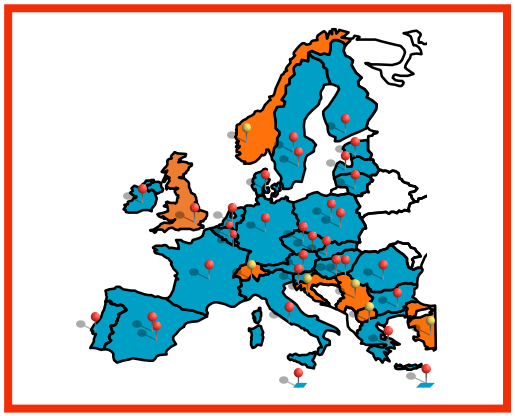
PT34

Detection of Shiga toxin-producing *E. coli* (STEC) in sprout spent irrigation water



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

Objectives and Design of the study

The objective of PT34 consisted in the examination of artificially contaminated sprout spent irrigation water

The participating Labs were requested to carry out the same pre-treatment procedure used in PT30 (EURL-VTEC_Method_09, available at the EURL-VTEC website), based on the centrifugation of the spent irrigation water and enrichment carried out at 41.5°C and then apply ISO TS 13136:2012 for detecting the presence of STEC

- Detection of the main STEC virulence genes (*eae* and *stx* genes)
- Isolation and characterization of STEC strains

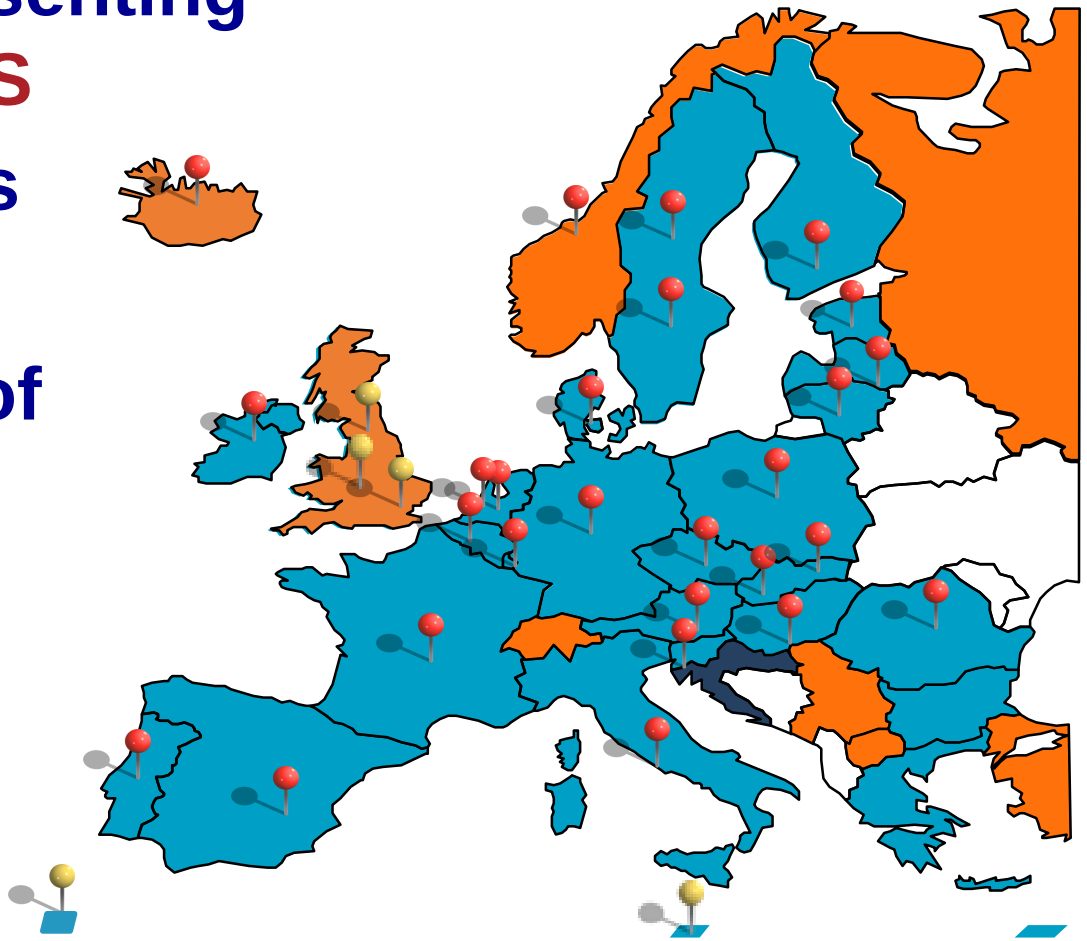
The PT34 was organized and run in October 2022

PT34: Participants

25 NRLs representing
22 EU MS
+ 2 EFTA NRLs

+ 3 Non-EU NRLs of
Chile
Egypy
UK (3 Labs)

+ 19 Italian OLs



Test Samples

The spent irrigation water used in the study was obtained from a local sprout producer who collected the irrigation water after 48h from the beginning of red radish sprout production process (Reg. (EU) 209/2013)

The presence of a natural background microflora has been evaluated ➡ $2x10^6$ CFU/ml Background microflora

➡ Water samples were negative at the Real Time PCR screening for the gene targets of STEC according to the ISO TS 13136:2012

Stability test:

- The stability test showed that all the samples were positive at the Real Time PCR screening after 5 days from the spiking
- Two specimens, each consisting of 200 ml of water in sterile plastic bottles, potentially contaminated with STEC, were sent in the blind to the participating laboratories.

PT34: Samples

Contaminant (*Genotype*)	Contamination level in:	
	Sample 1	Sample 2
C210-03 STEC O157 *(stx1+; stx2+, eae+)*	-	100 CFU/ml

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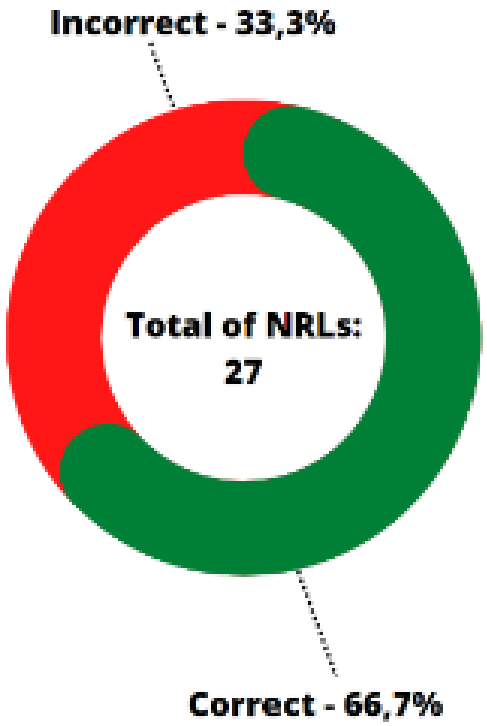
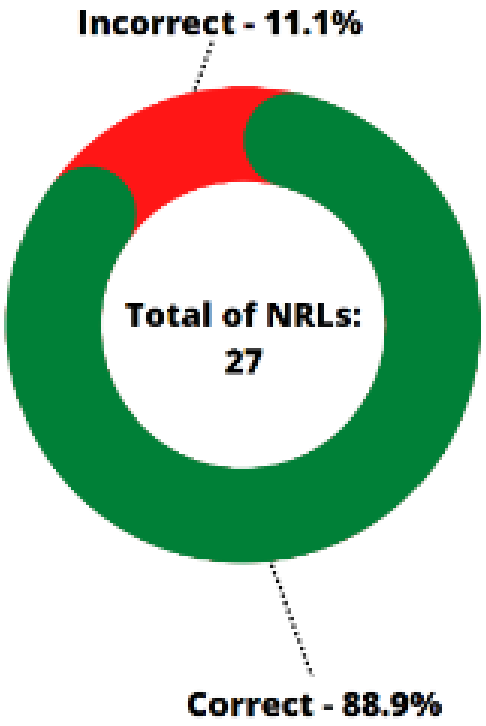
C210-03 STEC O157 (*stx1+; stx2+, eae+*)	-	100 CFU/ml

- ✓ The homogeneity test was performed on 6 randomly selected samples for each of the two sample types
- ✓ Samples labelled with randomly generated numerical codes were shipped refrigerated on the 17th of October 2022
- ✓ Results submitted through an on-line form from 27 labs

Percentage of Laboratories correctly detecting (a) and isolating (b) the STEC strain contaminating sample 2

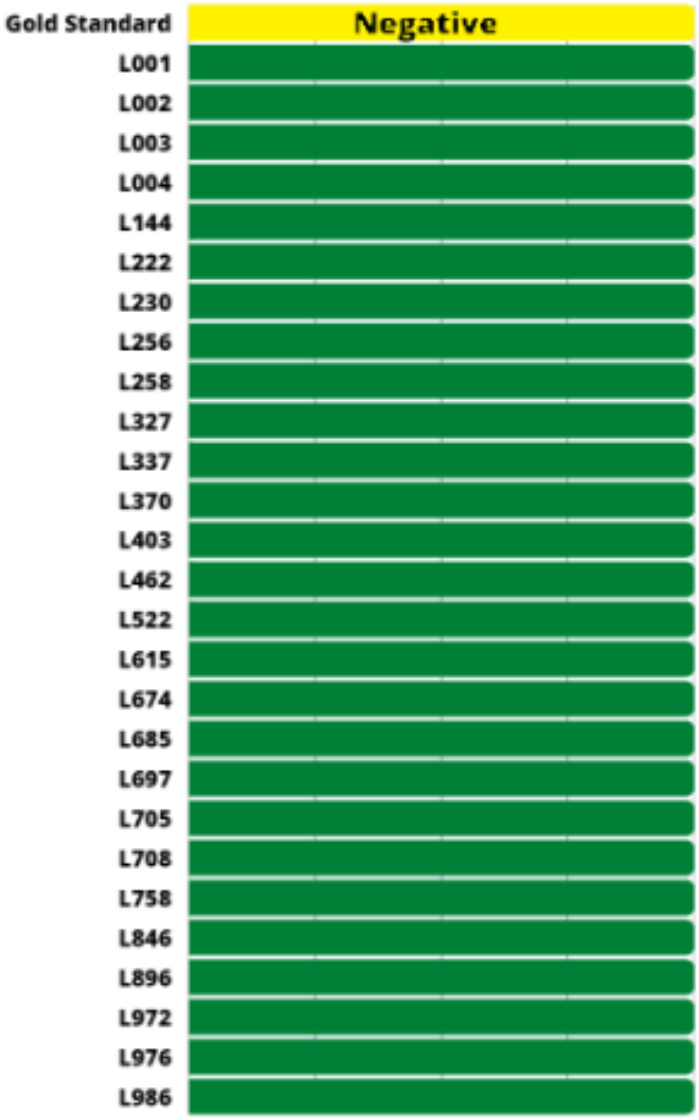


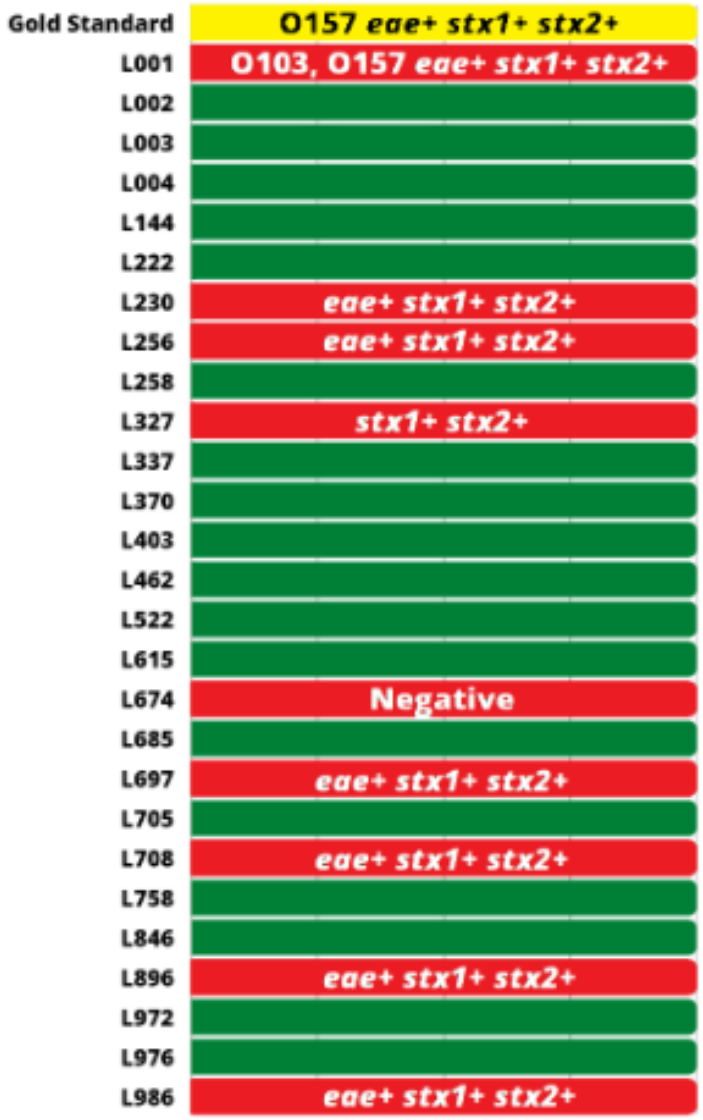
a. Screening Step



b. Isolation Step

Real-time PCR detection of virulence and serogroup associated genes in the enriched cultures from EU NRLs





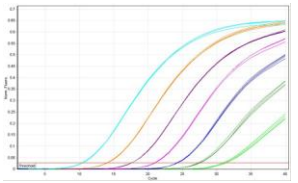
Isolation and genotyping of STEC strain from the spent irrigation water

Gold Standard	Not Done
L001	
L002	
L003	
L004	
L144	
L222	
L230	
L256	
L258	
L327	
L337	
L370	
L403	
L462	
L522	
L615	
L674	
L685	
L697	
L705	
L708	
L758	
L846	
L896	
L972	
L976	
L986	

Gold Standard	O157 *eae+ stx1+ stx2+*
L001	Not achieved
L002	
L003	
L004	
L144	
L222	
L230	
L256	
L258	
L327	
L337	
L370	
L403	
L462	
L522	
L615	
L674	Not done
L685	
L697	
L705	
L708	
L758	
L846	
L896	Not achieved
L972	
L976	
L986	

Most of the participants were able to isolate the contaminating STEC strain

Evaluation of the NRLs performance in the Real Time PCR screening step:

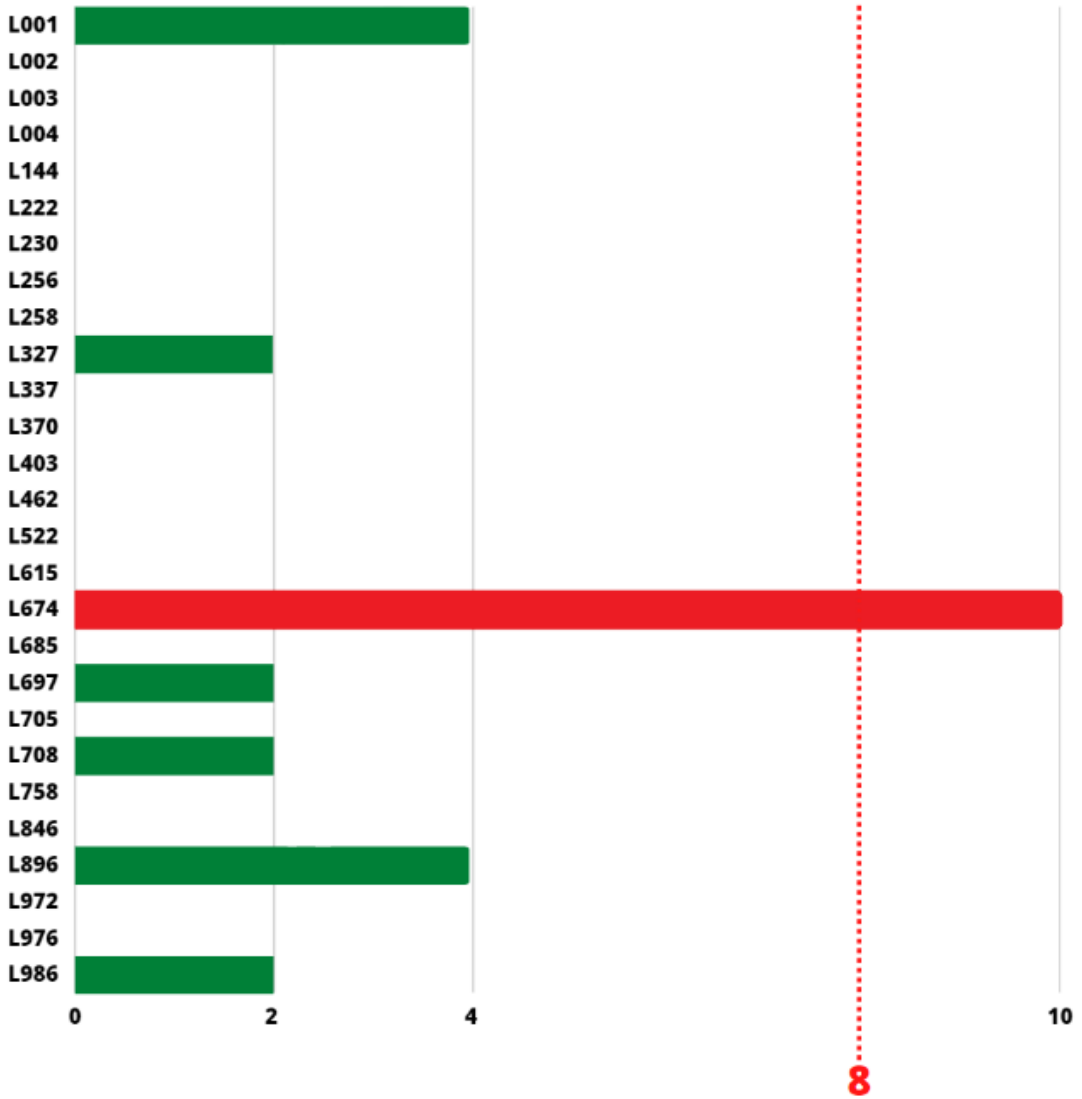


- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx1* and *stx2* genes
- **2 penalty points** for the incorrect detection of *eae* gene
- **2 penalty points** to each incorrect or missing result concerning the identification of the top-5 and O104 serogroups

Evaluation of the NRLs performance in the isolation of STEC strain:

- **2 penalty points** to the lack of isolation of STEC from sample 2
- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx1* and *stx2* genes
- **2 penalty points** for the incorrect detection of eae gene
- **2 penalty points** for the identification of a serogroup different from that of the STEC strain used to contaminate the samples (O157)

Evaluation of the EU NRLs performance in the PT procedures (screening + isolation steps)



The labs that scored higher than 8 were considered under-performant

Only one laboratory did not comply with the definition of satisfactory proficiency

Concluding remarks

- The analytical results, provided by 27 laboratories, confirmed the suitability of the treatment procedure for spent irrigation water developed by the EURL-VTEC
- The virulence genes of the contaminating STEC O157 strain were identified with satisfactory sensitivity in the spiked sample.
- Many laboratories didn't report the presence of the *rfbEO157* gene in the screening
- STEC O157 was isolated by the majority of laboratories detecting STEC
- One participating laboratory presented a non-satisfactory performance and it has been contacted

Thanks to all the participants in the study and thank you all for your attention!