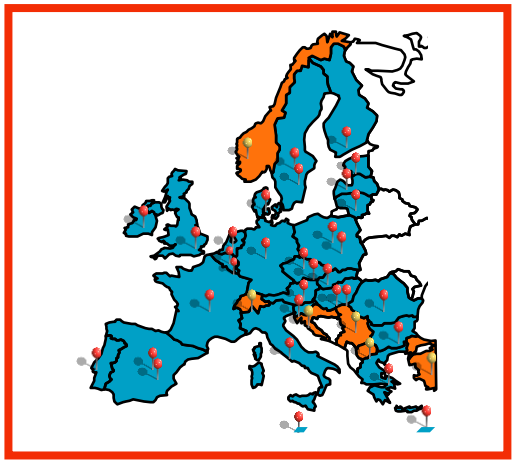
PT22

Detection of STEC in sprouts spent irrigation water (PT22)

November-December 2018



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European Reference Laboratory for *Escherichia coli*

The EURL for *E. coli* issued a procedure for pre-treatment of spent irrigation water, based on centrifugation. This methodology was assessed in two PT rounds

The outcome of PT19 run in 2017 on the detection and isolation of STEC in sprout spent irrigation water samples had underlined the need to revise the procedure for testing such a complex matrix

A modification of the procedure was proposed to the NRLs in the framework of PT22, which aimed at defining the performance of the method

The revision of the procedure included to carry out the enrichment step at 41.5 °C instead of 37°C

The **objectives** of PT221 were:

- ✓ To assess the performance of the revised method for testing spent irrigation water
- ✓ The proficiency of the participants was not evaluated

NRLs and Italian Official Laboratories (OLs) were invited to take part to the voluntary inter-laboratory study:

56 Laboratories enrolled

Test samples

A local sprout producer provided the spent irrigation water used for the study. The water was collected starting from 48 h after the beginning of the sprout production process, as prescribed in Reg. (EU) 209/2013.

Natural background microflora = 10^6 CFU/ml

Contaminant (Genotype)	Contamination level in:	
	Sample 1	**Sample 2**
C125-06 STEC O103 (*stx2*+, *eae*+)	**50 CFU/ml***	-

*The lowest contamination level used in PT19

Stability tests:

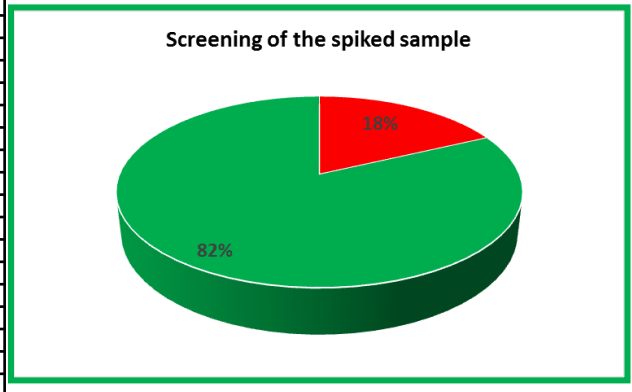
Real Time PCR screening was positive for the STEC target genes and **isolation** was successful even after 10 days from the spiking

Test Samples were prepared on 17 November and shipped on 19 of November 2018

10 bottles for each of the two contamination levels were randomly selected for homogeneity testing and analyzed according to the PT laboratory procedure. All the homogeneity tests gave the expected results.

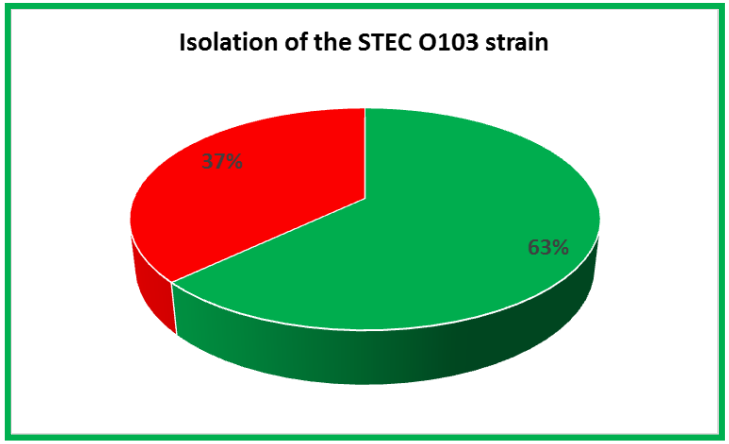
Results: Real Time PCR screening of the test samples

NRL	Detection of virulence and serogroup-associated genes in:							
	Sample 1				Sample 2			
	stx1	*stx2*	*eae*	wzx_{O103}	*stx1*	*stx2*	*eae*	wzx_{O103}
True value	-	+	+	+	-	-	-	-
L109								
L144								
L257								
L283								
L288								
L295								
L296								
L300								
L307		-						
L319								
L323								
L341								
L350								
L351								
L355								
L391		-				+	+	+
L400		-						
L429								
L439								
L441		-						
L446		-						
L504								
L521								
L542		-						
L576								
L590								
L598		-				+	+	+
L599								
L609								
L636								
L649								
L662								
L683								
L689								
L776	+			-				
L783								
L789								
L802								
L803								
L813								
L821		-						
L825								
L905								
L906		-						
L920		-						
L929		-						
L935								
L940								
L948								
L970								
L980								
L997								



Results: Isolation of the STEC O103

STEC strain isolation and genotyping from:					
NRL	Sample 1				Sample 2
	STEC O103 Isolation	Genotype			-
		stx1	*stx2*	*eae*	
True value	+	-	+	+	None
L109					
L144					
L257					
L283	-				
L288					
L295					
L296					
L300					
L319					
L323					
L341					
L350					
L351	-				
L355	-				
L391					O103, vtx1-, vtx2+, eae+
L429	ND				
L439	-				
L504	-				
L521					
L576	-				
L590					
L598					O103, vtx1-, vtx2+, eae+
L599	-				
L609					
L636					
L649	-				
L662					
L683					
L689	-				
L776	-				
L783					
L789					
L802					
L803	-				
L813	-				
L825	-				
L905					
L935	-				
L940					
L948					
L970					
L980					
L997					



Evaluation of the performance of the method

Screening Step

	Se	Sp
stx1	N.A.	99 %
stx2	84.7 %	100 %
eae	100 %	N.A.
wzx_{O103}	93.3 %	N.A.

Isolation Step

Se: 74.1 %
(around 22 % for the low contamination level in PT19)

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

Concluding remarks

The network of Laboratories working in the field of pathogenic *E. coli* is made up of very collaborative participants

the sensitivity of the method in the Real Time PCR screening was quite good

the isolation step showed a strong improvement when the enrichment temperature was raised at 41.5 °C, when compared to the results obtained in PT19 with the samples containing the same concentration of contaminant

The modification included in the revised protocol resulted effective

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