

16th Annual Workshop of the National Reference Laboratories for *E. coli* –
online event, 18-19 October 2021

**Update on the activities carried out by CEN TC463 WG2 on
Shiga Toxin producing *Escherichia coli***

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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)

In 2022 the EN ISO 13136 projects have been approved at ISO and CEN level

EN ISO 13136-1

Result of voting

CEN level: 10 Yes -1 No

ISO level: 21 Yes – 1 No

EN ISO 13136-2

Result of voting

CEN level: 10 Yes -1 No

ISO level: 19 Yes – 1 No

Comments on both parts have been received
Results of voting, as well as the comments to be addressed, have been shared with WG2 experts



Discussion on optionally reporting the Real Time PCR positive results in the notes field of test report: will be closed by mid-October

Second round of com

Update on CEN TC463 general meeting, held on 26 June 2023

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 new WG and call for experts;
- ✓ Option 5: to withdraw the project and reactivate it once the ILS will be conducted.

ISO/TC 34/SC 9 will be invited to launch the same consultations.

On 4 October 2023 CEN WG2 experts met to design the validation program

Nineteen experts participated (face-to-face and online)

The main hindrance for the validation (i.e. organization of Inter-laboratory studies) is the lack of a dedicated budget, and EURL for *E. coli* can't support the whole validation study on its budget

ISO 13136 Part 1

Experimental Design according to ISO 16140-2

Evaluation of Sensitivity and Specificity and LOD₅₀ (CI 95%)

- at least 10 sets of accepted data from 10 collaborators (at least 5 different organizations)
- 3 levels of contamination: blank, and two contamination levels (one of these should produce fractional positive results)
 - At least 8 blind replicates per level
 - 240 results per ILS
 - 5 different matrices in case of horizontal application of the method

Nr.	(Food) category
1	Minced meat
2	Raw Milk
3	Flour
4	Leafy vegetables
5	Fermented sausage

Volunteers to participate in any of the ILS?

We already have one candidacy for coordinating the validation program of ISO 13136-2 to the ISO/TC34/SC 9 ad hoc group

The design of the ILS shall be made by WG2 experts

For part 2 of ISO 13136, validation in accordance with ISO 16140-6 'Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures' is the most appropriate. Validation study as only strains shall be sent and tested instead of food samples

Targets:

Ten *stx* gene subtypes (*stx1a*, *stx1c*, *stx1d*, *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f*, *stx2g*)
eae and *aggR*

Serogroups O157, O26, O111, O145, O103, O121, O45

16 strains for inclusivity and 8 for exclusivity

Possibility to use data from EURL for *E. coli* PTs