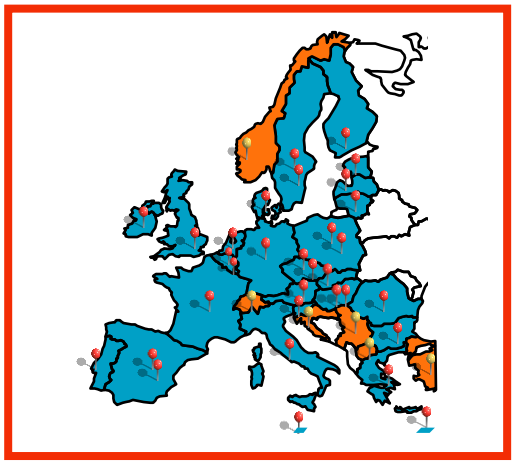
PT21

Detection of STEC in sprouts

(April-May 2018)



EU-RL VTEC

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Istituto Superiore di Sanità

Outline

Results of the inter-laboratory study (PT21)

Results of the voluntary inter-laboratory study on the effect of the enrichment temperature of 41.5 °C on the detection and isolation of STEC in sprouts

Organized in April-May 2018

The **objectives** of PT21 were:

- to improve the preparedness of the NRLs towards testing sprouts in compliance with Regulation (EU) No 209/2013;
- 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.

PT21

39 NRLs representing 27 EU Member States, Egypt, Iceland, Norway, Russia and Switzerland participated in the study

Three test samples (1, 2 and 3), each consisting of 25 g of red radish sprouts spiked with 0, 2 and 20 CFU/g of a STEC O26 strain were assayed

Contaminant (*Genotype*)	**Contamination level in:**		
	Sample 1	**Sample 2**	**Sample 3**
Strain C1188-02, STEC O26 *(stx1+, stx2+, eae+*)	-	**Low: 2 CFU/g**	**High: 20 CFU/g**

UoM 0.209 log CFU/ml

Stability tests:

Samples spiked as those used in PT21 were assayed by ISO TS 13136 after 3, 5 and 10 days since the initial contamination

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

Isolation was successful for the high level of contamination samples at all times tested,
but never achieved for the low-level contamination samples

Preparation of the PT21 samples

The artificial contamination of the samples was carried out on the 13th of April 2018

The red radish sprouts used possessed natural **background microflora** (about 6 x10^7 CFU/g)

Assessment of the **homogeneity**: ten replicates of the two levels of contamination assayed. The low level of contamination samples showed very high Ct.

Labeled with randomly generated numerical codes different for each NRL, immediately refrigerated and transferred into refrigerated safety packages that were shipped on 16 April 2018 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 wzx_{O26}

Two penalty points assigned to the lack of isolation of the STEC O26 in sample 3 only

Evaluation of sensitivity/specificity

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

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

limit of detection (LOD) has been calculated for the isolation step (Wilrich and Wilrich, 2009, Journal of AOAC international, Vol. 92 No. 6, 1763-1772)

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*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}
True value	-	-	-	-	+	+	+	+	+	+	+	+
L109												
L257												
L266												
L269												
L295					-							
L296												
L300												
L307												
L319												
L323												
L341												
L350												
L351												
L355										-		
L391	+	+	+	+	-	-						
L400*					-	-			-	-		
L429												
L446					-	-			-	-		
L494								ONT				
L521												
L542												
L576												
L598												
L599												
L609												
L649	+	+	+	+								
L662												
L689												
L789												
L802												
L803												
L813												
L906												
L920												
L935								ONT				ONT
L940					-							
L954												
L970												
L997												

***L400** received the test samples after 10 days from shipment and the temperature recorded was 25 °C.

Results: Isolation of the STEC O26 from the red radish sprouts

STEC strain isolation and genotyping from:									
NRL	Sample 1	Sample 2				Sample 3			
	-	STEC O26 Isolation	Genotype			STEC O26 Isolation	Genotype		
			stx1	*stx2*	*eae*		*stx1*	*stx2*	*eae*
True value	None	+	+	+	+	+	+	+	+
L109									
L257									
L266									
L269									
L295		-							
L296									
L300									
L307		-							
L319									
L323									
L341									
L350			-	-		-			
L351									
L355									
L391	O26 *vtx1 vtx2 eae*								
L400*									
L429		-							
L446*									
L494		-							
L521									
L542		-				-			
L576									
L598									
L599									
L609		-				-			
L649		-				-			
L662		-							
L689		-				-			
L789		-							
L802									
L803									
L813									
L954									
L970									
L997									

Sample 2: 23 (63.9 %) out of 36 NRLs detecting STEC in the screening step isolated the STEC O26 strain

Sample 3: 32 (86.4 %) Laboratories out of the 37 detecting the presence of STEC could isolate the contaminating strain

Four NRLs obtained a score equal or higher than 8

Evaluation of the performance of the method
Screening Step

	Se (low)	Se (high)	Sp
stx1	88.1 %	97.4 %	100%
stx2	95.0 %	95.0 %	100%
eae	100 %	100 %	N.A.
wzx_{O26}	94.6 %	97.3 %	N.A.

Isolation Step

Se: 76.1 % for the low contamination level.
Se: 88,1 % for the high contamination level.

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,i,L}$	$d_{0.5,i,U}$	$d_{0.95,i}$	$d_{0.95,i,L}$	$d_{0.95,i,U}$	$\lvert z_i \rvert$
1		0,007	0,204	3,848	2,557	5,792	16,631	11,050	25,032	0,000
Combined data		0,007	0,204	3,848	2,557	5,792	16,631	11,050	25,032	0,000

Voluntary inter-laboratory study on the effect of the enrichment temperature of 41.5 °C on the detection and isolation of STEC in sprouts

The use of BPW seems to be more suitable as enrichment broth rather than mTSB+N or mTSB+A

How to reduce the growth of background microflora in the absence of supplements

Discussion about the appropriateness of raising the incubation temperature to 41.5°C

The NRLs and Italian Official Labs were invited to participate in this study to evaluate the effect of the temperature of 41,5°C on STEC isolation as enrichment temperature

A total of 48 Laboratories including 31 NRLs and 17 Italian OLs participated in the voluntary study

The participants received two sets of three samples (25 g red radish sprouts each containing 0, 2 and 20 CFU/g of STEC O26)

Contaminant (*Genotype*)	Contamination level in:		
	Sample 1	Sample 2	Sample 3
Strain C1188-02, STEC O26 *(stx1+, stx2+, eae+)*	-	Low: 2 CFU/g	High: 20 CFU/g

One set to be analized applying ISO TS 13136 as such (enrichment at 37°C in BPW)

One set to be analized by ISO TS 13136 but enrichment carried out at 41.5°C in BPW

Stability tests:

The stability was assessed using either the enrichment temperature of 37°C or of 41.5 °C on samples with 2 and 20 CFU/g of STEC O26 and tested by ISO TS 13136:2012 after 3, 5 and 10 days since the initial contamination

The **Real Time PCR** screening was positive for the STEC target genes even after 10 days from the spiking for both the set of samples enriched at 37 °C or 41.5 °C. Ct obtained at 41.5°C were lower than those at 37°C

Isolation

	T1 (3 days)	T2 (5 days)	T3 (10 days)
Samples	**Isolation (no. of positive pools)**	**Isolation (no. of positive pools)**	**Isolation (no. of positive pools)**
2 CFU/g @37 ° C	-	-	-
2 CFU/g @41.5 ° C	+ (2 pools)	+ (1 pool)	+ (1 pool)
20 CFU/g @37 ° C	+ (1 pool)	+ (1 pool)	-
20 CFU/g @41.5 ° C	+ (2 pools)	+ (1 pool)	+ (1 pool)

Results: Real Time PCR screening of the test samples

Lab	Detection of virulence and serogroup-associated genes in:																							
	Sample 1 (37 ° C)				Sample 1 (41.5 ° C)				Sample 2 Low level contamination (37 ° C)				Sample 2 Low level contamination (41.5 ° C)				Sample 3 High level contamination (37 ° C)				Sample 3 High level contamination (41.5 ° C)			
	stx1	*stx2*	*eae*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}	*stx1*	*stx2*	*eae*	wzx_{O26}
True value	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L144																								
L257																								
L266																								
L269																								
L283																								
L288																								
L295									-															
L296																								
L300																								
L307																								
L319																								
L323																								
L339																								
L341																								
L350																								
L351																								
L391	+	+	+	+					-	-			-	-			-	-					-	ONT
L400									-	-			-	-			-	-					-	ONT
L439																								
L441																								
L446									-	-							-	-						
L521																								
L542																								
L598																								
L599																								
L609																								
L649	+	+	+	+																				
L662																								
L683																								
L689																								
L698																								
L765																								
L789																								
L793																								
L802																								
L803																								
L813																								
L821																								
L825																								
L875																								
L885																								
L906																								
L929																								
L940									-															
L954																								
L970																								
L979																								
L980																								

Results: Isolation of the STEC O26 from the red radish sprouts

Lab	STEC strain isolation and genotyping from:																			
	Sample 1 (37 ° C)	Sample 1 (41.5 ° C)	Sample 2 (37 ° C)				Sample 2 (41.5 ° C)				Sample 3 (37 ° C)				Sample 3 (41.5 ° C)			
	-	-	STEC O26 Isolation	Genotype			STEC O26 Isolation	Genotype			STEC O26 Isolation	Genotype			STEC O26 Isolation	Genotype		
				stx1	*stx2*	*eae*		*stx1*	*stx2*	*eae*		*stx1*	*stx2*	*eae*		*stx1*	*stx2*	*eae*
True value	None	None	+	+	+	+					+	+	+	+				
L144																		
L257																		
L266																		
L269																		
L283																		
L288																		
L295																		
L296																		
L300																		
L307																		
L319																		
L323																		
L339																		
L341																		
L350				-	-													
L351																		
L391	O26 *stx1 stx2 eae*																	
L400																		
L439																		
L441																		
L446																		
L521																		
L542																		
L598																		
L599																		
L609																		
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L683																		
L689																		
L698																		
L765																		
L789																		
L793																		
L802																		
L803																		
L813																		
L821																		
L825																		
L875																		
L885																		
L906																		
L929																		
L940				-														
L954																		
L970																		
L979																		
L980																		

following enrichment at 41.5 °C there was less background microflora on the plates and more colonies resembling *E. coli* were available for further confirmation.

Sample 2:
29/45 (64,4%) isolated @37°C
43/47 (91,5%) isolated @41.5°C

Sample 3:
35/46 (76.1 %) isolated @37°C
44/47 (93.6 %) isolated @41.5°C

Sensitivity

Real Time PCR

	Enrichment at 37 ° C				Enrichment at 41.5 ° C			
	stx1	***stx2***	***eae***	***wzx$_{O26}$***	***stx1***	***stx2***	***eae***	***wzx$_{O26}$***
Se (low level)	93.9 %*	97.9 %*	100 %	100 %	100 %	100 %	100 %	100 %
Se (high level)	97.9 %	97.9 %	100 %	100 %	100 %	100 %	100 %	100 %

Isolation

	Enrichment at 37 ° C	Enrichment at 41.5 ° C
Se (low level)	73.3 %	92.2 %
Se (high level)	80.7 %	94 %

LOD 37°C

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1		0,005	0,169	5,435	3,880	7,615	23,491	16,767	32,911	0,000
Combined data		0,005	0,169	5,435	3,880	7,615	23,491	16,767	32,911	0,000

LOD 41.5°C

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1		0,016	0,197	1,692	1,141	2,509	7,312	4,929	10,845	0,000
Combined data		0,016	0,197	1,692	1,141	2,509	7,312	4,929	10,845	0,000

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 step slightly increased when the enrichment temperature applied was 41.5 °C

the isolation step was strongly improved when the enrichment temperature was raised at 41.5 °C, being particularly beneficial for the low level contamination samples

LOD_{50} for the isolation step was much lower when the enrichment was carried out at 41.5 °C (1.692 CFU), compared to the enrichment at 37 °C (5.435 CFU)

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