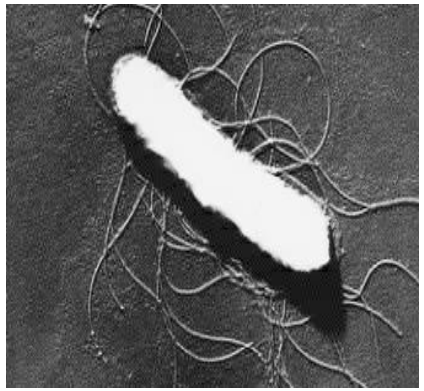
PT37

37TH INTER-LABORATORY STUDY ON THE DETECTION OF SHIGA TOXIN-PRODUCING *E. COLI* (STEC) IN SPROUTS (PT37) - 2023



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DESIGN AND OBJECTIVES OF THE STUDY

- To improve the preparedness of the NRLs towards testing sprouts in compliance with Regulation (EU) No 209/2013;
- To improve the preparedness of the NRLs towards the detection and isolation of STEC strains not belonging to the O157 serogroup
- To give further support to the NRLs for the accreditation of the ISO TS 13136:2012.
- The PT was organized in compliance with the requirements of ISO 17043:2010.

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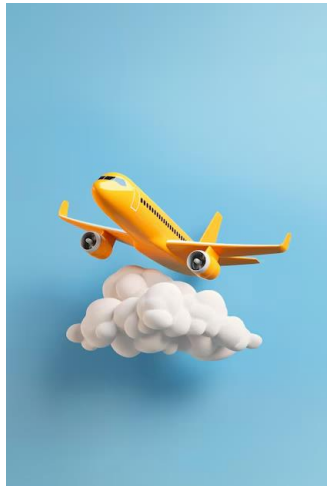
The study was run in October 2023

Invitation sent to NRLs in August 2023

Laboratories were requested to identify the presence of STEC using the ISO TS 13136:2012 method, taking into account the adaptation provided by the EU Reference Laboratory for *E. coli* (EURL-VTEC) for the specific detection of STEC O104:H4

PARTICIPANTS

33 NRLs from the 24 EU Member States and three EFTA Countries participated in the study.



The stability and homogeneity of the samples were evaluated according to the requirements of ISO 17043:2010.

Contamination ⇒ 20 October 2023
Shipping ⇒ 23 October 2023

all the NRLs received the samples within 24 hours

https://it.freepik.com/foto-vettori-gratuito/aereo-giallo

SAMPLES CHARACTERISTICS

- **3 samples of 25g each**

Sample 1	Sample 2	Sample 3
negative	Low : 5 CFU/g	High : 50 CFU/g

Contaminant (*Genotype*): **ED0773, STEC O187:H28 (*stx1*-, *stx2*+, *eae*-), originally isolated from seeds**

-The sprouts used have been acquired as a single batch from a local producer and contained a natural background microflora of $9.3X10^4$ bacterial CFU per gram of sprouts ($1X10^4$ CFU of enterobacteria per gram of sprouts)

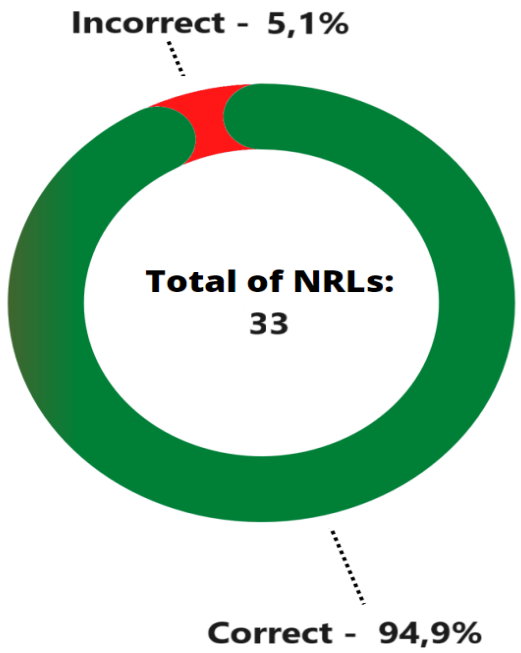
- Two 25 g portions were tested for the absence of STEC: both samples were negative for all the target genes

Stability: sprouts samples spiked on the 13th of September 2023 and tested by ISO TS 13136:2012 after 0, 5, 7, and 12 days since the initial contamination. Real Time PCR screening was positive for *stx2* even after 12 days from the spiking. Isolation was successful at all time points for the high level of contamination, and by day 5 for the low level

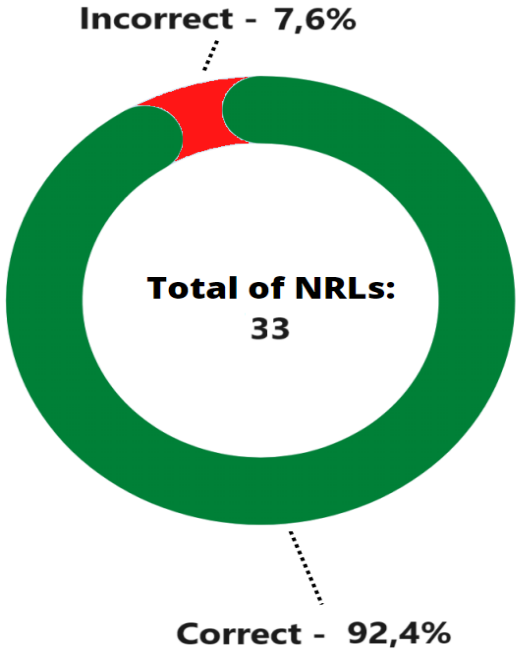
Homogeneity: evaluated on the 24th of October, 10 replicates of each sample tested – expected results were obtained

RESULTS-I

PERCENTAGE OF LABORATORIES REPORTING THE CORRECT SCREENING RESULTS (A) AND ISOLATING (B) THE STEC STRAIN (GREEN: CORRECT RESULT; RED: INCORRECT RESULT).



a) Screening Step: % of Laboratories correctly detecting STEC in the spiked sample (green: correct results; red: incorrect results).



b) Isolation Step: % of Laboratories that successfully isolated the STEC strain detected in the screening step (green: correct results; red: incorrect results).

Results-II

Real-time PCR detection of virulence and serogroup-associated genes in the enrichment cultures

(green boxes: correct results and red boxes: incorrect results)

	Sample 1	Sample 2	Sample 3
Gold standard	Negative	*stx2*	*stx2*
L001			
L002			
L004			
L006		none	
L014			
L016			
L017			
L018			
L025			
L144			
L222			
L230			
L256			
L258			
L327		*stx1; stx2*	*stx1; stx2*
L337			
L370			
L403			
L462			
L522			
L615			
L674			
L685			
L697			
L705			
L708			
L758			
L846			
L896			
L972			
L976			
L986			
L993	*stx2*		none

RESULTS-III

ISOLATION AND GENOTYPING OF STEC STRAINS FROM THE SPROUTS SAMPLES

(GREEN BOXES: CORRECT RESULTS AND RED BOXES: INCORRECT RESULTS)

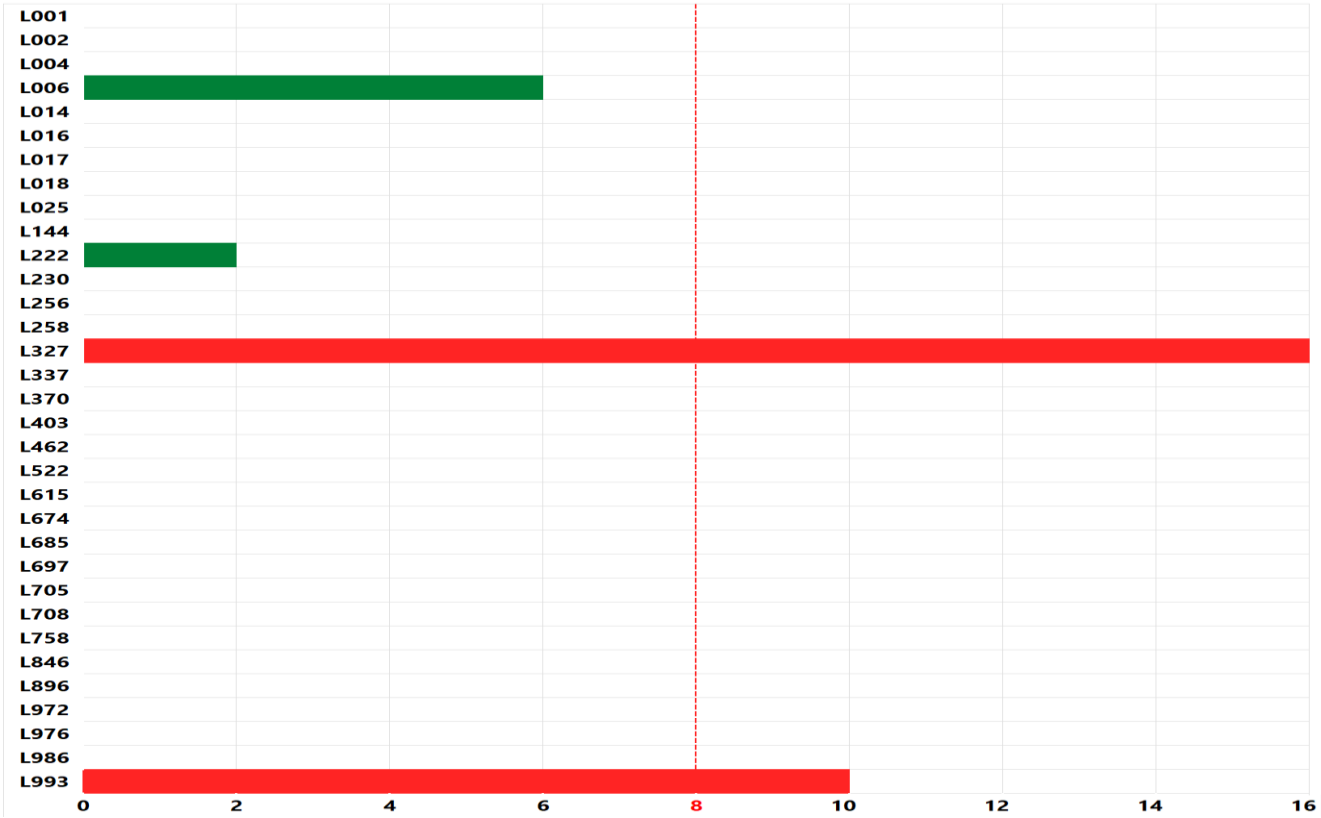
	Sample 1	Sample 2	Sample 3
Gold standard	Not done	*stx2* ; OND (O187)	*stx2* ; OND (O187)
L001			
L002			
L004			
L006		isolation not achieved	
L014			
L016			
L017			
L018			
L025			
L144			
L222		isolation not achieved	
L230			
L256		*	*
L258			
L327		*stx1; stx2*	*stx1; stx2*
L337			
L370			
L403		*	*
L462			
L522			
L615			
L674			
L685			
L697			
L705			
L708		*	*
L758			
L846			
L896			
L972			
L976			
L986		*	*
L993	isolation not achieved		isolation not done

Some NRLs (indicated with a star in this figure) correct reported that the isolated STEC was of O187 serogroup

COLLECTION AND EVALUATION OF RESULTS

- The results were collected through a dedicated online form.
- Participants were asked to indicate their laboratory code (Lab code) in the form.
- The competence of the Laboratories in identifying the stx1 and stx2 genes in the enrichment cultures of the two samples was evaluated by assigning four penalty points for each incorrect result.
- The performance of each Laboratory in isolating and characterizing the STEC strain was evaluated by assigning two penalty points in case of failure to isolate STEC from samples 2 and 3.
- The sum of the penalty points obtained in the different phases of the procedure produced a total score, used to evaluate the overall performance of the participating Laboratories.

EVALUATION OF THE NRLS PERFORMANCE IN THE PT PROCEDURES (SCREENING AND ISOLATION STEPS).



The proficiency of laboratories that obtained a score above 8 was considered unsutisfactory

EVALUATION OF THE PERFORMANCE OF THE METHOD

- The calculation of ***Se* in the screening step** was performed based on the results provided by 33 and 32 participating NRLs, respectively. The results reported for sample 3 by L993 were excluded, as an inversion of samples 1 and 3 was suspected.
- The ***Sp* in the screening step** was calculated on the results provided by 32 participating NRLs. The results from L993 were excluded.

Table 1. Sensitivity and Specificity of the method.

	Se (low level)	Se (high level)	Sp
stx1	NA	NA	98.0%
stx2	97.0%	100%	100%
eae	NA	NA	100%

Sensitivity: *Se* = [true positive / (true positive+ false negative)] x 100
Specificity: *Sp* = [true negative/ (true negative+ false positive)] x 100

CONCLUSIONS FOR EU-NRL PT37

The analysis of the results provided by 33 Laboratories participating in PT37 induces the following conclusions:

1. A high participation rate was observed, confirming the consolidation of the network of National Reference Laboratories for *E. coli*;
2. The virulence genes of the contaminating STEC strain were identified with satisfactory sensitivity in the spiked samples.
3. The majority of the laboratories could isolate the STEC from both the samples with low level and high level of contamination.
4. Four laboratories were able to correctly characterize the isolated strain as belonging to O187 serogroup.

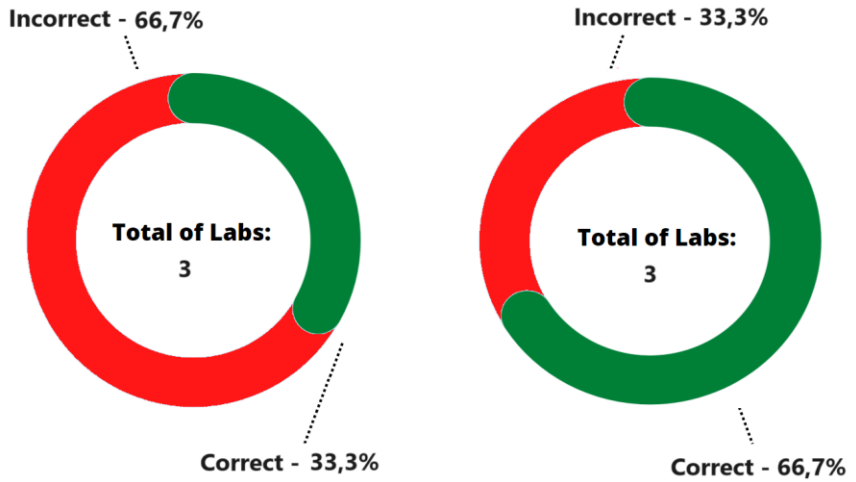
NON-EU NRLS PARTECIPANTS

- Three non-EU NRLs participated in the study:

- Egypt, The Central Laboratory of residues analysis of pesticides and heavy metals in food
- UK, United Kingdom Health Security Agency (UKHSA), Food, Water & Environmental Microbiology - London
- UK, United Kingdom Health Security Agency (UKHSA), Food, Water & Environmental Laboratory - Porton

NON-EU NRLS PARTECIPANTS

Figure 1. Percentage of Laboratories reporting the correct screening results (a) and isolating (b) the STEC strain (green: correct result; red: incorrect result).

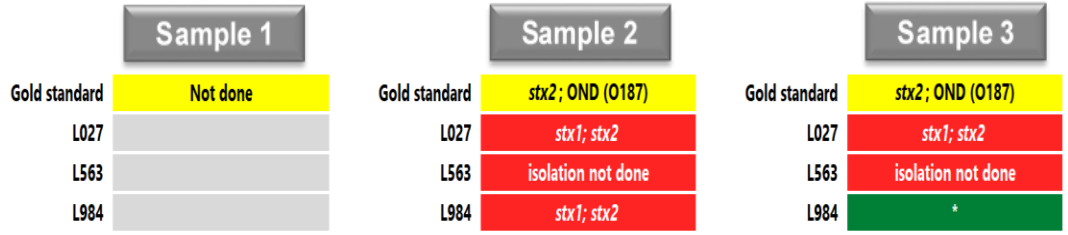


Screening Step: % of Laboratories correctly detecting STEC in the spiked sample (green: correct results; red: incorrect results).

Isolation Step: % of Laboratories that successfully isolated the STEC strain detected in the screening step (green: correct results; red: incorrect results).

Figure 2. Real Time PCR detection of virulence and serogroup-associated genes in the enrichment cultures (yellow boxes represent the gold standards; green boxes: correct results and red boxes: incorrect results).

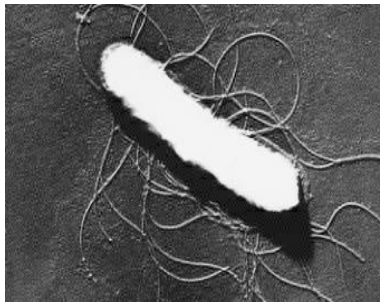
Figure 3. Isolation and genotyping of STEC strains from the sprouts samples (Yellow boxes represent the gold standards; green boxes: correct results and red boxes: incorrect results; *: correct identification of O187 serogroup).



Evaluation of the non NRLs performance in the PT procedures (screening and isolation steps).

Conclusions for non-EU NRL PT37

- The presence of a contaminating STEC in samples 2 and 3 was correctly identified by all of them at the screening step.
- Two laboratories reported the presence of both *stx1* and *stx2* genes, but were using a Real Time PCR detection kit not able to discriminate between the two types
- One laboratory (L563) uncorrectly reported the presence of eae in the two spiked samples. Moreover, this Lab did not attempt isolation of the contaminating strain from samples 2 and 3.
- L984 performed WGS on the strain isolated from sample 3, and correctly identified both the presence of *stx2* virulence gene and the O187:H28 serotype. Therefore, despite obtaining several penalty points, L027 and L984 were able to identify and isolate a contaminating STEC strain from both the spiked samples.



- To all the laboratories that participated
- To the colleagues of the LNR National Reference Laboratory for *E. coli*
- And thanks to all of you for your attention