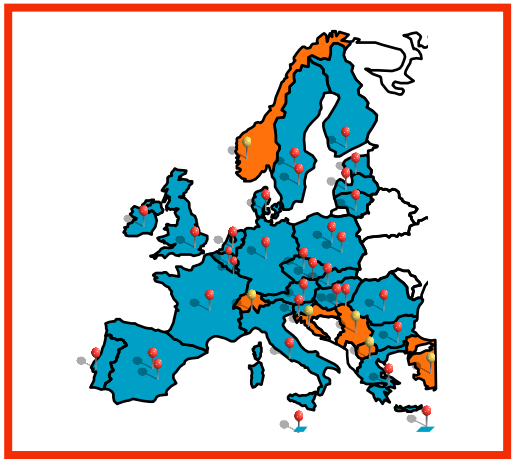
PT20

Detection of STEC in rocket salad

(November-December 2017)



EU-RL VTEC

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

Istituto Superiore di Sanità

- In the past few years, a growing number of foodborne illnesses have been traced back to fruits and produce, increasing the concern that vegetables might be more important as a vehicle for human enteric pathogens, including STEC

- rocket salad has been recently implicated in an outbreak of STEC O157 infection

- this matrix had never been proposed in previous PTs organized by the EURL-VTEC

The **objectives** of the study were:

- to improve the preparedness of the NRLs towards testing food commodities for the presence of STEC, by applying to the ISO TS 13136:2012;
- to improve the preparedness of the NRLs towards the detection and isolation of STEC strains not belonging to the O157 serogroup;
- to give further support to the NRLs and the Official Laboratories for the accreditation of the ISO TS 13136:2012

PT20

40 NRLs representing 27 EU Member States, Egypt, Iceland, Norway, Russia and Switzerland agreed to participate in the study, 37 submitted the results

Three 25 g rocket samples spiked with 0, 4 and 40 CFU/g of a STEC O111 strain were assayed

Contaminant (*Genotype*)	**Contamination level in:**		
	Sample 1	**Sample 2**	**Sample 3**
Strain ED476, STEC O111 *(stx1+, stx2+, eae+*)	-	**Low: 4 CFU/g**	**High: 40 CFU/g**

UoM 0.138 log CFU/ml

Stability tests: Real Time PCR screening positive also after 13days from spiking, Isolation was achieved up to 10 days from spiking

The rocket salad used possessed natural **background microflora** (about 7 x10^4 CFU/g)

The artificial contamination of the samples was carried out on the 10th of November 2017

Assessment of the **homogeneity**: eight replicates of each level of contamination

Labeled with randomly generated numerical codes different for each NRL, immediately refrigerated and transferred into refrigerated safety packages that were shipped on 13 November 2017 by courier

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 $wbdl_{O111}$

Two penalty points assigned to the lack of isolation of the STEC O111

Evaluation of sensitivity/specificity

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

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

Results: Real Time PCR screening of the test samples

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample 1				Sample 2 Low level contamination				Sample 3 High level contamination			
	stx1	*stx2*	*eae*	*wbdI*$_{O111}$	*stx1*	*stx2*	*eae*	*wbdI*$_{O111}$	*stx1*	*stx2*	*eae*	*wbdI*$_{O111}$
True value	-	-	-	-	+	+	+	+	+	+	+	+
L128												
L136												
L163												
L174												
L178												
L226												
L318												
L404												
L446												
L527	+	+		ONT			-	ONT				
L546												
L552												
L562					-				-			
L600												
L607					-		-					
L621												
L653												
L659												
L700												
L703												
L712												
L723												
L724												
L738												
L744												
L783												
L792												
L825												
L827												
L843												
L862												
L894												
L925												
L944												
L950												
L979												
L987												

Results: Isolation of the STEC O111 from the rocket salad

STEC strain isolation and genotyping from:									
NRL	Sample 1	Sample 2				Sample 3			
	-	STEC O111 Isolation	Genotype			STEC O111 Isolation	Genotype		
			stx1	*stx2*	*eae*		*stx1*	*stx2*	*eae*
True value	None	+	+	+	+	+	+	+	+
L128						-			
L136									
L163									
L174									
L178									
L226									
L318									
L404									
L446									
L527					-				
L546									
L552									
L562		-					-		
L600									
L607		-							
L621									
L653									
L659									
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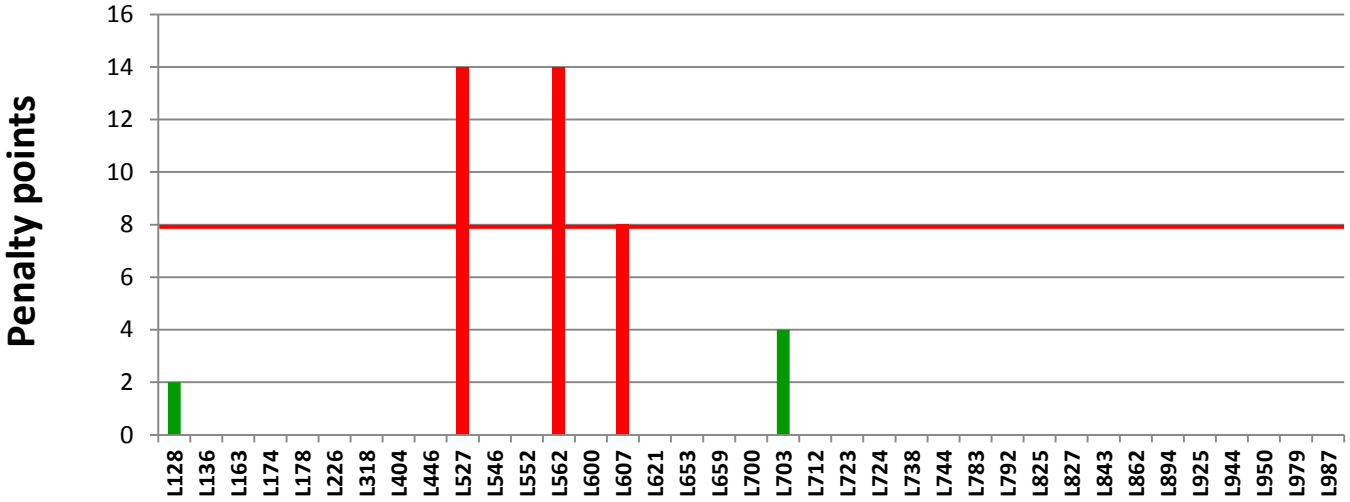
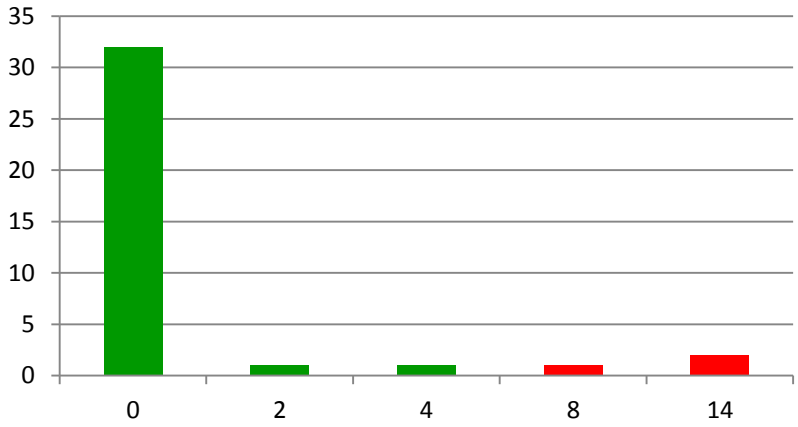
L987									

Evaluation of the NRLs proficiency



No of Laboratories per penalty score



3 Labs obtained a score higher than 8

Evaluation of the performance of the method
Screening Step

	Se (low)	Se (high)	Sp
stx1	97.2 %	100%	97.3%
stx2	100%	100%	100%
eae	94.6 %	100 %	N.A.
$wbdl_{O111}$	97.3 %	90.5 %	N.A.

Isolation Step

Se: 94.4 % for the low contamination level.
Se: 97.2 % for the high contamination level.

PT20: Concluding remarks

- PT19 was meant to expand the scope of PTs in terms of couples food matrix/contaminating STEC strains.
- High participation rate was observed
- The study confirmed that the ISO TS 13136:2012 method represents a suitable tool for the detection of STEC in the food commodity most regarded as vehicles of human infections
- Three out of the 37 NRLs that contributed results showed an unsatisfactory performance
- Performance parameters were calculated, providing support for accreditation