

**16<sup>th</sup> Annual Worksop of the National Reference Laboratories for *E. coli* –  
online event, 18-19 October 2021**

## **Update on the revision of ISO 13136**

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CEN/TC 275/WG 6 “Microbiology of the food chain” and all its TAGs have been disbanded in late 2019.

WG6 has been transformed to a new structure CEN/TC 463 “Microbiology of the food chain”. This new TC met on 25<sup>th</sup> of November 2019 , created new WGs and among them WG2 "Shiga toxin producing Escherichia coli (STEC)" to conduct the revision of CEN ISO/TS 13136:2012, convenor: Rosangela Tozzoli.

National standardization bodies nominated experts for the CEN/TC 463/ WG2 and the work on STEC is now being conducted under this structure.



## Part 1



+ enrichment broth (BPW)

↓  
Incubation (41,5° C)

↓  
Real Time PCR for *stx* genes

↓  
Positive to *stx* genes:  
Streak enrichment culture onto  
solid media to isolate the STEC  
(up to 50 colonies tested)

Based on isolation results:  
STEC detected in XX g

## Part 2



↓  
STEC serogroup determination (top 5  
+ O45 and O121), virulotyping  
(including detection of *eae* and  
*aggR/aaiC* and *stx* genes subtyping)

EN ISO 13136-1 "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)"

Submitted to CEN in April 2021

EN ISO 13136-2 "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"

Outline prepared and submitted to CEN in December 2020

# Outline of EN ISO 13136-2

In line with the recent opinion issued by EFSA in 2020 «Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC»

**Stx genes subtyping**  
 (stx1a, stx1c, stx1d; stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, stx2g; EU-RL VTEC\_Method\_006\_Rev 1, PCR-based)

In alternative WGS  
 (Validation according to ISO 16140)

**Colonization factors**  
 Determination of the presence of adhesion genes  
 (Real Time PCR for the identification of the presence of eae gene – Møller Nielsen and Andersen 2003, aggR gene or aggR/aaIC genes – EU-RL VTEC\_Method\_005\_Rev 1)

In alternative WGS  
 (Validation according to ISO 16140)

**Serogroups identification**  
 Determination of the presence of genes associated with O157, O26, O111<sup>\*</sup>, O103<sup>^</sup>, O145<sup>§</sup>, O45 and O121<sup>§</sup>  
 (Real Time PCR for the identification of the presence of rfbEO157<sup>\*</sup>, wzxO26<sup>\*</sup>, wbd/O111<sup>\*</sup>, wzxO103<sup>^</sup>, wzxO145<sup>§</sup>, wzxO45<sup>§</sup>, wzxO121<sup>§</sup>)  
<sup>\*</sup>Perelle et al 2004  
<sup>^</sup>Perelle et al 2005  
<sup>§</sup>USDA 2012

In alternative slide agglutination or WGS  
 (Validation according to ISO 16140)

The document is currently being drafted, will be submitted to CEN/TC 463 WG2 experts and then to CEN TC463 by November 2021

CEN/TC 463 will launch the NWIP votes on both parts when draft EN ISO/NP 13136-2

The full characterization of the isolated STEC strains is achieved by performing all the modules described here. The characterization scheme is not consequential, and the different modules can be applied based on specific needs (regulatory needs, Competent Authority's requests, clients' requests). In each box, reference to the available methods is made. Alternative methods can be used, including WGS (reference the ISO/DIS 23418:2020 Whole genome sequencing for typing and genomic characterization of foodborne bacteria — General requirements and guidance), provided that these are verified according to the reference standard ISO 16140.