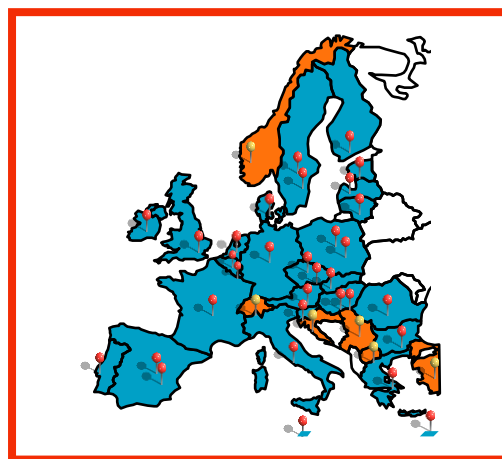
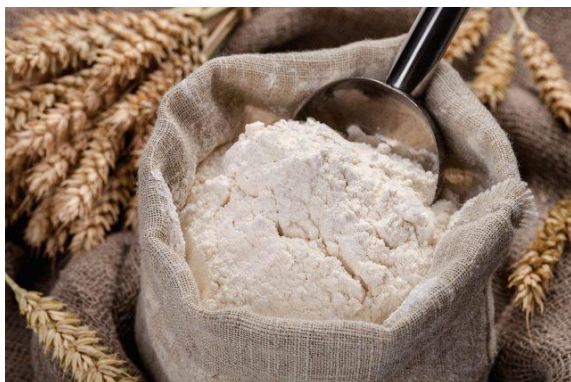


PT25

Detection of STEC in flour October-November 2019



Flour has recently been described as a novel vehicle for transmission of STEC, and was involved in a number of outbreaks too



EURL-VTEC decided to organize an inter-laboratory study, Proficiency Test 25 (PT25), on this particular matrix, in order to enhance the preparedness of NRLs in testing flour for the presence of STEC by applying the ISO TS 13136:2012

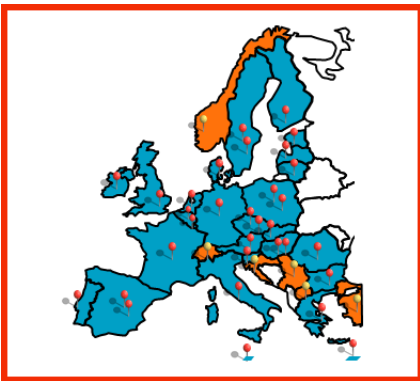


The inter-laboratory study (PT25) was organized and run in October-November 2019

Test samples

The flour used in the study was purchased from a local retailer

Two samples consisting of 25 g of flour have been assayed for the presence of STEC according to ISO TS 13136:2012 and were negative at the PCR screening for the STEC-associated gene targets.



43 Laboratories participated

Stability tests (September 2019):

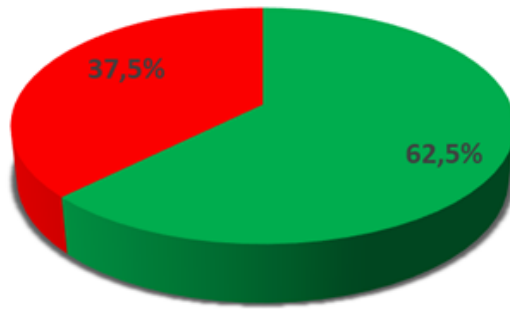
STEC O121 concentration	T0 Replicate 1		T0 Replicate 2		T1 (4 days) Replicate 1		T1 (4 days) Replicate 2	
Test	Real Time PCR	Isolation	Real Time PCR	Isolation	Real Time PCR	Isolation	Real Time PCR	Isolation
1 CFU/25 g	+	+	+	+	+	+	+	+
5 CFU/25 g	+	+	+	+	+	+	+	+
10 CFU/25g	+	+	+	+	+	+	+	+

STEC O121 concentration	T2 (7 days) Replicate 1		T2 (7 days) Replicate 2		T3 (11 days) Replicate 1		T2 (11 days) Replicate 2	
Test	Real Time PCR	Isolation	Real Time PCR	Isolation	Real Time PCR	Isolation	Real Time PCR	Isolation
1 CFU/25 g	-	Not done	-	Not done	+	+	-	Not done
5 CFU/25 g	+	+	+	+	+	+	+	+
10 CFU/25g	+	+	+	+	+	+	+	+

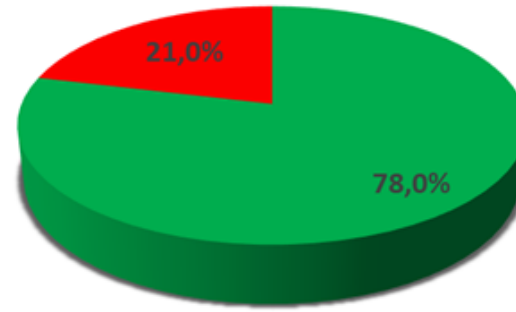
PT25 spiked samples

Contaminant (<i>Genotype</i>)	Contamination level in:		
	Sample 1	Sample 2	Sample 3
ED898 STEC O121 (<i>stx2+</i> , <i>eae+</i>)	0 CFU	1 CFU/25 g (0,04 CFU/g)	5 CFU/25 g (0,2 CFU/g)

Screening step: Percentage of Laboratories correctly detecting STEC in the spiked samples



Sample 2 (1 CFU/25 g)



Sample 3 (5 CFU/25 g)

One participant presented results compatible with an exchange of sample 1 with sample 2: the results reported by this Lab were excluded from the *Se* and *Sp* evaluation

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

NRL	Detection of virulence and serogroup-associated genes in:										
	Sample 1			Sample 2 Lower level contamination				Sample 3 Higher level contamination			
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>Top-5 O-genes</i>	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>Top-5 O-genes</i>
True value	-	-	-	-	+	+	-	-	+	+	-
L136											
L157									-		
L175											
L187					-						
L203					-						
L229											
L240					-						
L258									-		
L286					-						
L295											
L337									-		
L355					-				-		
L375											
L376											
L383											
L413											
L417		+	+		-						
L421											
L424					-						
L443											
L513											
L519									-		
L537					-						
L543					-						
L546											
L556					-						
L574											
L676					-						
L693											
L695									-		
L734											
L737											
L775					-						
L791					-						
L810									-		
L825											
L840											
L843					-				-		
L912											
L925					-						
L967											
L969											
L986					-				-		

Isolation and genotyping of STEC strains from the flour samples

NRL	STEC strain isolation and genotyping from:								
	Sample 1	Sample 2				Sample 3			
	-	STEC O121 Isolation	Genotype			STEC O121 Isolation	Genotype		
			stx1	stx2	eae		stx1	stx2	eae
True value	None	+	-	+	+	+	-	+	+
L136									
L157									
L175									
L187									
L203									
L229		ONT				ONT			
L240									
L258									
L286									
L295									
L337									
L355									
L375									
L376									
L413		ONT				-			
L417									
L421									
L424									
L443									
L513									
L519									
L537									
L543									
L546									
L556									
L676									
L693									
L695									
L734									
L737									
L775									
L791									
L810									
L825									
L840									
L843									
L912									
L925									
L967									
L969									
L986									

All the laboratories detecting STEC in the enrichment culture of sample 2 were able to isolate the contaminating strain. The same applies for sample 3, with the exception of one laboratory

Evaluation of the performance of the method

Screening Step

	Se (Lower level)	Se (Higher level)	Sp
stx1	N.A.	N.A.	100 %
stx2	72.7 %	82 %	100 %
eae	100 %	100 %	N.A.

Limit of detection (LOD) of the isolation step

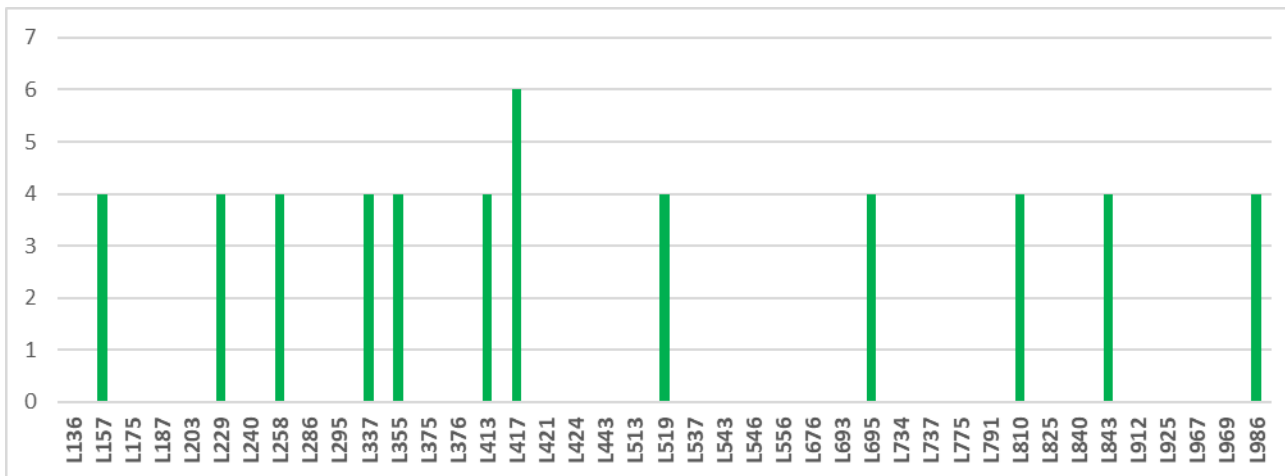
No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	LOD _{50%} ¹			LOD _{95%} ²			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
<i>i</i>	<i>matrix_i</i>	<i>F_i</i>	<i>s_{fi}</i>	<i>d_{0.5,i}</i>	<i>d_{0.5,i,L}</i>	<i>d_{0.5,i,U}</i>	<i>d_{0.95,i}</i>	<i>d_{0.95,i,L}</i>	<i>d_{0.95,i,U}</i>	<i> z_i </i>
1	Flour	0,463	0,161	0,060	0,043	0,083	0,259	0,188	0,357	4,234

Sensitivity: $Se = [\text{true positives} / (\text{true positives} + \text{false negatives})] \times 100$

Specificity: $Sp = [\text{True negatives} / (\text{true negatives} + \text{false positives})] \times 100$

Evaluation of NRLs Proficiency

- 4 penalty points for incorrect stx detection in sample 3 and 2 penalty points for incorrect determination of ese gene in the screenong
- 2 penalty points to the lack of isolation from the sample 3. No penalty points were instead assigned to the lack of isolation from sample 2 (lower level of contamination), as the contamination level was determined as being close to the limit of detection of the procedure.
- As for strain characterization, 2 penalty points were assigned to the laboratories not identifying the O121 serogroup in the STEC isolated strain.
- Sum of 8: underperformance



Concluding remarks

A high participation was recorded for PT25, confirming the eagerness and collaboration of the network.



Two Laboratories received the samples but did not report the results to EURL-VTEC and this represents an issue that needs to be followed up.

3The levels of contamination used in this PT were very low and the results obtained allowed the determination of the LOD50 of the analyte (STEC O121) with the flour matrix as 0.043 CFU/g. This value is very close to the lowest level of contamination used in sample 2 for this PT25 (0.04 CFU/g), therefore no penalty points were assigned for the incorrect detection of STEC in Sample 2.

None of the participating Laboratories obtained a score equal or higher than eight.

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