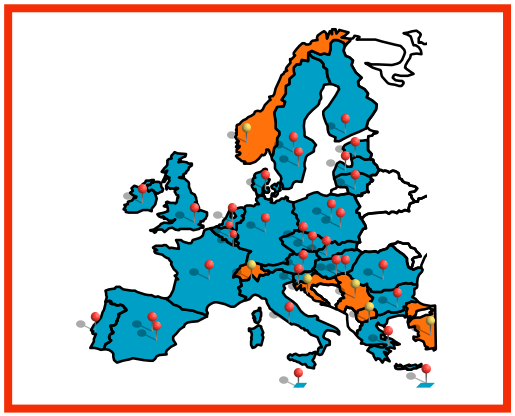
PT27

Detection of Shiga toxin-producing *E. coli* (STEC) in basil



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Objectives and Design of the study

Herbs have recently been described as a food vehicle for transmission of STEC, and were responsible for a number of outbreaks too

SCIENTIFIC OPINION

EFSA Journal

Implicated food vehicle category (number of reported strong evidence outbreaks; number of reporting countries)	**Human cases**	**Hospitalisations**	**Deaths**
Bovine meat and products thereof (15; 7)	**143**	**76**	**0**
Milk and dairy products[a] (14; 8)	**94**	**43**	**2**
Tap water, including well water (8; 4)	**75**	**7**	**0**
Vegetables, fruit and products thereof[b] (7; 3)	**575**	**73**	**2**
Pig meat and products thereof (2; 1)	6	2	0
Other or mixed red meat and products thereof (2; 2)	10	0	0
Sheep meat and products thereof (1; 1)	27	9	0
Unspecified meat[c] (1; 1)	2	1	0
Fish and seafood[d] (1; 1)	5	0	0
Herbs and spices (1; 1)	50	3	0
Total	**987**	**214**	**4**

Number of human cases, hospitalisations and deaths per implicated food vehicle category reported in strong evidence STEC food-borne outbreaks from 2012 to 2017

Objectives and Design of the study

The objective of PT27 consisted in the examination of artificially contaminated basil samples, in order to enhance the preparedness of NRLs in testing herbs for the presence of STEC by applying the ISO TS 13136:2012.

- The detection of the main STEC virulence genes (*eae* and *stx* genes)
- The isolation and characterization of STEC strains

The inter-laboratory study (PT27) was organized and run in November 2020

PT27: Participants

23 NRLs representing
19 EU MS

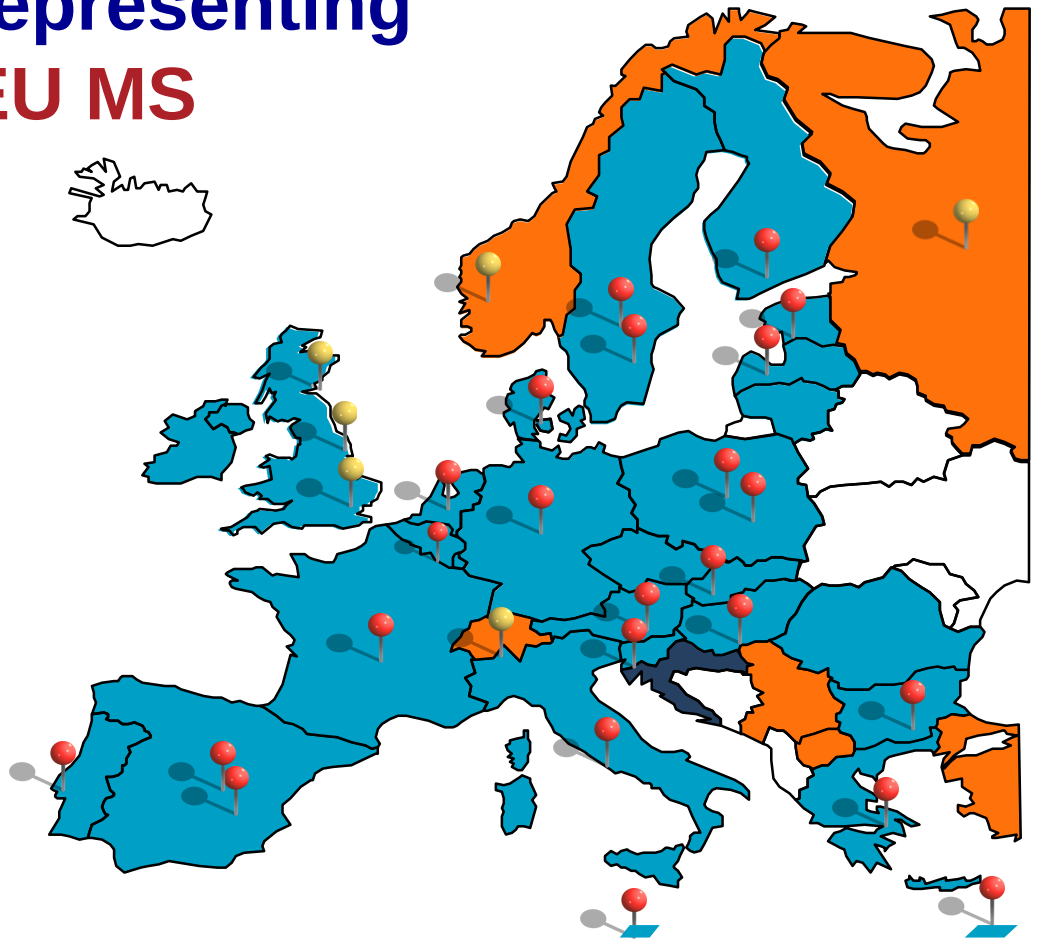
+ Non-EU NRLs of

Norway

Russia

Switzerland

UK (3 Labs)



Test Samples

The basil 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 10 g of basil ➡ No growth was observed

Two samples consisting of 25 g of basil have been assayed for the presence of STEC according to the ISO TS 13136:2012 ➡ Both samples were negative at the Real Time PCR screening for the gene targets of STEC

Stability tests (September 2020):

STEC O88 Concentration	T0 Replicate 1		T1 (3 days) Replicate 1		T2 (7 days) Replicate 1		T3 (10 days) Replicate 1		T4 (14 days) Replicate 1	
Test	RT PCR	Isolation	RT PCR	Isolation	RT PCR	Isolation	RT PCR	Isolation	RT PCR	Isolation
5 CFU/g	+	+	+	+	+	+	+	+	-	-
50 CFU/g	+	+	+	+	+	+	+	+	-	-

PT27: Samples

Contaminant (*Genotype*)	Contamination level in:		
	Sample 1	Sample 2 (interval)	Sample 3 (interval)
ED049 STEC O88 (*stx1+, stx2+*)	0 CFU	5 CFU/g (4.7-8.3)	50 CFU/g (47-83)

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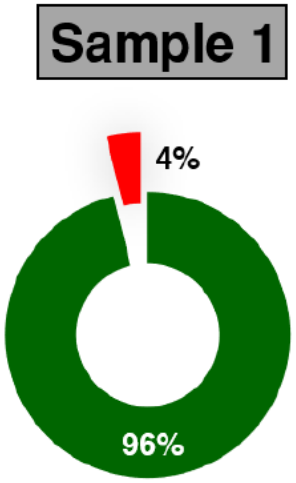
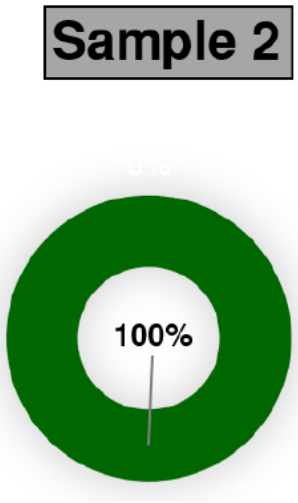
- ✓ Basil Samples were contaminated on the 6 of November 2020
- ✓ The homogeneity test was performed on 6 randomly selected samples of the three contamination levels
- ✓ Samples labelled with randomly generated numerical codes were shipped on the 9 of November 2020
- ✓ Results submitted through an on-line form from 27 NRLs

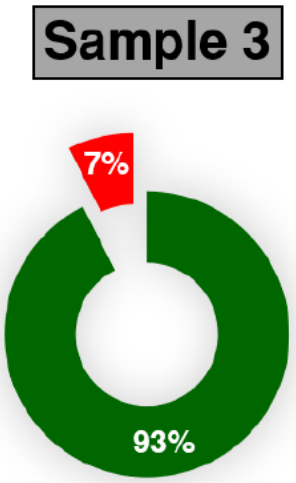
Screening step: Proportion of Laboratories correctly detecting the presence or absence of STEC in the samples



Sample 1 (0 CFU/g)



Sample 2 (5 CFU/g)



Sample 3 (50 CFU/g)

One participant recoded the samples but failed in connecting the codes newly assigned to the original ones provided by the EURL for *E. coli*. The results reported by this Lab were excluded from the analysis.

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

Isolation and genotyping of STEC strains from basil samples

All the participants were able to isolate the contaminating STEC strain. Most of the labs did not detect the O88 serogroup in the isolated STEC strain

Evaluation of the performance of the method

Sensitivity: Se = [true positives / (true positives + false negatives)] x 100
Specificity: Sp = [True negatives / (true negatives + false positives)] x 100

Screening step

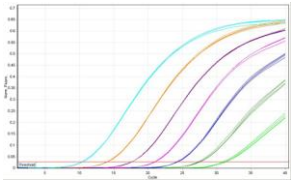
	Se (Lower level)	*Se* (Higher level)	*Sp*
stx1	100%	96.3%	96.3%
stx2	100%	96.3%	96.3%
eae	N. A.	N. A.	98.1%

Isolation step

***Se* = 96.1%** ➡ **Sample2 (Lower level)**

***Se* = 96.3%** ➡ **Sample3 (Higher level)**

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

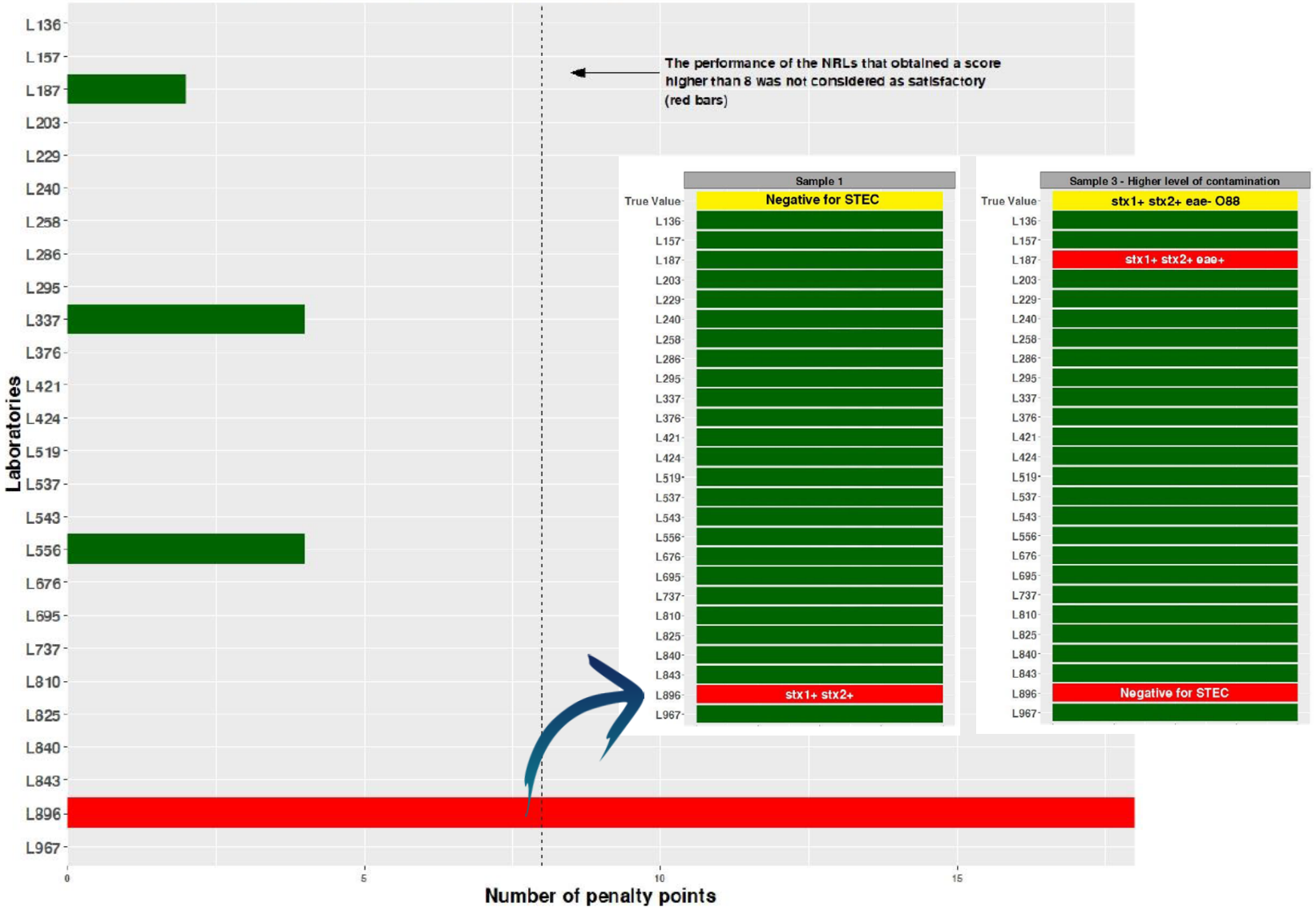


- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx1* and *stx2* genes
- **2 penalty points** to each result indicating the presence of *eae* gene
- **2 penalty points** to each result concerning the identification of one of the top five serogroups

Evaluation of the NRLs performance in the isolation of STEC:

- **2 penalty points** to the lack of isolation from sample 2 and 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 indicating the presence of *eae* gene
- **2 penalty points** to each incorrect result concerning the identification of O88 serogroup
- **No penalty points** were assigned for reporting the serogroup of isolated STEC strain as ONT

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



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

Concluding remarks

- A lower participation was recorded for PT27 compared with the previous rounds of PTs. However, considering the COVID-19 pandemic, the level of participation observed confirmed the eagerness and collaboration of the network.
- Only one participant was considered under-performant, due to incorrect registration of results
- The identifcation of the O88 serogroup was problematic and only achieved by laboratories performing WGS confirming the usefulness of this approach.
- The O88 serogroup is not comprised in the field of application of the ISO TS 13136 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!