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English Version

Microbiology of food and animal feed - Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens - Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups (ISO/TS 13136:2012)

Microbiologie des aliments - Méthode basée sur la réaction de polymérisation en chaîne (PCR) en temps réel pour la détection des micro-organismes pathogènes dans les aliments - Méthode horizontale pour la détection des Escherichia coli producteurs de Shigatoxines (STEC) et la détermination des sérogroupes O157, O111, O26, O103 et O145 (ISO/TS 13136:2012)

Mikrobiologie von Lebensmitteln und Futtermitteln - Real-time-Polymerase-Kettenreaktion (PCR) zum Nachweis von pathogenen Mikroorganismen in Lebensmitteln - Horizontales Verfahren für den Nachweis von Shiga-Toxin bildenden Escherichia coli (STEC) der Serogruppen O157, O111, O26, O103 und O145 (ISO/TS 13136:2012)

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Foreword

This document (CEN ISO/TS 13136:2012) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods” the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

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Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are pathogenic *E. coli*, which can cause diarrhoea as well as more severe diseases in humans such as haemorrhagic colitis and haemolytic uremic syndrome (HUS). Although STEC may belong to a large number of serogroups, those that have been firmly associated with the most severe forms of the disease, in particular HUS, belong to O157, O26, O111, O103, and O145 (Reference [1]).

The following nomenclature has been adopted in this Technical Specification:

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

— Stx: Shiga toxin (synonymous with Vtx: Verocytotoxin);

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

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Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

IMPORTANT — It is necessary to consider any STEC as pathogenic to humans and potentially to cause severe disease depending on both the risk profile of the food commodity (ready-to-eat foods vs. foods intended to be consumed after technological treatment such as pasteurization, cooking etc. used to reduce any bacteria present in the food) and the health status of the subject ingesting the food.

Moreover, given the high genomic plasticity of this bacterial species, it is possible that novel arrangements of virulence features can give rise to novel sero-pathogroups such as the Shiga toxin-producing enteroaggregative *E. coli* O104 that caused the HUS outbreaks in Germany and France in 2011-05/06. Novel atypical *E. coli* sero-pathogroups can arise from the acquisition of an *stx*-converting bacteriophage by an *E. coli* strain belonging to pathogroups different from STEC.

Such atypical strains fall in the scope of this method and can be efficiently detected as they are positive for the presence of the *stx* genes.

1 Scope

This Technical Specification describes the identification of Shiga toxin-producing *Escherichia coli* (STEC) by means of the detection of the following genes:

a) the major virulence genes of STEC, *stx* and *eae* (References [2][3]);

b) the genes associated with the serogroups O157, O111, O26, O103, and O145 (References [3][4]).

In any case, when one or both of the *stx* genes is/are detected, the isolation of the strain is attempted.

The isolation of STEC from samples positive for the presence of the genes specifying the serogroups in the scope of this method can be facilitated by using serogroup-specific enrichment techniques (e.g. immunomagnetic separation, IMS).

The protocol uses real-time PCR as the reference technology for detection of the virulence and serogroup-associated genes.

This Technical Specification is applicable to:

1) products intended for human consumption and the feeding of animals;

2) environmental samples in the area of food production and food handling;

3) environmental samples in the area of primary production.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*