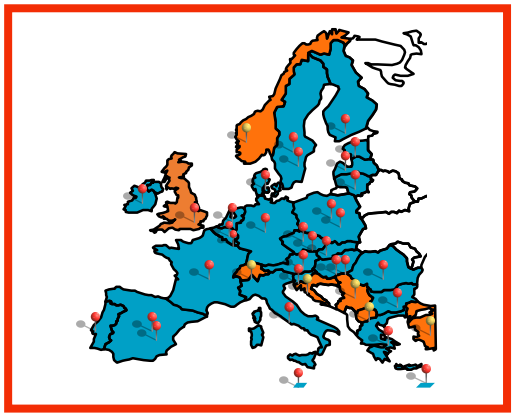
PT30

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 PT30 consisted in the examination of artificially contaminated sprout spent irrigation water expanding the observations related with the testing of sprout spent irrigation water

The participating Labs were requested to carry out the pre-treatment procedure developed by the EURL for *E. coli* and then apply ISO TS 13136:2012 for detecting the presence of STEC in sprout irrigation water, carrying out the enrichment at 41.5°C

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

The voluntary inter-laboratory study (PT30) was organized and run in October 2021

PT30: Participants

18 NRLs representing
16 EU MS

+ 4 Non-EU NRLs of

Egypt

Iceland

Norway

UK (2 Labs)

+ 23 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 radish sprout production process

The presence of a natural background microflora has been evaluated ➡ $4x10^5$ 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

Beside the presence of the bacterial background microflora, the water resulted heavily contaminated with protozoa

Stability tests:

- Tests carried out on spent irrigation water of the same nature but collected during another cycle of sprout production
- Tests carried out using different levels of contaminations
- the STEC O157 strain could be isolated from the 50 CFU/ml spiking level after seven days from contamination.

PT30: Samples

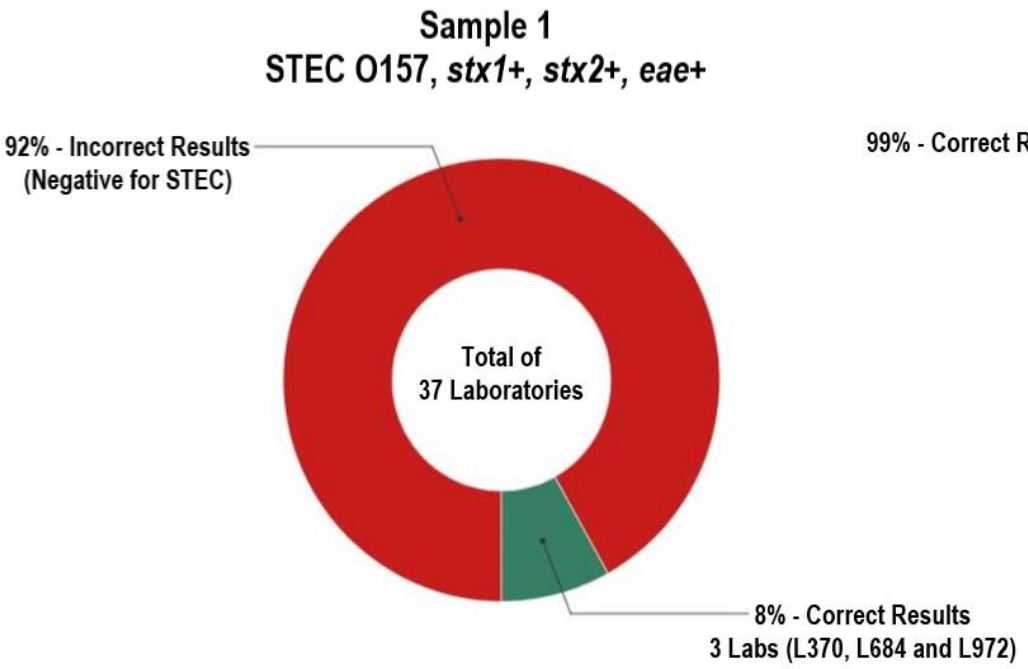
Contaminant (*Genotype*)	Contamination level in:	
	Sample 1	**Sample 2**
C210-03 STEC O157 (*stx1+, stx2+, eae+*)	50 CFU/ml	-

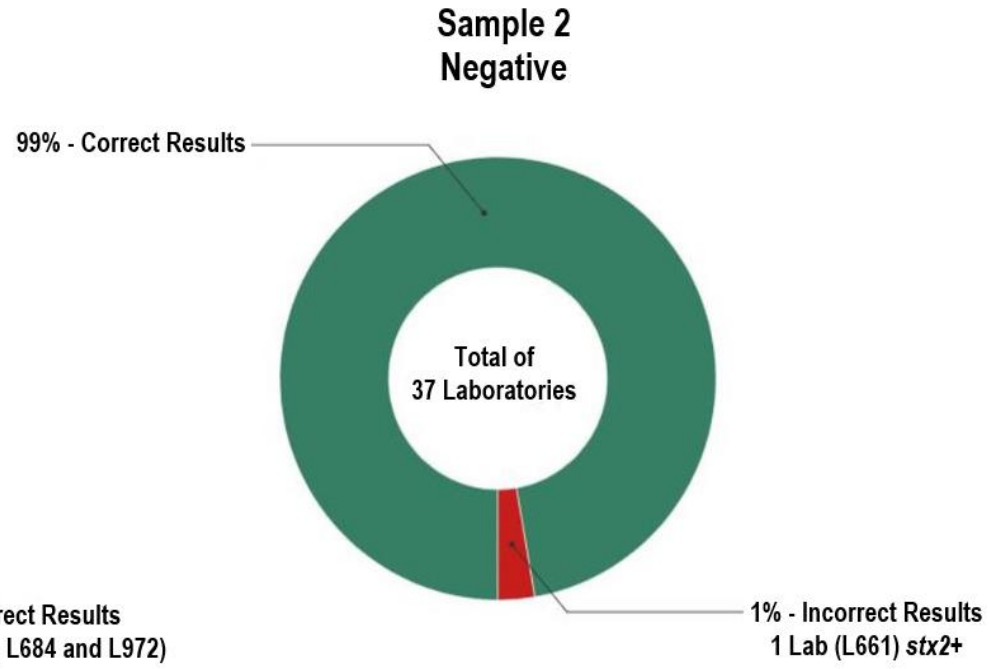
PT30: Samples

Contaminant (*Genotype*)	Contamination level in:	
	Sample 1	Sample 2
C210-03 STEC O157 *(stx1+, stx2+, eae+)*	50 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 on the 4th of October 2021
- ✓ Results submitted through an on-line form from 37 labs

Screening step: Proportion of Laboratories correctly detecting the presence or absence of virulence and serogroup-associated genes in the enrichment cultures





Two out of the three labs detecting the presence of STEC in sample 1 could isolate the STEC O157 *eae*+ *stx1*+ *stx2*+ contaminating strain

Concluding remarks

- PT30 was not meant to assess the proficiency of the laboratories but it was rather a collaborative study to verify the appropriateness of the procedure for the pre-treatment and analysis of spent irrigation water samples for the presence of STEC

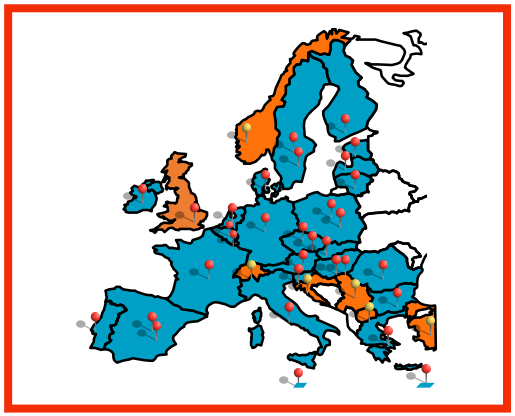
- This study confirmed the complexity of testing the sprout spent irrigation water and highlighted the necessity to refine the pre-treatment procedure

- The preliminary tests showed the water samples contained a large number of free-living amoebae

- All the information coming from this study will be used to improve the testing process for spent irrigation water

PT33

Detection of Diarrhoeagenic *E. coli*, including STEC in cheese



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 PT33 consisted in the examination of artificially contaminated cheese samples, in order to enhance the preparedness of NRLs in testing cheese for the presence of Diarrhoeagenic *E. coli*, including STEC.

- The detection of the main virulence genes associated with pathogenic *E.coli*
- The isolation and characterization of the strains

The inter-laboratory study (PT33) was organized and run in June 2022

PT33: Participants

26 NRLs representing
22 EU MS

+ 5 Non-EU NRLs of

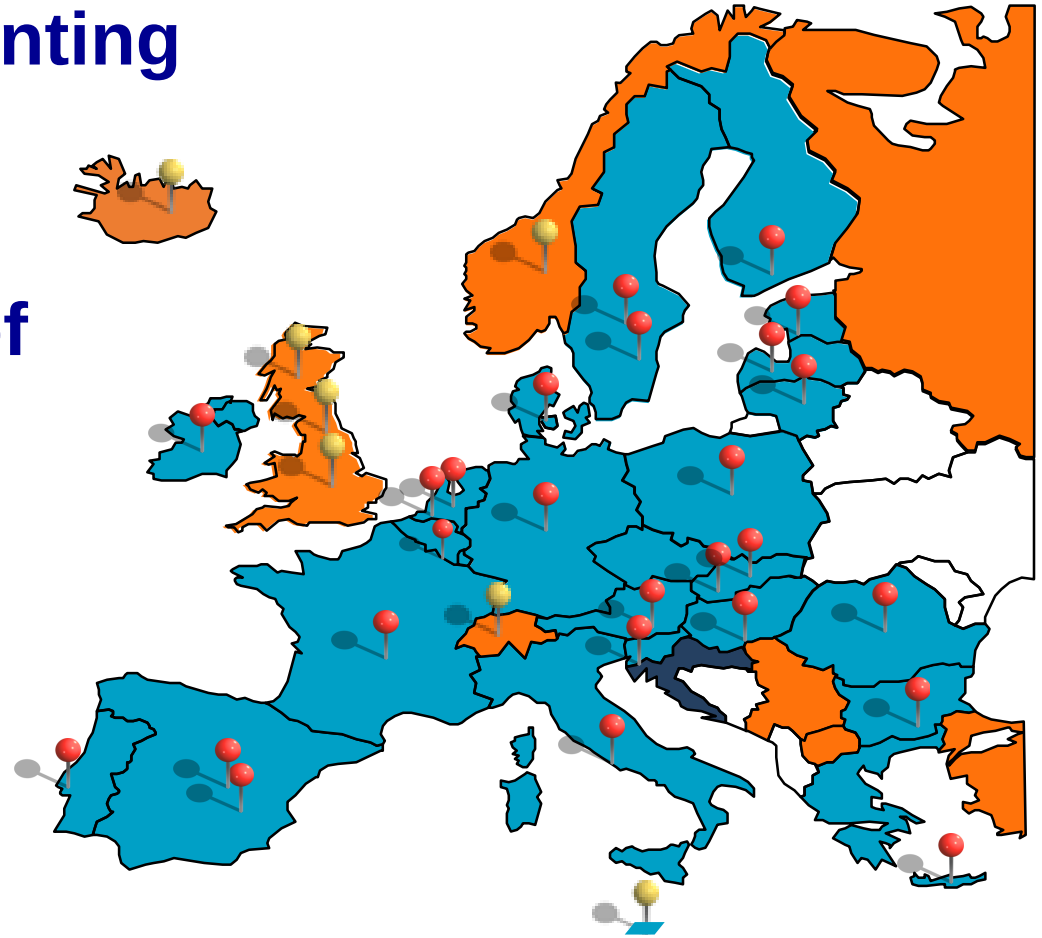
Egypy

Iceland

Norway

Switzerland

UK (3 Labs)



Test Samples

The cheese used in the study was purchased from a local retailer

The presence of a natural background microflora has been evaluated by plating on two different media (TSA and MacConkey agar) serial dilutions of 25 g of cheese in BPW ➡ No growth was observed

Two samples consisting of 25 g of cheese have been assayed for the presence of STEC and EAEC ➡ Both samples were negative at the Real Time PCR screening for the gene targets of STEC and EAEC

Stability tests (May 2022):

EAEC O104 Concentration	T0		T1 (3 days)		T2 (7 days)		T3 (10 days)	
Test	RT-PCR	Isolation	RT-PCR	Isolation	RT-PCR	Isolation	RT-PCR	Isolation
2 CFU/g	+	+	+	+	+	+	+	+
STEC O78 Concentration	**T0**		**T1 (3 days)**		**T2 (7 days)**		**T3 (10 days)**	
Test	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**
2 CFU/g	+	+	+	+	+	+	+	+

PT33: Samples

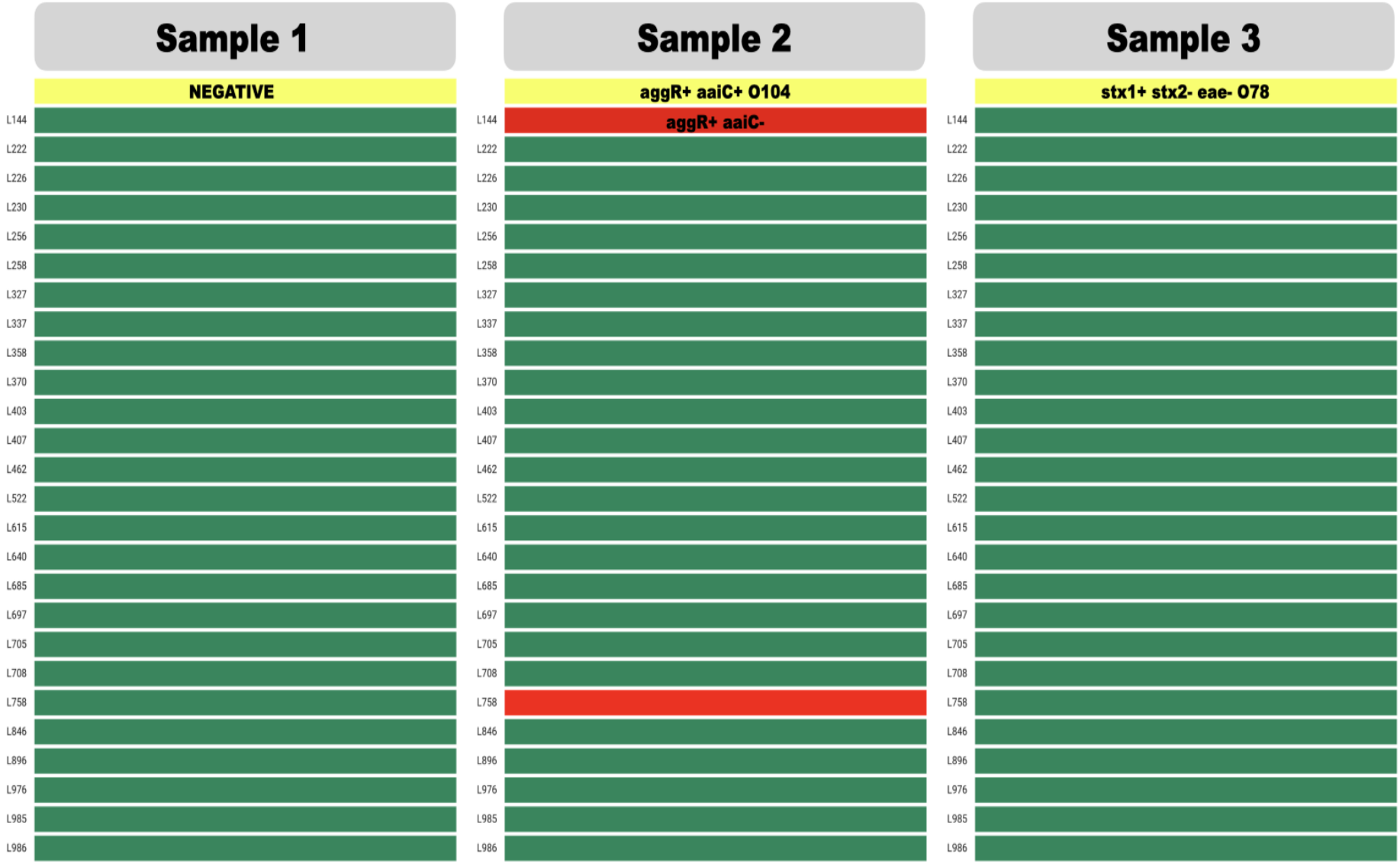
Cheese Samples	Contamination
Sample 1	Negative
Sample 2	2 CFU/g EAEC O104 *aggR, aaiC**
Sample 3	2 CFU/g STEC O78 *stx1***

PT33: Samples

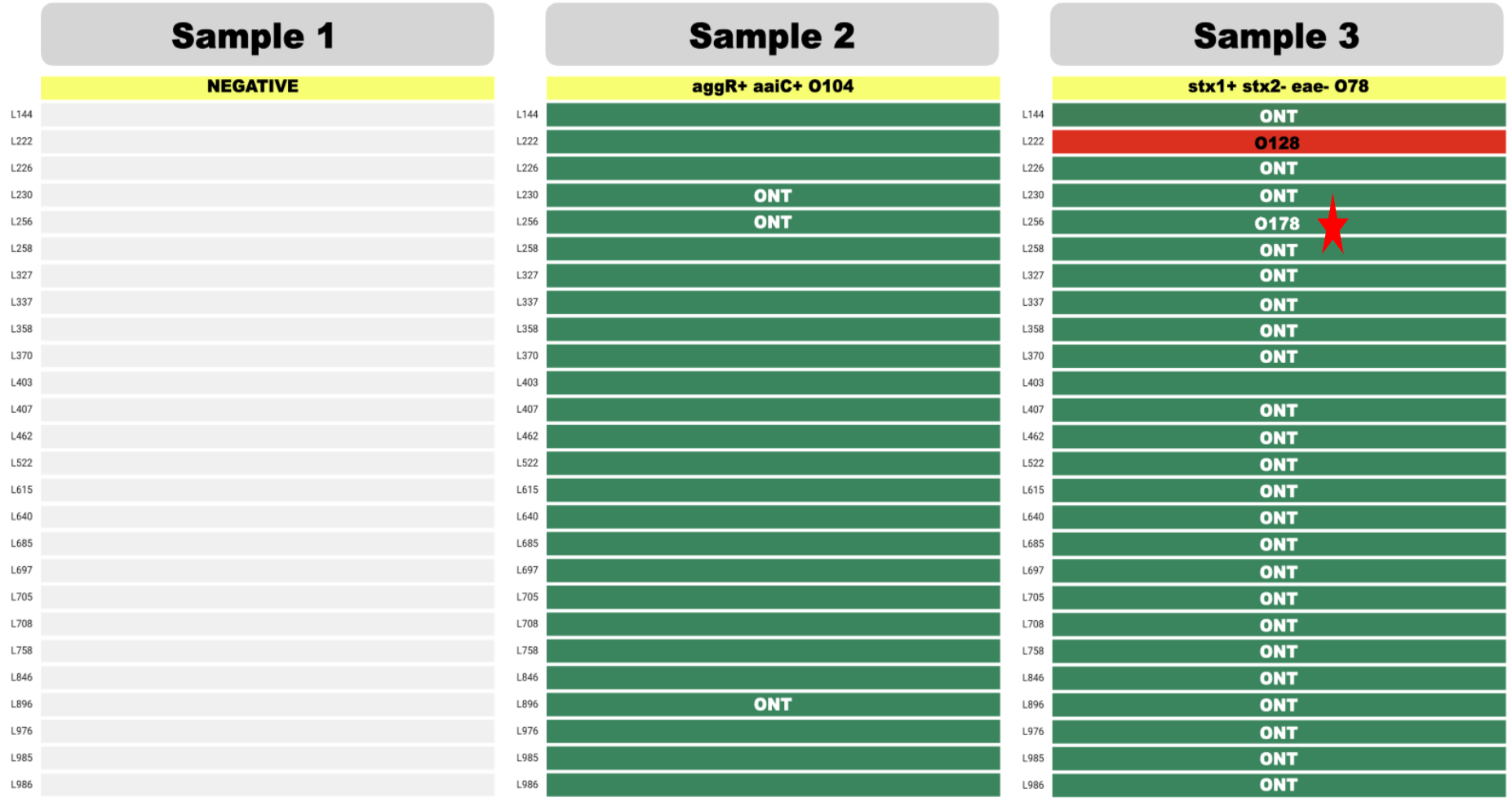
Cheese Samples	Contamination
Sample 1	Negative
Sample 2	2 CFU/g EAEC O104 *aggR, aaiC**
Sample 3	2 CFU/g STEC O78 *stx1***

- ✓ Cheese Samples were contaminated on the 24 th of June 2022
- ✓ The homogeneity test was performed on 6 randomly selected samples for each of the three cheese sample types
- ✓ Samples labelled with randomly generated numerical codes were shipped on the 27 th of June 2022
- ✓ Results submitted through an on-line form from 33 NRLs

Real-time PCR detection of virulence genes in the enrichment cultures from EU NRLs

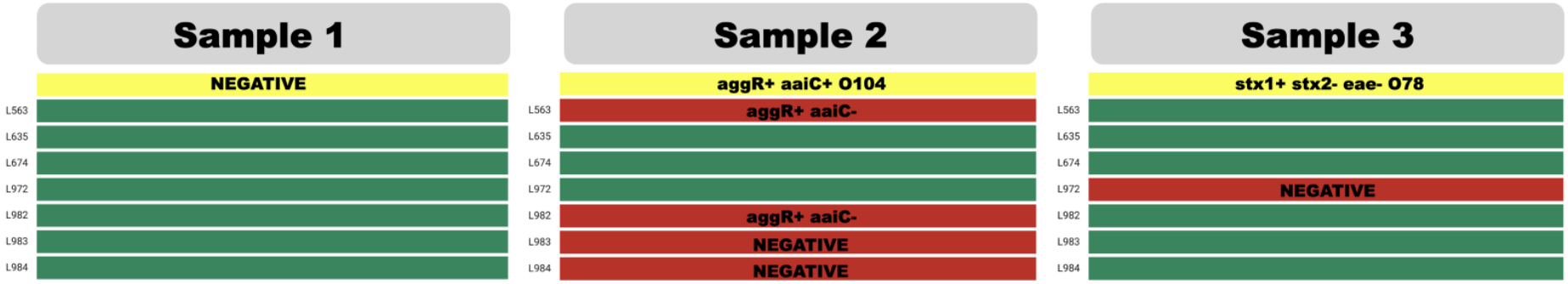


Isolation and genotyping of STEC and EAEC strains from cheese samples

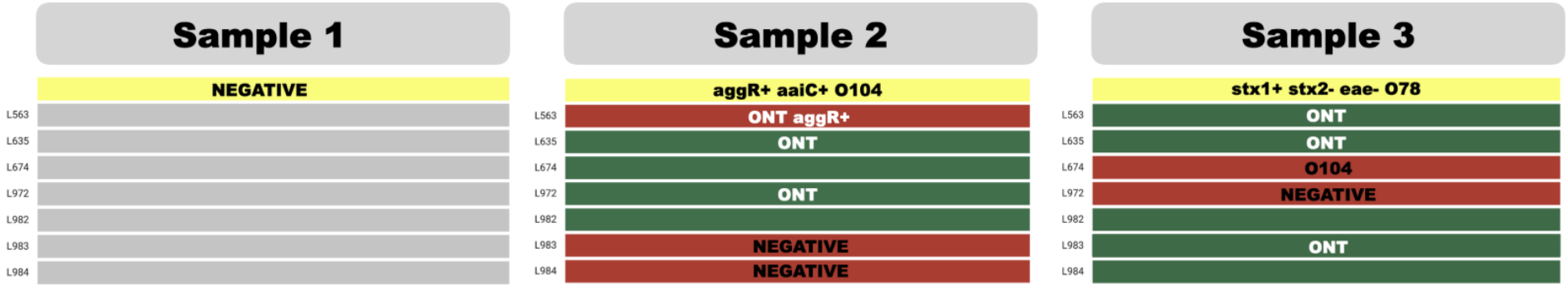


All the participants were able to isolate the contaminating STEC and EAEC strains. Most of the labs did not detect the O78 serogroup in the isolated STEC strain

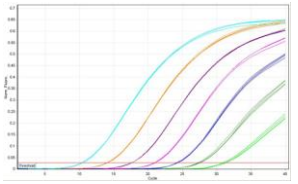
Real-time PCR detection of virulence genes in the enrichment cultures from Non-EU NRLs



Isolation and genotyping of STEC and EAEC strains from cheese samples



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** to each result incorrectly reporting the presence of *eae* gene
- **2 penalty points** to each incorrect or missing result concerning the identification of the *aggR* and *aaiC* genes

Evaluation of the NRLs performance in the isolation of STEC and EAEC strains:

- **2 penalty points** to the lack of isolation of EAEC from sample 2 and of STEC from sample 3 or to the isolation from sample 1
- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx1* and *stx2* genes
- **2 penalty points** to each result incorrectly reporting the presence of *eae* gene
- **2 penalty points** to each incorrect or missing result concerning the identification of the *aggR* and *aaiC* genes

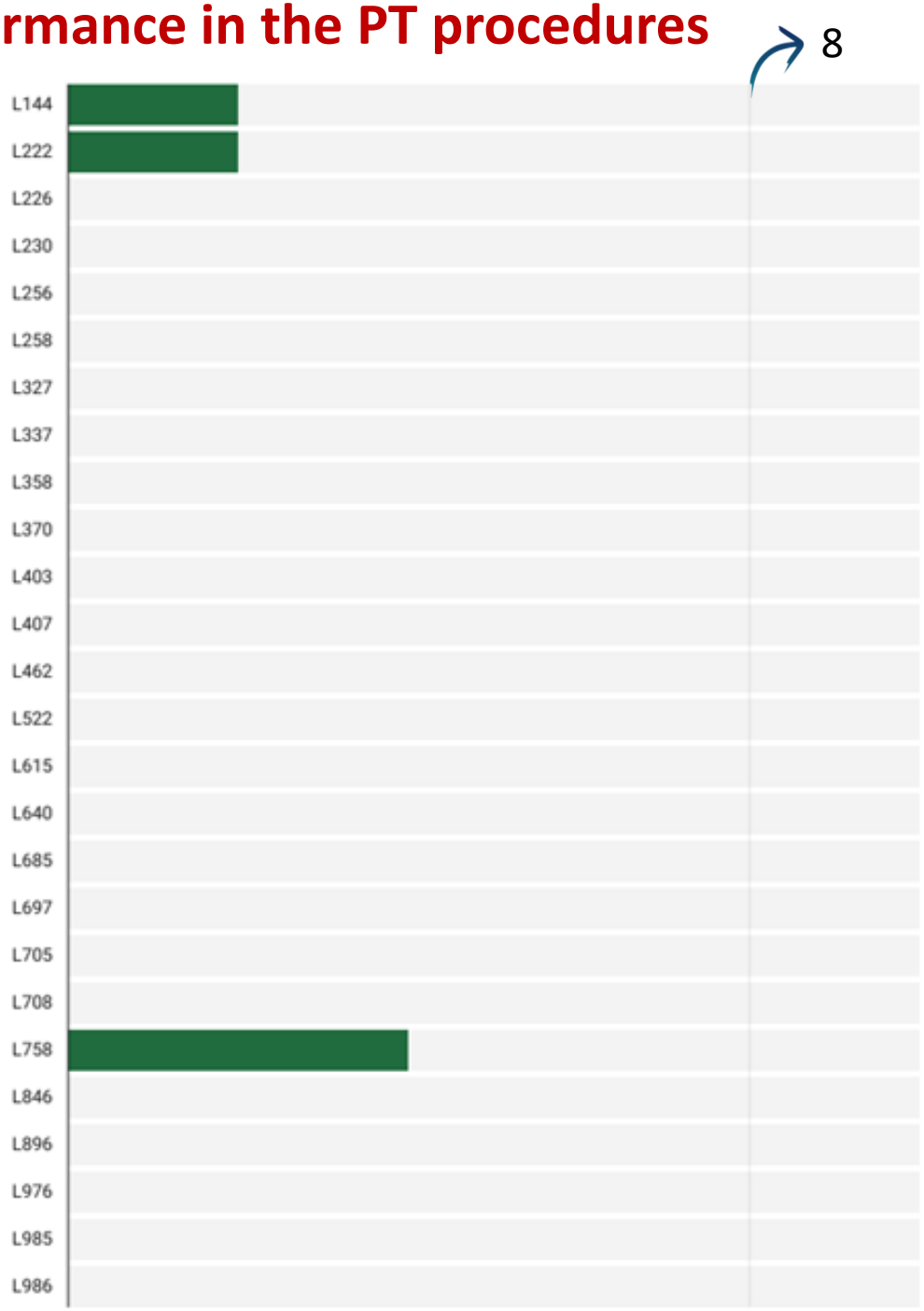
Evaluation of the NRLs performance in the isolation of STEC and EAEC strains:

- **2 penalty points** to each incorrect result concerning the identification of O78 serogroup of the STEC strain
- **No penalty points** were assigned for reporting the serogroup of the isolated STEC strain as ONT (the O78 serogroup is not in the list of the top-14 serogroups)
- The serogroup of the EAEC strain was not the subject of the performance assessment

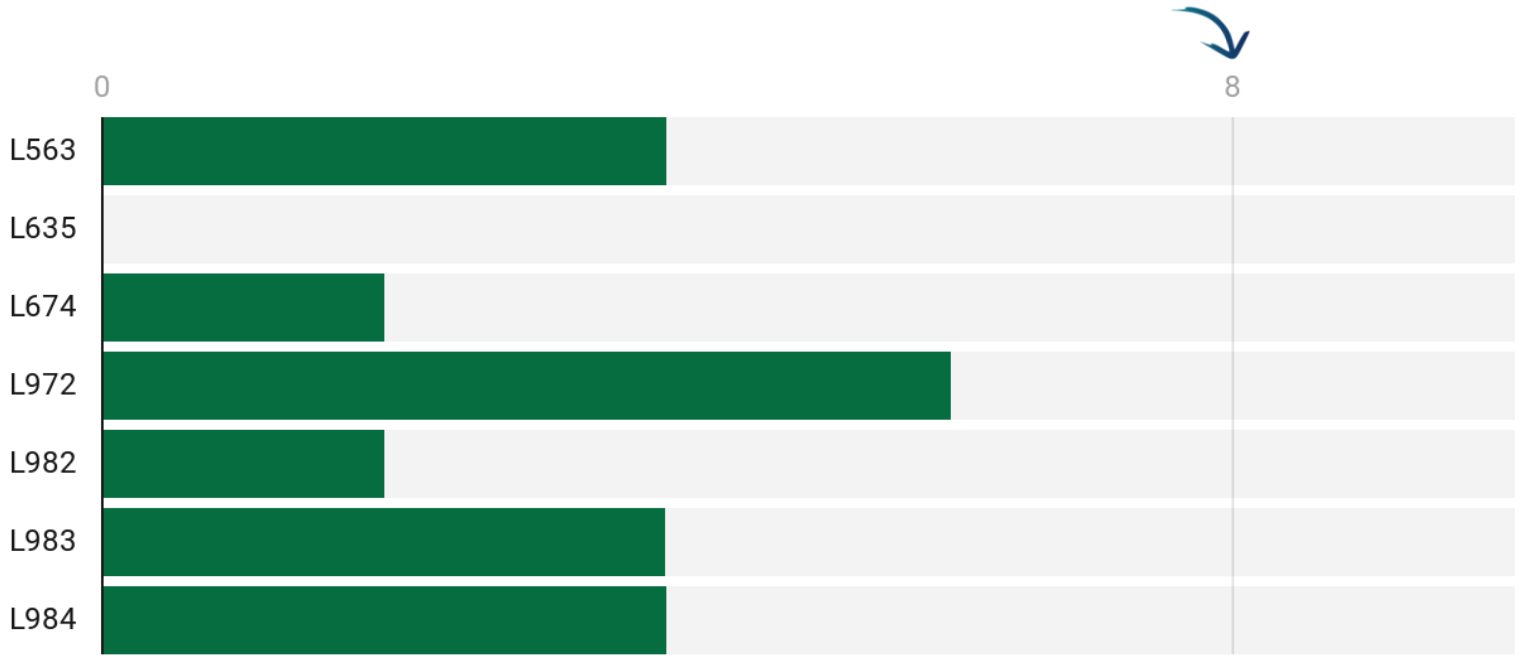
Evaluation of the EU NRLs performance in the PT procedures (screening + isolation steps)

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

All the labs complied the definition of satisfactory proficiency



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



All the labs complied the definition of satisfactory proficiency

Concluding remarks

- A high participation was recorded for PT33. The level of participation observed confirmed the eagerness and collaboration of the network.
- No NRL was considered under-performant. The capability of the NRLs in identifying the STEC and other pathogenic *E. coli* virulence genes is highly satisfactory.
- The identification of the O78 serogroup was problematic and achieved only by three participants (one EU NRL and two Non-EU NRLs).
- The O78 serogroup is not comprised in the top-14 serogroups and thus has not been used to assess the proficiency of the laboratories in the determination of this feature.

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