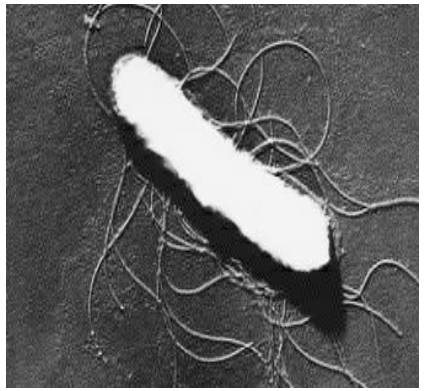
PT39

REPORT OF THE 39TH INTER-LABORATORY STUDY ON THE DETECTION OF SHIGA TOXIN-PRODUCING *E. COLI* (STEC) IN CHEESE (PT39) - 2024



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DESIGN AND OBJECTIVES OF THE STUDY

- To improve the preparedness of the NRLs towards testing cheese samples for the detection and isolation of STEC according to the ISO TS 13136:2012;
- To improve the preparedness of the NRLs towards the detection and isolation of STEC strains not belonging to the O157 serogroup;
- To give support to the NRLs for the accreditation of the ISO TS 13136:2012.

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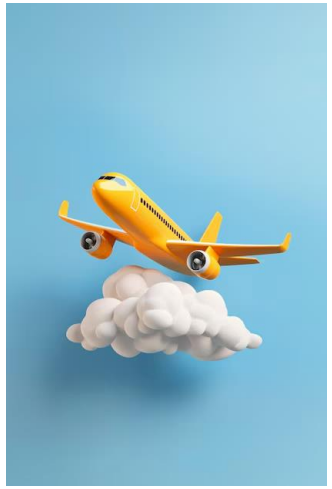
The study was run in May 2024

Invitation were sent to NRLs in February 2024

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

30 laboratories consisting in twenty-seven NRLs from 22 EU Member States and three NRLs from EFTA Countries, participated in the study.



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

Contamination ⇒ 17 May2024
Shipping ⇒ 20 May 2024

all the NRLs received the samples within 24 hours

SAMPLES

- 2 samples of 25g each

Contaminant (*Genotype*)	Contamination level in:	
	Sample 1	Sample 2
Strain C1188-02, STEC O26:H11 (*stx1+, stx2+, eae+*)	-	2 CFU/25 g

Two samples consisting of 25 g of cheese have been assayed for the presence of STEC by applying the ISO TS 13136:2012 method. Both samples were negative for all the target genes at the screening.

The cheese was purchased from a local retailer. The presence of natural background microflora was evaluated by plating on TSA and MacConkey agar but no growth was observed on both media.

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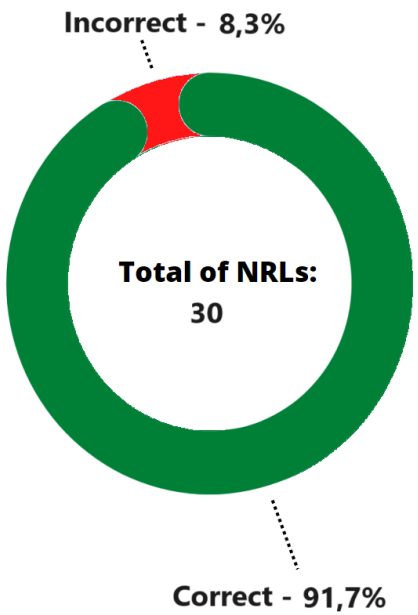
Stability was assessed using samples spiked on 13th February 2024
and tested by ISO TS 13136:2012 after **0, 2, 6, and 9 days** since the initial contamination.

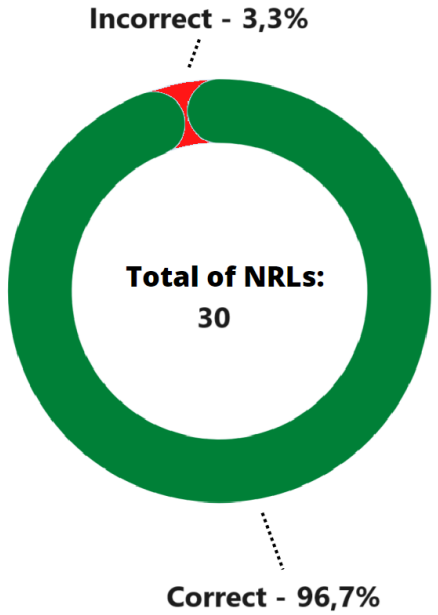
The Real Time PCR screening for the STEC target genes and isolation were both successful for all the samples spiked at all the time pointstarget genes and isolation were both successful for all the samples spiked at all the time points.

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 per laboratory (values in the yellow boxes are the gold standards; green boxes: correct results; red boxes: incorrect results).

	Sample 1	Sample 2
Gold standard	Negative	*stx1; stx2; eae;* O26
L001		
L002		*stx1; stx2; eae*
L003		
L014		
L017		
L018		
L025		
L144		
L222		
L230		
L258		
L327		
L337		
L370		
L403		
L462		
L522		
L615		
L674	*stx1; stx2; eae;* O26	
L685		
L697		
L705		*stx1; stx2; eae*
L708		
L758		*stx1; stx2; eae*
L846		*stx1; stx2; eae*
L896		
L972		
L976		
L986		
L993		

RESULTS-III

ISOLATION AND GENOTYPING OF STEC STRAINS FROM THE CHEESE SAMPLES (VALUES IN THE YELLOW BOXES ARE THE GOLD STANDARDS; GREEN BOXES: CORRECT RESULTS; RED BOXES: INCORRECT RESULTS).

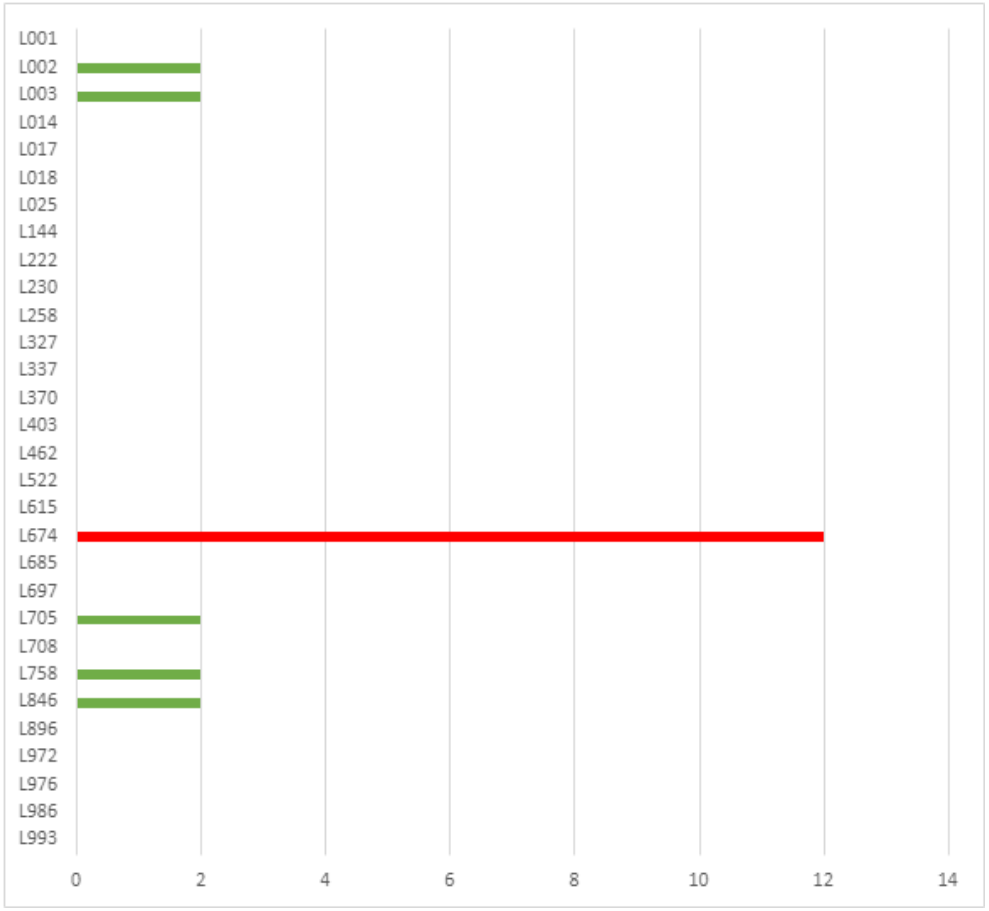
	Sample 1
Gold standard	Not done
L001	
L002	
L003	
L014	
L017	
L018	
L025	
L144	
L222	
L230	
L258	
L327	
L337	
L370	
L403	
L462	
L522	
L615	
L674	isolation not achieved
L685	
L697	
L705	
L708	
L758	
L846	
L896	
L972	
L976	
L986	
L993	

	Sample 2
Gold standard	*stx1; stx2; eae;* O26
L001	
L002	
L003	isolation not achieved
L014	
L017	
L018	
L025	
L144	
L222	
L230	
L258	
L327	
L337	
L370	
L403	
L462	
L522	
L615	
L674	
L685	
L697	
L705	
L708	
L758	
L846	
L896	
L972	
L976	
L986	
L993	

COLLECTION AND EVALUATION OF RESULTS

- The results were submitted through a dedicated Microsoft Form.
- The participating laboratories had to indicate in the Form their Lab code, provide the information on the arrival date, temperature, and quality of the samples, as well as the results obtained for each test of the blind samples.
- The results reported were categorized by assigning penalty points.
- **Four penalty points** to each incorrect or missing result concerning the identification of *stx1* and *stx2* genes, and **two penalty points** for the incorrect identification of *eae* gene as well as the top-5 serogroups-associated genes.

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



The sum of the penalty points obtained in the different steps of the procedure originated a total score, used to evaluate the performance of the NRLs in the PT

The results reported were categorized by assigning penalty points.

EVALUATION OF THE PERFORMANCE OF THE METHOD

- Sensitivity (*Se*) and Specificity (*Sp*) were calculated for the PCR screening of *stx1*, *stx2* and *eae* genes, and for the isolation of the STEC strain according to the following formulas:

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

Table 1. Sensitivity and Specificity of the method.

	Se	Sp
stx1	100%	**96.7%**
stx2	100%	**96.7%**
eae	**100%**	**96.7%**

The calculation of ***Se* in the isolation step** was based on the results provided for Sample 2 by all the 30 participating NRLs. The Sensitivity was calculated as 96.7%

CONCLUSIONS FOR EU-NRL PT

The analysis of the results provided by 30 Laboratories participating in PT39 induces the following conclusions:

1. A high participation rate was observed, confirming the consolidation of the network of National Reference Laboratories (NRLs) for *E. coli*;
2. The virulence genes of the contaminating STEC strain were identified with 100% sensibility in the spiked sample.
3. Four NRLs did not detect O26 serogroup for Sample 2 in the screening step.
4. The majority of the laboratories could isolate the STEC from the spiked sample.
5. Only one participating laboratory presented a non-satisfactory performance, possibly due to contamination of Sample 1 during the analysis.

NON-EU NRLS PARTECIPANTS

- Four non-EU NRLs participated in the study:

✓ Macedonia, Faculty of Veterinary Medicine Skopje

✓ Egypt, The Central Laboratory of residues analysis of pesticides and heavy metals in food

✓ UK, United Kingdom Health Security Agency (UKHSA), Porton

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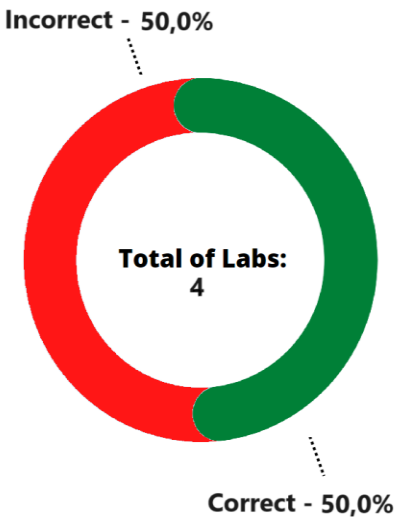
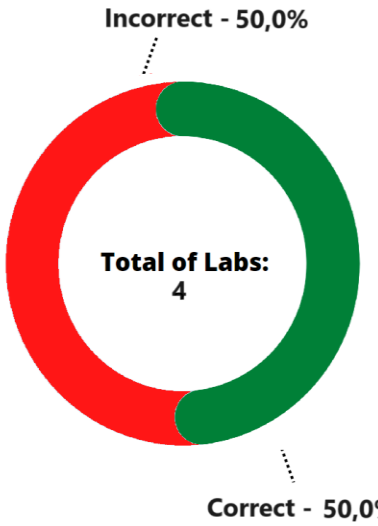
NON-EU NRLS PARTECIPANTS

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 per laboratory (values in the yellow boxes are the gold standards; green boxes: correct results; red boxes: incorrect results).

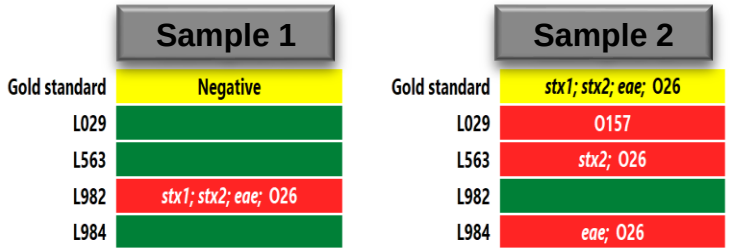
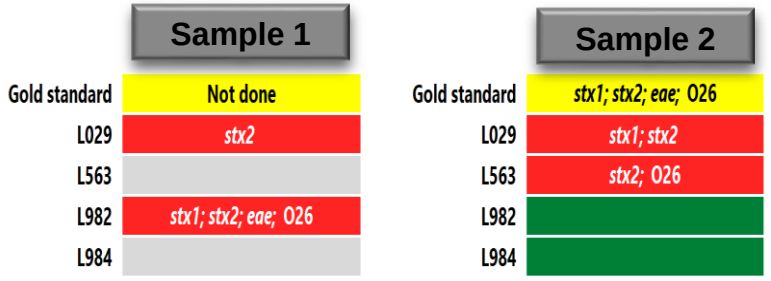
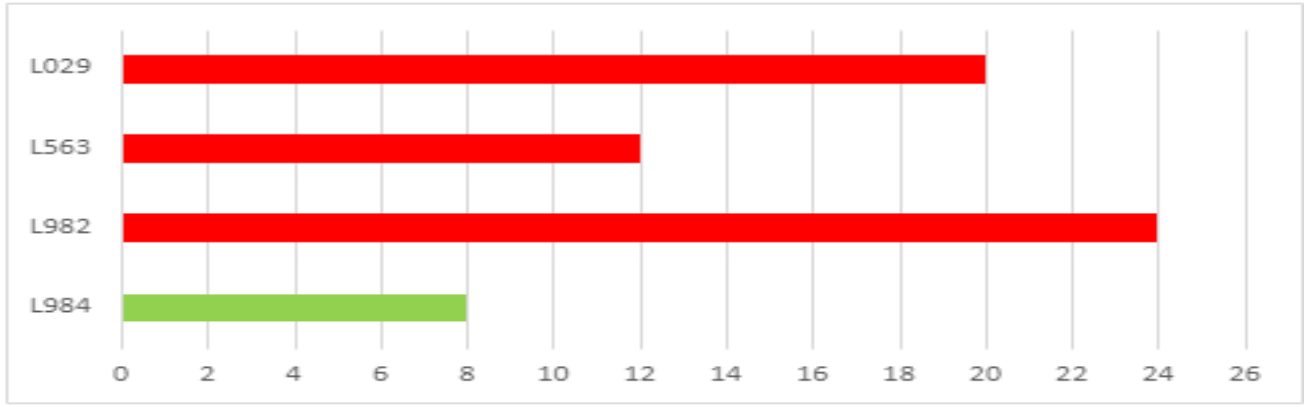


Figure 3. Isolation and genotyping of STEC strains from the cheese samples (values in the yellow boxes are the gold standards; green boxes: correct results; red boxes: incorrect results).

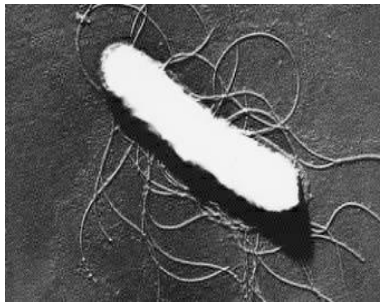


Evaluation of the non NRLs performance in the PT procedures (screening and isolation steps).



CONCLUSION for non EU NRL PT

1. The majority of the participants obtained scores corresponding to unsatisfactory performance.
2. One laboratory (L982) detected the presence of STEC and could isolate it from both test samples. This NRL reported in the notes field that it was evident that the amount of STEC in one of the two samples was much higher than the amount in the other, suggesting that cross contamination may have occurred.
3. The remaining three laboratories provided incorrect results concerning the detection of the virulence genes either in the screening step, or in the isolated STEC strain. In addition, one laboratory mis-detected the serogroup-associated gene in the screening.
4. It has to be considered that the participants received the test samples in a range of 3 to 7 days from the shipment, the latter being at the limit or out of the estimated range of stability (9 days). The samples were received not refrigerated, but all the NRLs reported to have isolated a STEC strain from one of the two samples, suggesting that the samples were still stable for the analysis.
5. The possibility to attend training stages is open to non-EU NRLs too, and the underperforming Laboratories may exploit the participation to these trainings to fix technical problems they may encounter when dealing with STEC analyses.



- 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