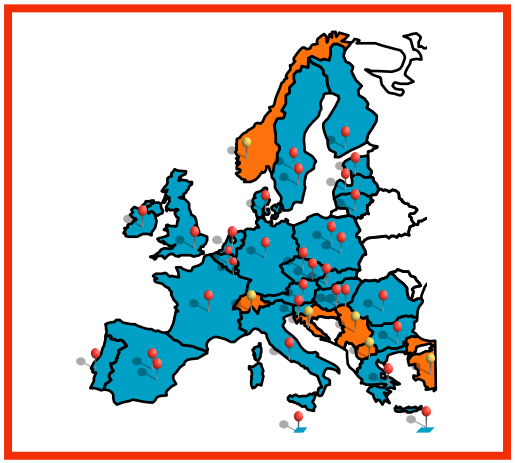
PT19

Detection of STEC in sprout irrigation water



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

- ✓ Regulation (EU) No 209/2013 has introduced for the first time microbiological criteria for STEC in the EU legislation (sprouts)
- ✓ ISO TS 13136:2012 and the EURL procedure for the identification of VTEC O104:H4 are prescribed for the detection of STEC in sprouts by Reg. 209/2013
- ✓ Reg. (EU) 209/2013 also gives the possibility to replace the sampling and testing of sprouts with the analysis of five samples of 200 ml of the water that has been used for their irrigation
- ✓ However, testing spent irrigation water for the presence of VTEC or other enteric pathogens may pose technical problems, due to some characteristics of this particular matrix.
- ✓ In 2015 EURL-VTEC issued a simple procedure for pretreatment of spent irrigation water, which was evaluated in an inter-laboratory study

PT16 Procedure

The samples pre-treatment procedure was provided by EURL VTEC

200 ml irrigation water samples had to be treated as follows:

Centrifugate at 4,500 g for 30 minutes at + 4°C

Decant Supernatant

Resuspend the pellet in 10X BPW of its volume/weight

Enrichment carried out over night

DNA extraction from 1 ml and test for the presence of STEC

Real Time PCR for STEC
ISO/TS 13136 and adaptation for *E. coli* O104:H4

Negative → STOP

Positive → Go for isolation

PT16

A total of 51 laboratories, 30 NRLs of EU member States, 4 NRLs of non EU countries, and 17 Italian Official Laboratories accepted to participate in the study

Three spent irrigation water samples spiked with 0, 200 and 500 CFU/ml of a STEC O157 strain were assayed

The presence of the VTEC O157 genes was identified correctly by 48 laboratories (96 %) in sample A (high level of contamination) and by 47 laboratories (94 %) in sample B (low level of contamination)

The contaminating VTEC O157 strain was isolated from both samples by the majority of the laboratories (88 % for sample A and 84 % for sample B)

The results were satisfactory and the procedure was evaluated as an effective tool for the verification of the conformity of the end product (sprouts)

PT19 in 2017

- to improve the preparedness of the NRLs towards testing spent irrigation water for the presence of STEC

- To test the EURL-VTEC procedure with samples of spent irrigation water contaminated with non-O157 STEC

PT19 – Design of the study

- ✓ Three 200 ml spent water samples potentially contaminated with STEC were sent to the participating laboratories
- ✓ The samples were spiked with three different levels of contamination of the same non-O157 STEC strain (High, Low, 0)
- ✓ Organized according to the requirements of ISO 17043:2010

PT19 – Participants

33 NRLs representing

24 EU countries

+ the NRLs of

Norway

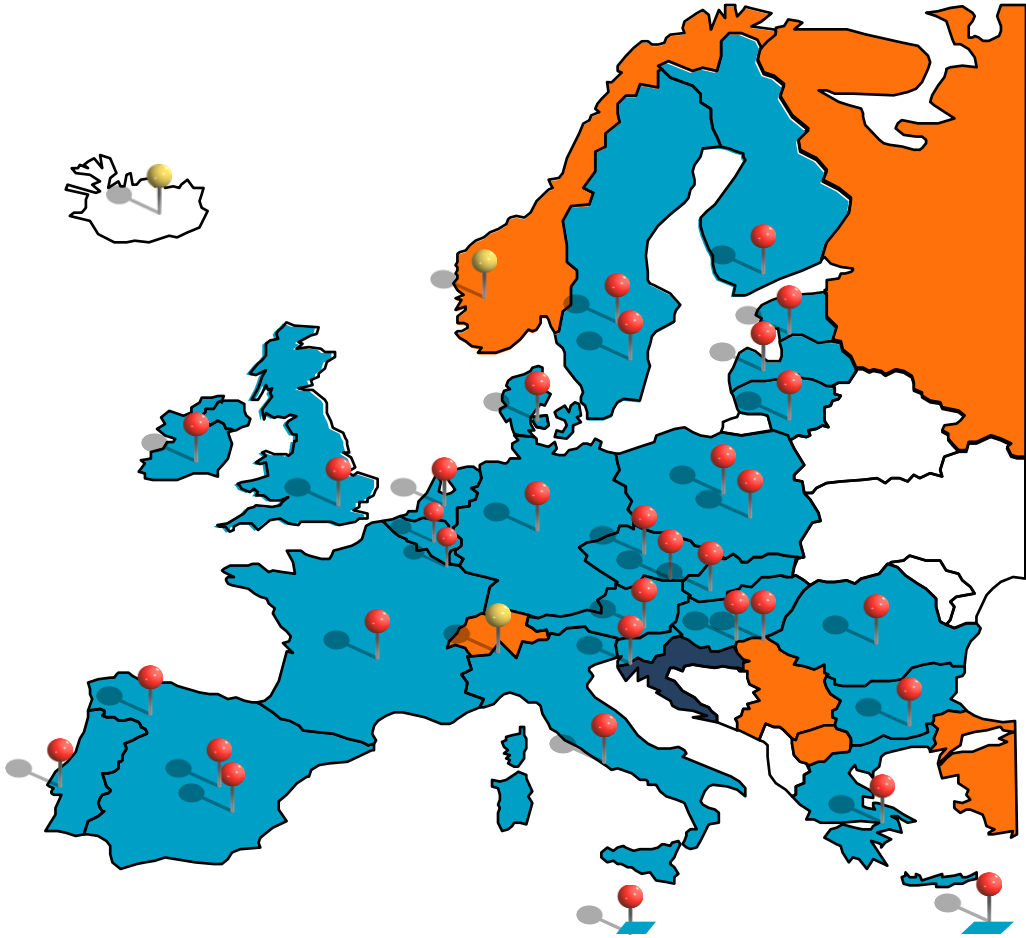
Switzerland

Iceland

Chile

Russia

Egypt



PT19 – Samples

Contaminant (*Genotype*)	Contamination level in:		
	Sample 1	**Sample 2**	**Sample 3**
STEC O145 *(stx1+, eae+)*	-	Low: 50 CFU/ml	High: 500 CFU/ml

UoM: 0.37 log CFU/ml

Stability: 4, 6 and 11 days since the initial contamination

Test samples were prepared on the 31st of March, immediately refrigerated, assessed for homogeneity (10 bottles per level of contamination) and sent on the 3rd of April into refrigerated safety boxes

Analysis of the NRLs results

Evaluation of proficiency in the Real Time PCR screening step:

- 4 penalty points assigned for incorrect results for *stx1* and *stx2*
- 2 penalty points assigned for incorrect results for *eae* and $ihp1_{O145}$

NO PENALTY POINTS ASSIGNED FOR THE LACK OF ISOLATION OF STEC O145

Evaluation of sensitivity/specificity in the screening step

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

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

PT19 Results: Detection of virulence and serogroup-associated genes

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample 1				Sample 2 Low level contamination				Sample 3 High level contamination			
	stx1	*stx2*	*eae*	*ihp1*$_{O145}$	*stx1*	*stx2*	*eae*	*ihp1*$_{O145}$	*stx1*	*stx2*	*eae*	*ihp1*$_{O145}$
True value	-	-	-	-	+	-	+	+	+	-	+	+
L136												
L163					-					+		
L174												
L178												
L230												
L318												
L504												
L546					-							
L552												
L562												
L600												
L607					-							
L621					-							
L653												
L654	+		+	+					-			
L659												
L712												
L723												
L724								ONT				ONT
L738					-				-			
L744					-							ONT
L783												
L792												
L827							ND	ND				
L843								ONT				
L862					-							
L894					-				-			
L925												
L944												
L979						+				+		

Sample 2:
20 laboratories (66.7%) identified correctly the presence of the virulence genes *stx1* and *eae*, and the absence of *stx2* gene

Eight NRLs couldn't detect the presence of *stx1* gene and therefore didn't conduct any other test on the sample (detection of *eae* and serogroup-associated genes)

21 NRLs carried out the detection of serogroup-associated genes and 19 correctly identified the presence of *ihp1*$_{O145}$

PT19 Results: Detection of virulence and serogroup-associated genes

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample 1				Sample 2 Low level contamination				Sample 3 High level contamination			
	stx1	*stx2*	*eae*	*ihp1*$_{O145}$	*stx1*	*stx2*	*eae*	*ihp1*$_{O145}$	*stx1*	*stx2*	*eae*	*ihp1*$_{O145}$
True value	-	-	-	-	+	-	+	+	+	-	+	+
L136												
L163					-					+		
L174												
L178												
L230												
L318												
L504												
L546					-							
L552												
L562												
L600												
L607					-							
L621					-							
L653												
L654	+		+	+					-			
L659												
L712												
L723												
L724								ONT				ONT
L738					-				-			
L744					-							ONT
L783												
L792												
L827							ND	ND				
L843								ONT				
L862					-							
L894					-				-			
L925												
L944												
L979						+				+		

Sample 3:
25 laboratories (83.3 %) identified correctly the presence of the virulence genes *stx1* and *eae*, and the absence of *stx2* gene

Three NRLs couldn't detect the presence of *stx1* gene and therefore didn't conduct any other test on the sample (detection of *eae* and serogroup-associated genes)

28/30 laboratories identified correctly the presence of the *ihp1*$_{O145}$ gene

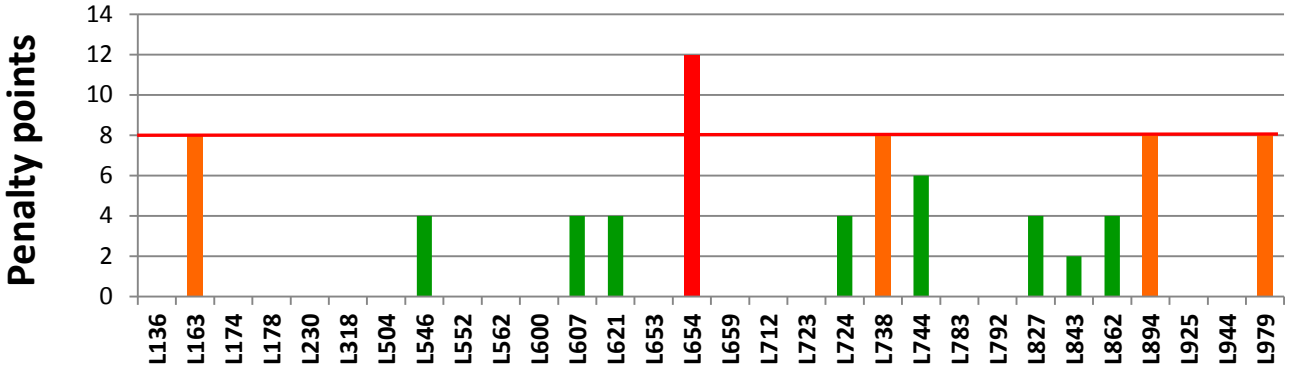
PT 19 Results: isolation of the STEC O145 strain in the irrigation water samples

NRL	Sample 1	Sample 2				Sample 3			
	-	STEC O145 Isolation	Genotype			STEC O145 Isolation	Genotype		
			stx1	*stx2*	*eae*		*stx1*	*stx2*	*eae*
True value	None	+	+	-	+	+	+	-	+
L136									
L163		*							
L174									
L178									
L230									
L318									
L504									
L546		*							
L552									
L562									
L600									
L607		*							
L621		*							
L653									
L654	O145, *stx1+*, *eae+*					*			
L659									
L712									
L723									
L724									
L738		*				*			
L744		*							
L783									
L792									
L827		O157	-		-				
L843									
L862		*							
L894		*				*			
L925									
L944									
L979									

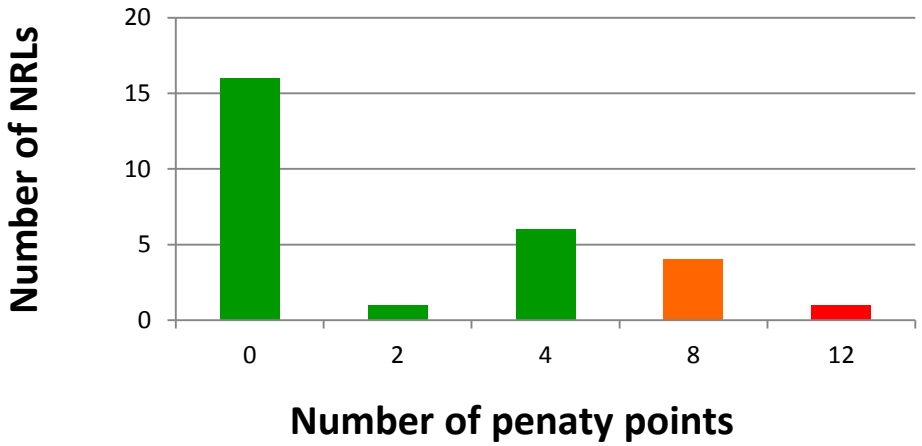
22 laboratories had detected the presence of STEC in Sample 2 (low level of contamination), only five (22. 7%) could isolate STEC O145

In sample 3 (high level of contamination) the isolation was achieved six NRLs out of the 27 laboratories (22.2 %) detecting the presence of STEC in the screening step

Evaluation of the NRLs proficiency (Screening step only)



Performance higher than 8 was considered as unsatisfactory – 1 lab



Evaluation of the performance of the method (Screening Step)

	Se (high)	Se (low)	Sp
stx1	93.1 %	73,3 %	100 %
stx2	N.A.	N.A.	96.7 %
eae	100 %	100 %	N.A.
$ihp1_{O145}$	93.3 %	90.5 %	N.A.

PT19: Concluding remarks

PT19 was meant to expand the range of STEC serogroups by including a STEC non-O157 to further prepare NRLs in testing spent irrigation water samples.

The virulence genes of the contaminating STEC O145 strain were identified with satisfactory sensitivity in both high and low level of contamination

STEC O145 was isolated only by a few laboratories representing about 22 % of the participants

The results of the present PT underlined the difficulty in isolating the contaminant microorganism in such matrix when it does not belong to O157 serogroup

Adjustments to the procedure are needed

The problem of low isolation rates from spent irrigation water