) and the haemolytic uremic syndrome (HUS).

The Stx are the definative virulence factor of STEC, and belong to a heterogenous family of AB_5 toxins, including two antigenically distinct types, Stx1 and Stx2. STEC harbor *stx1* and/or *stx2* genes. Large variability in the *stx* gene sequences has been described and both *stx1* and *stx2* can be divided into several subtypes, with some of these subtypes having a greater association with the most severe forms of STEC infection (References [1, 2]). More than 180 serogroups of *E. coli* have been identified (Reference [3])), and a small group of these serogroups have been historically associated with STEC strains of major public health concern, being frequently isolated from patients with the most severe forms of infection (Reference [4]). Nevertheless, the range of STEC serogroups associated with human disease is much broader and continuously evolving, and in more recent scientific literature all STEC isolates are considered human pathogens, with the potential to cause at least diarrhoea (Reference [5]).

Beside the serogroup, other markers of STEC associated with HC and HUS cases have been described, such as the locus of enterocyte effacement (LEE) pathogenicity island (indicated by the presence of the *eae* gene, encoding the adhesin intimin) or, more recently, the determinants of the enteroaggregative adhesion typically present in Enteroaggreagative *E. coli* (References [5, 6]). Such features, and particularly the *eae* gene, are now regarded as aggravating factors for STEC infections, but may not be essential for the occurrence of severe diseases (Reference [5]). Additionally, greater diversity in the genetic features associated with STEC pathogenicity continues to be described, such as strains carrying virulence factors associated with more than one *E. coli* pathotype, referred to as hybrid or cross-pathotypestrains (References [7-9]), highlighting the heterogeneity of this *E. coli* pathogroup and the need for a more detailed characterization of the isolates.

The present standard method describes the characterization of STEC isolates, obtained either by the application of ISO 13136-1, or any other method. This characterization includes the determination of the following features:

The subtype of Stx- encoding genes

— The presence of the genes *eae* and *aggR* that are involved in the colonization of hosts by STEC.

— Serogroup[1)]

The following nomenclature has been adopted in this standard method:

stx: Shiga toxin encoding gene (synonymous with *vtx*);

Stx: Shiga toxin (synonymous with VT);

STEC: Shiga toxin-producing *Escherichia coli* (synonymous with VTEC: Verocytotoxin-producing *Escherichia coli*), *E. coli* strains possessing Stx-coding genes

eae: gene encoding the adhesin intimin, causing the 'Attaching and Effacing' adhesion;

aggR: gene encoding the transcriptional activator AggR, activating several factors involved in enteroaggregative adhesion;

stx1a, *stx1c*, *stx1d*: genes encoding the three subtypes of *stx1*, respectively;

stx2a, *stx2b, stx2c, stx2d, stx2e, stx2f, stx2g*: genes encoding subtypes of *stx2*.

The technical changes listed in the Foreword, introduced in this document compared to ISO/TS 13136:2012, are considered as major (see ISO 17468). These technical changes have a major impact on the performance characteristics of the method.

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1) The serogroups concerned by the methodology specified in this standard are O157, O26, O111, O145 and O103 (historically termed as top-5 serogroups), O45 and O121 (which, together with the aforementioned five serogroups, are often isolated from patients with STEC infection in the United States).

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WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting and characterizing STEC are only undertaken in properly equipped laboratories, by skilled microbiologists, and that great care is taken in the disposal of all materials that have potentially come into contact with bacterial cultures. 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, as well as working in compliance with national and international regulations concerning the handling and containment of biological agents.

1 Scope

This document is applicable to pure cultures of STEC. The present standard describes the characterization of STEC strains, isolated from any source. In particular, the characterization of STEC strains described here regards:

— Subtyping of the Stx-encoding genes

— Determination of the presence of the *eae* gene

— Determination of the presence of the *aggR* gene

— Identification of the presence of genes associated with the following serogroups: O157, O26, O111, O103, O145, O121 and O45.

The full characterization of the isolated STEC strains is achieved by performing all the modules described here. The characterization scheme is not sequential, and the different modules can be implemented separately based on specific needs (e.g. Regulatory needs, Competent Authority's requests, clients' request). Alternative methods may be used, including Whole Genome Sequencing (WGS), provided these are validated according to the most relevant part of reference standard series ISO 16140.

2 Normative references

The following documents are referred to in the text in such a way that some (or all) of 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 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*

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

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 23418:2022, *Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance*

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/

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

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

3.2
stx1* and *stx2
Genes encoding Stx1 and Stx2, respectively

3.3
eae
Gene encoding the adhesin intimin, located on the Locus of enterocyte effacement and consisting in a key factor for the 'Attaching and Effacing' colonization mechanism

3.4
aggR
Gene encoding the transcriptional activator AggR, activating several factors involved in the enteroaggregative adhesion characteristic of enteroaggregative *E. coli* (EAEC). EAEC are a pathogroup of diarrheagenic *E. coli*, and hybrid strains of EAEC/STEC have been reported in association with severe disease, such as the STEC O104:H4 responsible for a large outbreak occurring in the EU in 2011.

3.5
***stx* genes subtypes**
The two antigenically distinct types of Stx, Stx1 and Stx2, are each divided into different subtypes, namely three subtypes for Stx1 (Stx1a, Stx1c and Stx1d) and at least seven subtypes for Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) (Refrence [1]). New Stx subtypes are continuously identified, including Stx1e, Stx2h, Stx2i, Stx2j, Stx2k, Stx2l, Stx2m, and Stx2o (References [10-14]). The present standard regards the determination of three *stx1* genes subtypes (*stx1a*, *stx1c* and *stx1d*) and the seven *stx2* genes subtypes *stx2a* to *stx2g*.

3.6
O-antigen
Serogroups or "O" antigens are identified by numbers, counting from 1 to 188 at the time of the preparation of this document, but the serogroups' list is evolving constantly.