

19th Annual Workshop of the National Reference Laboratories for *E. coli* –
30 September – 1 October 2024

Revision of ISO 13136

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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 (TBX+other) to isolate the STEC
(up to 50 colonies tested)

Based on isolation results:
STEC detected in XX g

Part 2



stx genes subtyping
Detection of *eae* and *aggR*
STEC serogroup determination (top 5
+ O45 and O121)

Last Year: ISO 13136-1 and -2 had passed the CD stage

June 2023 CEN TC463 general meeting Action 16/2023: projects EN ISO 13136-1 & -2

According to CEN rules, a draft containing validation data should be issued by December 2023. This is not feasible for ISO 13136-1 and ISO 13136-2 projects, therefore CEN TC463 will launch a consultations to define the future of the projects. The options (reported as Action 16/2023) include:

- ✓ Option 1: to go to enquiry stage without validation data, but to organize an ILS before the final stage (FDIS draft). A pre-FDIS consultation would be organized;
- ✓ Option 2: to go to enquiry stage (DIS draft) without validation data and to publication without validation data. An amendment to include validation data could be prepared at a later stage;
- ✓ Option 3: to change the type of deliverable and go for DTS vote and publication of a TS without validation data. A revision as full standard could be possible after the completion of the ILS;
- ✓ Option 4: to change the lead of the project to ISO lead to get the possibility to ask for an on-hold period to conduct the ILS. However, this will conduct to the creation of a

The amended documents concerning ISO 13136-1 and ISO 13136-2 have been prepared and submitted to CEN secretariat in December 2023, to make them available for the DIS/CEN Consultation

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

On 5 June 2024 an online meeting of CEN TC463 WG2 was organised

Aim: discussing comments received during DIS consultation
19 experts participating

Several comments concerning ISO 13136-1 have been addressed together

Some WG2 experts proposed to carry out an internal ballot, once all the comments will be addressed, to collect the consensus on the responses and the finalized documents prepared

Workplan:

reply to comments and prepare the amended documents
Share them with WG2 group
launching the internal ballot

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

The forms describing the ILS design have been prepared and submitted to the to the joint subgroup of WG 2 “Statistics” and WG 3 “Method validation” for their review in December 2023 – the comments received are available in the CEN platform

Particular care on the definition of the spiking levels taking into account that for one level fractionary results should be collected

Reconsider to carry out studies on larger test portions

Discuss the opportunity to include primary production stage samples

Concerning ISO 13136-2, depending on how the strains are chosen, the data could be used for more than 1 feature

Possibility to use data from PTs run from EURL for *E. coli* for the *stx* subtyping module of ISO 13136-2

Plan for validation

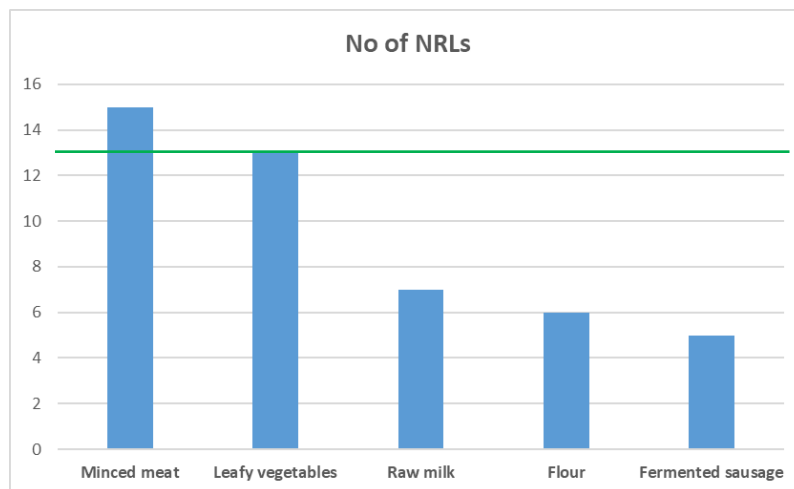
There is one candidate for the organization of the ILS only for one Matrix
EURL for *E. coli*, on minced meat, in 2025

The optimum would be that Laboratories in different continents could organize different ILS

Possibility to have ILS participants supporting also the expenses of samples' preparation?

One company had candidated to support the organization of ILS for ISO 13136 Part 2
withdrew the candidacy

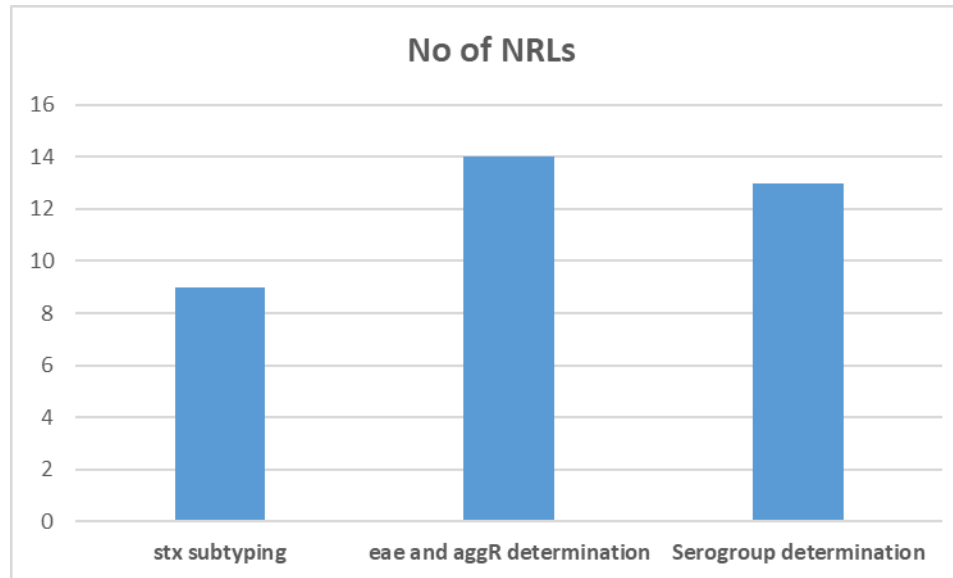
NRLs for *E. coli* expression of interest in participating in ILS



No of Labs to
be enrolled in
each ILS=13

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

NRLs for *E. coli* expression of interest in participating in ILS



No of Labs to
be enrolled in
each ILS=13

Interest in participating in the ILS for the validation of EN ISO 13136-2

CEN TC463 Plenary Meeting in June 2024

WG 2 Shiga toxin producing Escherichia coli (STEC)

Decision 05/2024: *project EN ISO 13136 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 STEC*
Part 2: Horizontal method for the characterization of STEC isolates

CEN/TC 463 decides to transfer the lead from CEN to ISO to produce validation data by ILS.

CEN/TC 463 recommends ISO/TC 34/SC 9 to keep Rosangela Tozzoli as convenor of the future WG to be created at ISO/TC 34/SC 9 level, and as Project leader of projects EN ISO 13136-1&-2.

CEN/TC 463 asks the convenor to download the documents from WG 2 as “zip” files to the future WG at ISO/TC 34/SC 9 level.

CEN/TC 463 invites NSBs to nominate their current WG 2-experts within the future WG at SC 9 level.

CEN/TC 463/WG 2 is asked to keep working on the DIS-comments during the transition from CEN to ISO.

CEN/TC 463 invites ISO/TC 34/SC 9 to consider an intermediate “pre-FDIS” step regarding projects EN ISO 13136-1&-2.

CEN/TC 463 asks WG 2 to consider the conditions of use of patented primers and probes in Annex D.

35 Members

3 Document Monitor

1 Technical programme manager

First meeting: 2 October 2024 –hybrid event