

20<sup>th</sup> Annual Workop of the National Reference Laboratories for *E. coli* –  
2-3 October 2025

# Revision of ISO 13136

**Rosangela Tozzoli**

**EU Reference Laboratory for *E. coli***

**Istituto Superiore di Sanità**

**Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Department,  
European Union Reference Laboratory for *Escherichia coli***



# DIS enquiry

Both projects have been approved

## ISO/DIS 13136-1

Ballot started on 14/15 February 2024 and ended on 08 May 2024

CEN level:  
National Members Approving 14 Disapproving 4  
77,778% Approvals

ISO level:  
P-Members Approving 23 – Disapproving 5  
85% Approvals

304 comments received – 140 Editorial

## ISO/DIS 13136-2

Ballot started on 28/29 February 2024 and ended on 22 May 2024

CEN level:  
National Members Approving 15 Disapproving 3  
83,333% Approvals

ISO level:  
P-Members Approving 24 – Disapproving 3  
85% Approvals

262 comments received – 160 Editorial



## Projects ISO 13136-1 and ISO 13136-2

the lead was transferred from CEN to ISO to gain time to produce validation data for the two standards under development (Decision 05/2024).

A new WG has been created at ISO/TC 34/SC 9 level, WG38. Experts from the previous WG and new ones have been enrolled in WG38, created in late June 2024, to continue working on the finalization of the draft EN ISO 13136-1&2.

**Convenor: Rosangela Tozzoli, Convenor Supporting team: Matthias Fischer, 36 Committee Members**



## WG38 activities

During the first WG38 meeting the experts discussed the opportunity to change the reagents for the identification of the *stx* genes indicated in ISO 13136 Part 1, taking into account the fact that they are not able to identify different subtypes of *stx2*, in particular *stx2f*

A first survey among experts highlighted the consensus to indicate the primers and probes described in the literature by Pavlovic et al, 2010

The NRL for *E. coli* Germany has used these reagents for a long time as they are indicated in the Official Method applied at national level and made performance data available

An internal WG38 ballot was launched in November 2024  
To gather the opinion of experts on the opportunity to replace the reagents for the identification of *stx* genes



# Ballot's result

Ballot launched from 15/11/2024 to 12/12/2024 – Results: in favor of the decision to change the primers and probes for *stx*. Resolution 01/2025 and decision 01/2025

Q1: "Are you in favour of replacing the primers and probes for *stx* described in Perelle et al. 2004 (currently indicated in the ISO TS 13136:2012) with those described in Pavlovic et al 2010?" **17 Yes 3 No**

Q2: "Are you in favour of having the Annex describing the primers and probes for *stx* detection as:"  
**13 Normative 7 Informative**

FOODBORNE PATHOGENS AND DISEASE  
Volume 7, Number 7, 2010  
© Mary Ann Liebert, Inc.  
DOI: 10.1089/fpd.2009.0457

Development of a Multiplex Real-Time Polymerase  
Chain Reaction for Simultaneous Detection  
of Enterohemorrhagic *Escherichia coli*  
and Enteropathogenic *Escherichia coli* Strains

Melanie Pavlovic<sup>1</sup>, Ingrid Huber<sup>1</sup>, Henrike Skala<sup>1</sup>, Regina Konrad<sup>1</sup>,  
Herbert Schmidt<sup>2</sup>, Andreas Sing<sup>1</sup> and Ulrich Busch<sup>1</sup>

**The new set of reagents for *stx* detection has now been indicated in the ISO 13136-1**



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Based on the discussion during the WG38 meetings, all requests raised during the DIS inquiry have been considered and ISO 13136-1 and ISO 13136-2 documents have been prepared, modified where appropriate.

The documents were sent to SC9 for a pre-FDIS consultation launched at the end of February 2025 and lasting six weeks.

Pre-FDIS comments addressed – June 2025

During the DIS and pre-FDIS stages the text of ISO 13136-1 and -2 underwent several improvements

Files submitted to ISO TC34 SC9 secretariat

No further technical modifications shall be made



Other modifications during the DIS and pre-FDIS stage

**Possibility to apply the Conventional PCR for testing pools and colonies withdrawn – no more applicable since more stx subtypes are detected with the new primers**

**Informative annex describing the main selective/differential principles of media available for *E. coli* growth**

**WGS is no more indicated as an alternative method for the STEC characterization in ISO 13136-2**



# Structure of the ISO 13136

## Part 1 – Detection and Isolation of STEC



+ enrichment broth (BPW)



Incubation (41,5°C)



Real Time PCR for *stx* genes (Pavlovic et al 2010)



Based on isolation results:  
STEC detected in XX g

## Part 2 – Characterization of STEC



STEC serogroup determination (top 5  
+ O45 and O121), virulotyping  
(including *stx* genes subtyping)

- **CD stage** ✓
- **DIS stage** ✓
- **Pre-FDIS** ✓

During the CEN TC 463 general meeting (2 June 2025)  
CEN TC463 WG2 has been disbanded

**Update on ISO TC34 SC9 general meeting,  
held on 3-6 June 2025**

**Resolution N 2025/42**

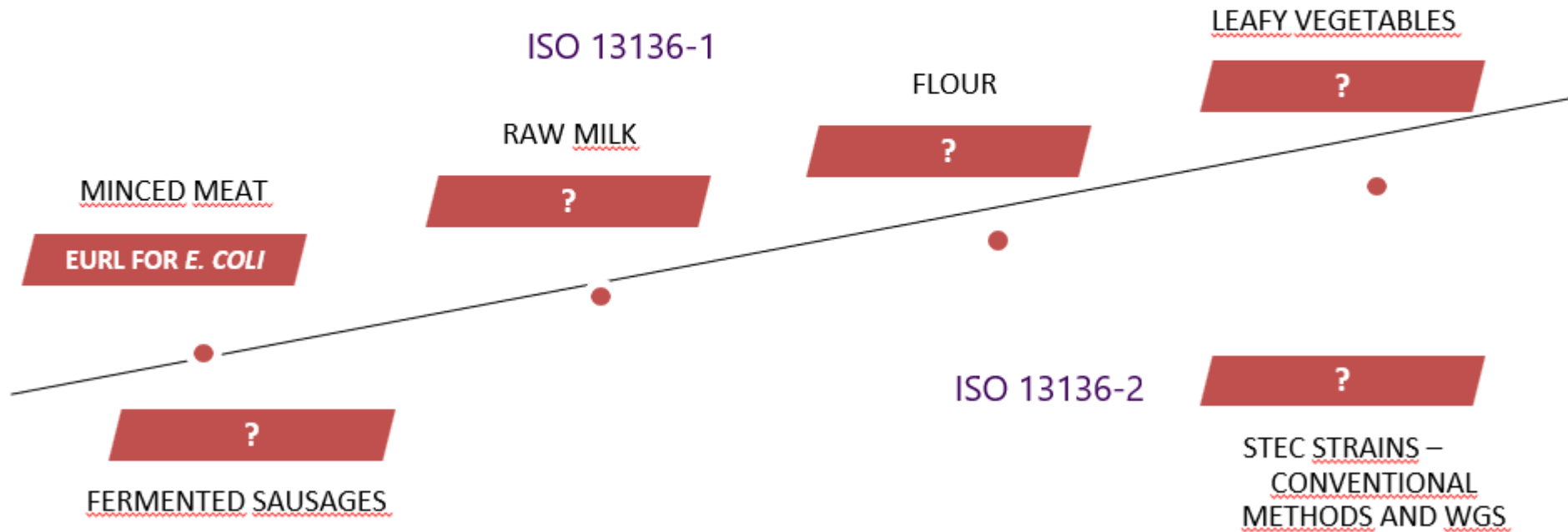
Launch a call at SC9 level for ILS organisers and/or participating laboratories to be ended by the latest mid-September 2025.

SC 9 agreed to put projects ISO 13136-1&-2 on hold for a period of 18 months to perform the validation studies.

In early July 2025 both projects have been put on-hold for 18 months

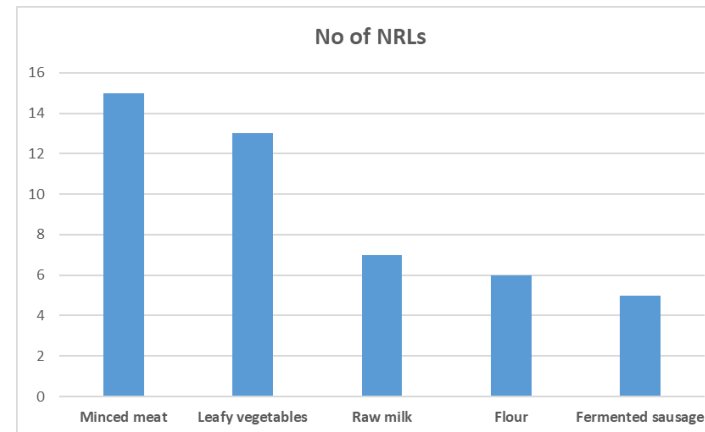


Former CEN TC463 WG2 has proposed a design for the ILS for ISO 13136-1 and ISO 13136-2 validation, submitted to Subgroup WG2-WG3 "Review evaluation/validation protocols for ISO standards" and feedback received



ILS on minced meat - Invitation should be sent in November 2025

In 2023 the EURL for *E. coli* sent a call for collecting interest among NRLs to participate in ILS



Interest in participating in the ILS for the validation of ISO 13136-1

What about the rest of the ILS for validation??



For ISO 13136 Part 1  
Minced meat,  
Raw milk,  
Flour,  
Leafy vegetables,  
Fermented sausages  
spiked with STEC strains isolated from specific matrices.

For ISO 13136 Part 2, the studies will regard all the characterizations included, which can be regarded as separated modules:

- *stx* genes subtyping,
- *eae* and *aggR* determination,
- identification of a set of serogroups

Costs: No dedicated fundings are available, the costs can be shared between ILS organizer and participants.

(Eisma call doesn't apply to validation of standards under ISO lead)

### ISO TC34 SC9 enquiry

WG38 convenor/convenor supporting team will prepare a supporting document for launching the ISO TC34 SC9 call for organizers/participants

The option of laboratories refunding the costs for their participation to the ILS will be explored during the call



Thank  
you!

