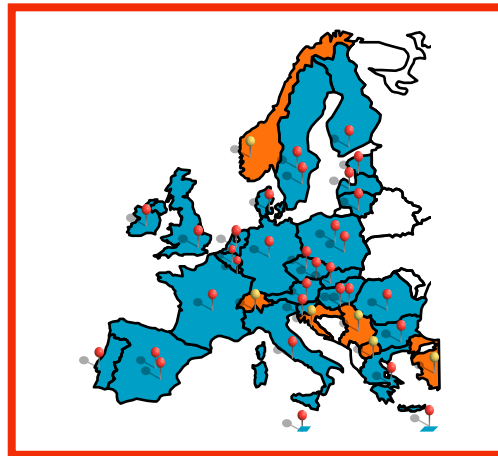


PT16

Detection of STEC in sprout irrigation water



PT16 – OBJECTIVES

- ✓ Regulation (EU) No 209/2013 has introduced for the first time microbiological criteria for VTEC in the EU legislation (sprouts)
- ✓ CEN/ISO/TS 13136:2012 and the EU-RL procedure for the identification of VTEC O104:H4 are prescribed for the detection of VTEC 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

The objective of the study was the evaluation of a procedure for the pre-treatment of sprout spent irrigation water samples to be entered in the analytical flow of the ISO TS 13136:2012

enteric
of this

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

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 – Design of the study

- ✓ The water used in this study had been obtained from a sprout producer
- ✓ 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 STEC strain (High, Low, 0)

PT16 - Analysis of the results

No assignment of **penalty points** for incorrect results

- ✓ **Evaluation of the performances of the method**
 - ✓ Sensitivity (**Se**) and Specificity (**Sp**) were differentially calculated for the various STEC characters considered in the studies
 - ✓ **LOD** of the isolation step was calculated

51 NRLs representing

30 EU countries

+ the NRLs of
Norway

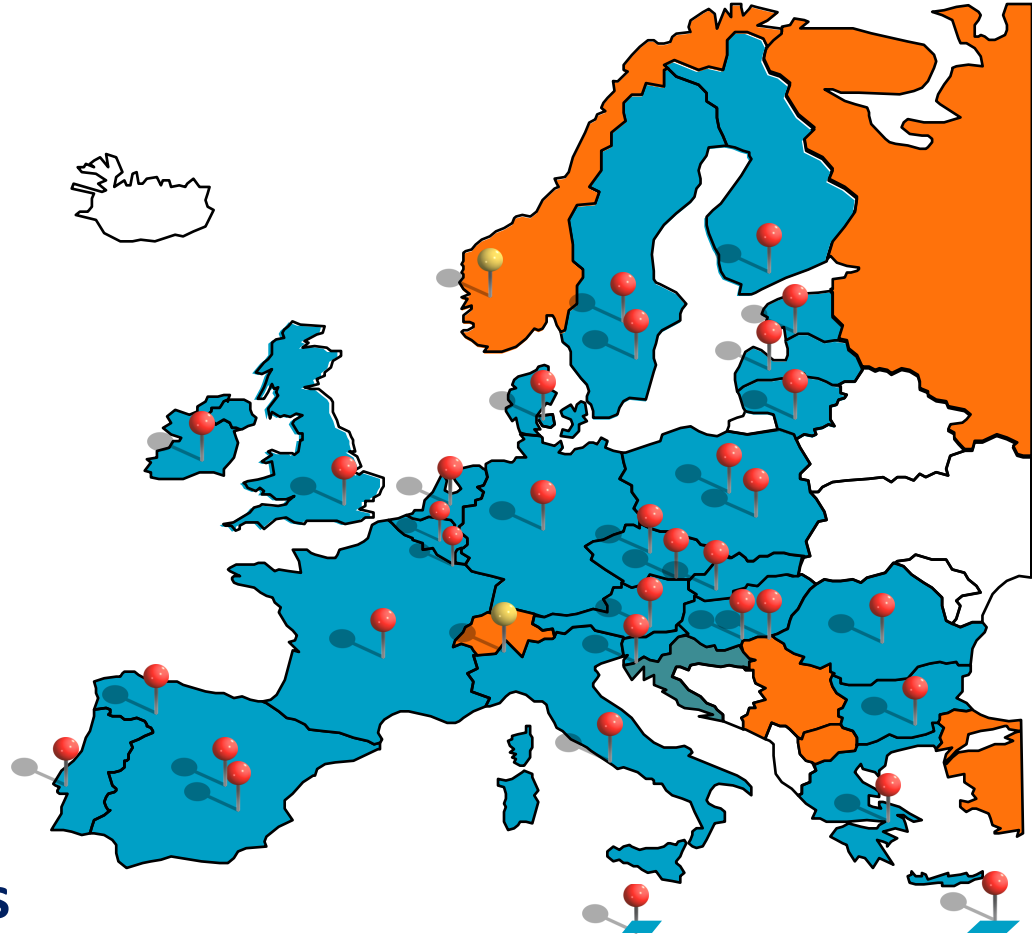
Switzerland

Russia

Egypt

+10 Italian OLs

Results reported from 50 labs



PT16 – Characteristics of the sprout irrigation water samples

Contaminant (<i>Genotype</i>)	Contamination level in:		
	Sample A	Sample B	Sample C
STEC O157 (<i>vtx1+</i> , <i>vtx2+</i> , <i>eae+</i>)	High: 500 CFU/ml	Low: 200 CFU/ml	-

UoM: 0,27 log CFU/ml

Test samples were immediately refrigerated and sent on November the 30th into refrigerated safety boxes

PT16 – assessment of stability and homogeneity

Stability: *ad hoc* spiked samples were tested in a total 9 days time span (T=0, 3, 7 and 9 days)

Homogeneity: When the test samples were prepared, 10 bottles for each of the three samples were randomly selected for homogeneity testing and analyzed according to the PT laboratory procedures

All the homogeneity and stability tests gave the expected results

PT16 Results: Detection of virulence and serogroup-associated genes

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample A				Sample B				Sample C			
	High level contamination				Low level contamination							
	vtx1	vtx2	eae	wzx _{O157}	vtx1	vtx2	eae	wzx _{O157}	vtx1	vtx2	eae	wzx _{O157}
True value	+	+	+	+	+	+	+	+	-	-	-	-
L130												
L140												
L180												
L186												
L190												
L243												
L244												
L257												
L261												
L271												
L285												
L303												
L320												
L324												
L327												
L415												
L469												
L470												
L476												
L528												
L545												
L546												
L547												
L551												
L559												
L568												
L574												
L583												
L615												
L617												
L627												
L656												
L658												
L660												
L714												
L725												
L733												
L756												
L761												
L782												
L813												
L831												
L836												
L849												

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample A				Sample B				Sample C			
	High level contamination				Low level contamination							
	vtx1	vtx2	eae	wzx _{O157}	vtx1	vtx2	eae	wzx _{O157}	vtx1	vtx2	eae	wzx _{O157}
True value	+	+	+	+	+	+	+	+	-	-	-	-
L885												
L887												
L936												
L952												
L968												
L997												

Sample A (High level): 48 labs identified correctly *vtx1*, *vtx2* and *eae* genes in the screening (2 labs reported a total of 3 incorrect results). 48 labs identified *rfbE*_{O157}

Sample B (Low level): 47 labs identified correctly *vtx1*, *vtx2* and *eae* genes in the screening (3 labs reported a total of 4 incorrect results). 48 labs identified *rfbE*_{O157}

Samples A and B: One lab incorrectly identified the presence of *wzx*_{O104}

PT 16 Results: isolation of the STEC O157 strain in the sprout irrigation water samples

STEC strain isolation and genotyping from:									
NRL	Sample A				Sample B				Sample C
	VTEC O157 Isolation	Genotype			VTEC O157 Isolation	Genotype			-
		stx1	stx2	eae		stx1	stx2	eae	
True value	+	+	+	+	+	+	+	+	None
L130									
L140									
L180									
L186									
L190									
L243									
L244									
L257									
L261									
L271									
L285									
L303									
L320									
L324									
L327									
L415									
L469									
L470									
L476									
L528									
L545									
L546									
L547									
L551									
L559									
L568									
L574									
L583									
L615									
L617									
L627									
L656									
L658									
L660									
L714									

STEC strain isolation and genotyping from:									
NRL	Sample A				Sample B				Sample C
	VTEC O157 Isolation	Genotype			VTEC O157 Isolation	Genotype			-
		stx1	stx2	eae		stx1	stx2	eae	
True value	+	+	+	+	+	+	+	+	None
L725									
L733									
L756									
L761									
L782									
L813									
L831									
L836									
L849									
L885									
L887									
L936									
L952									
L968									
L997									

Sample A: 44/50 labs isolated STEC O157

Sample B: 42/50 labs isolated STEC O157

Evaluation of the performance of the method

Real Time PCR screening

Sensitivity

<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>rfbE</i> _{O157}
HL 97.9%	HL 100%	HL 97.9%	HL 97.9%
LL 97.9%	LL 97.9%	LL 97.9%	LL 97.9%

Specificity

<i>stx1</i>	<i>stx2</i>
100%	100%

Isolation step

Se: 88% (high level) and 84% (low level)

- LOD_{50%}: 104.9 CFU per gram (c.i. 79,4-138,7)
- LOD_{95%}: 789.1 CFU per gram (c.i. 27.6-70)

PT 16: CONCLUDING REMARKS

- ✓ 51 Labs (34 NRLs and 17 Italian OLS) joined the study, results sent by 50
- ✓ No Penalty points were assigned since the study aimed at evaluating the performances of the method (including the pre-treatment step)
- ✓ The analytical results, provided by 50 laboratories, confirmed the suitability and fit-for-purpose of the developed treatment procedure for spent irrigation water, based on a simple centrifugation step
- ✓ The presence of the STEC O157 virulence 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).

Soon the procedure will be made available to the Network by preparing a SOP to be published on the EU-RL VTEC website

PT17

Detection of STEC in ground beef



Why this matrix?

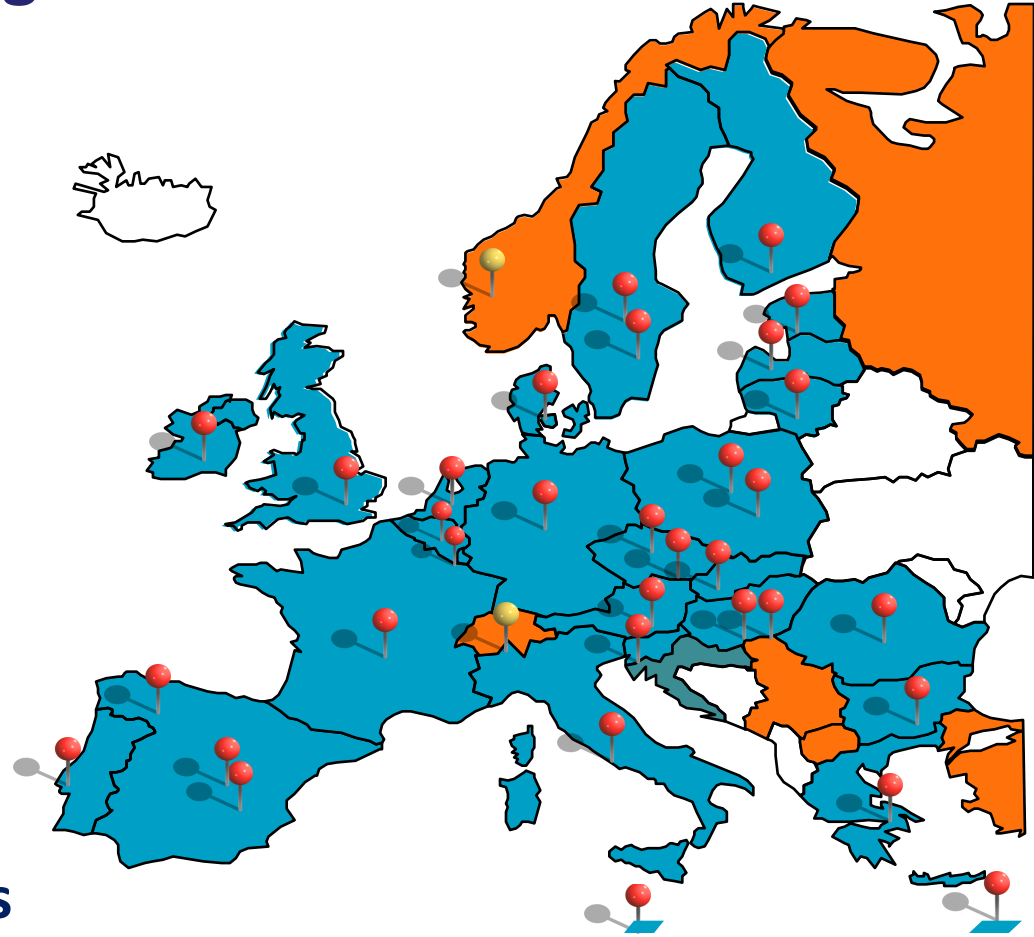


- Cattle is the major reservoir of STEC.
- Beef meat represents a food commodity traditionally associated with STEC infection.
- Minced meat has been recognized as the vehicle of STEC infections in numerous outbreaks, including the first epidemic episode occurred during the 80s when STEC O157 was identified for the first time.
- Even though microbiological criteria are not in place for this food commodity, bovine meat samples are continuously analyzed for the presence of STEC
- This matrix had never been proposed before in PTs organized by the EURL-VTEC.

37 NRLs representing
26 EU countries

+ the NRLs of
Norway
Switzerland
Russia
Chile
Egypt

Results reported from 36 labs



PT17 – Characteristics of the minced meat samples

Test portion: 25 g ground beef

Contaminant (<i>Genotype</i>)	Contamination level in:		
	Sample A	Sample B	Sample C
Strain ED 76, STEC O91 (<i>stx1+</i> , <i>stx2+</i> , <i>eae-</i>)	-	Low: 5 CFU/g	High: 50 CFU/g

UoM: 0,121 log CFU/ml

Spiked samples were prepared on 15 April, immediately refrigerated and sent on April the 19th into refrigerated safety boxes

PT17 – assessment of stability and homogeneity

Stability: *ad hoc* spiked samples were tested in a total 12 days time span (T=0, 4, 7 and 12 days)

Homogeneity: When the test samples were prepared, six bags for each of the three samples were randomly selected for homogeneity testing and analyzed according to the PT laboratory procedures

All the homogeneity and stability tests gave the expected results

PT17 Results: Detection of virulence and serogroup-associated genes

36
Labs

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample A				Sample B Low level contamination				Sample C High level contamination			
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	Top-5 and O104 associated genes	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	Top-5 and O104 associated genes	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	Top-5 and O104 associated genes
True value	-	-	-	-	+	+	-	-	+	+	-	-
L107												
L124												
L148												
L170											+	O26
L177												
L181												
L208								O104				O104
L280												
L328											+	O145
L343												
L356	+	+					+	O26				
L360												
L390												
L417												
L427								O104			+	O26
L435												
L444												
L524												
L597												
L614												
L630												
L653							+	O26			+	O26
L675							+	O26				
L705												
L721		+	+	O26	-		+		-		+	
L782												
L788												
L789												
L817								O104				
L838												
L844												
L873												
L886												
L907												
L912								O104				O104
L975							+	O26			+	

Sample A: one lab reported the presence of *stx1* and *stx2* and one lab reported *stx2*, *eae* and *WZX*_{O26}

Samples B and C: all but one lab detected the presence of STEC

Sample B: *eae* gene detected by 5 labs, in 4 cases together with *wzx*_{O26}

Sample C: *eae* gene detected by 6 labs, in 3 cases together with *wzx*_{O26} and in one case with *ihp1*_{O145}

Few labs detected the presence of *wzx*_{O104} (4 in sample B and 2 in sample C)

PT 17 Results: isolation of the STEC O91 strain

NRL	Sample A	Sample B								Sample C							
	-	STEC O91 Isolation	Genotype			Other E. coli	Genotype			STEC O91 Isolation	Genotype			Other E. coli	Genotype		
			stx1	stx2	eae		stx1	stx2	eae		stx1	stx2	eae		stx1	stx2	eae
True value	None	+	+	+	-					+	+	+	-				
L107																	
L124																	
L148																	
L170		ONT								ONT				O26	-	-	+
L177		ND								ND							
L181																	
L208		ND								ND							
L280																	
L328		ND								ND				O145	-	-	+
L343																	
L356	ONT, H4+																
L360		ND								ND							
L390																	
L417																	
L427																	
L435																	
L444														O157	-	-	-
L524		ONT								ONT							
L597																	
L614		ONT								ONT							
L630		O26								O26							
L653		ND				O26	+	+	+	ND				O26	+	+	+
L675		ND								ND							
L705																	
L721			-								-						
L782																	
L788						O103	-	+	-								
L789																	
L817		ND								ND							
L838																	
L844		ND								ND							
L873	O26, eae+	ND								ND							
L886																	
L907																	
L912		ND								ND							
L975		ONT				O26	-	-	+	ONT							

25 labs isolated STEC from both samples B and C, 24 correctly genotyped the strain, one missed *stx1* detection

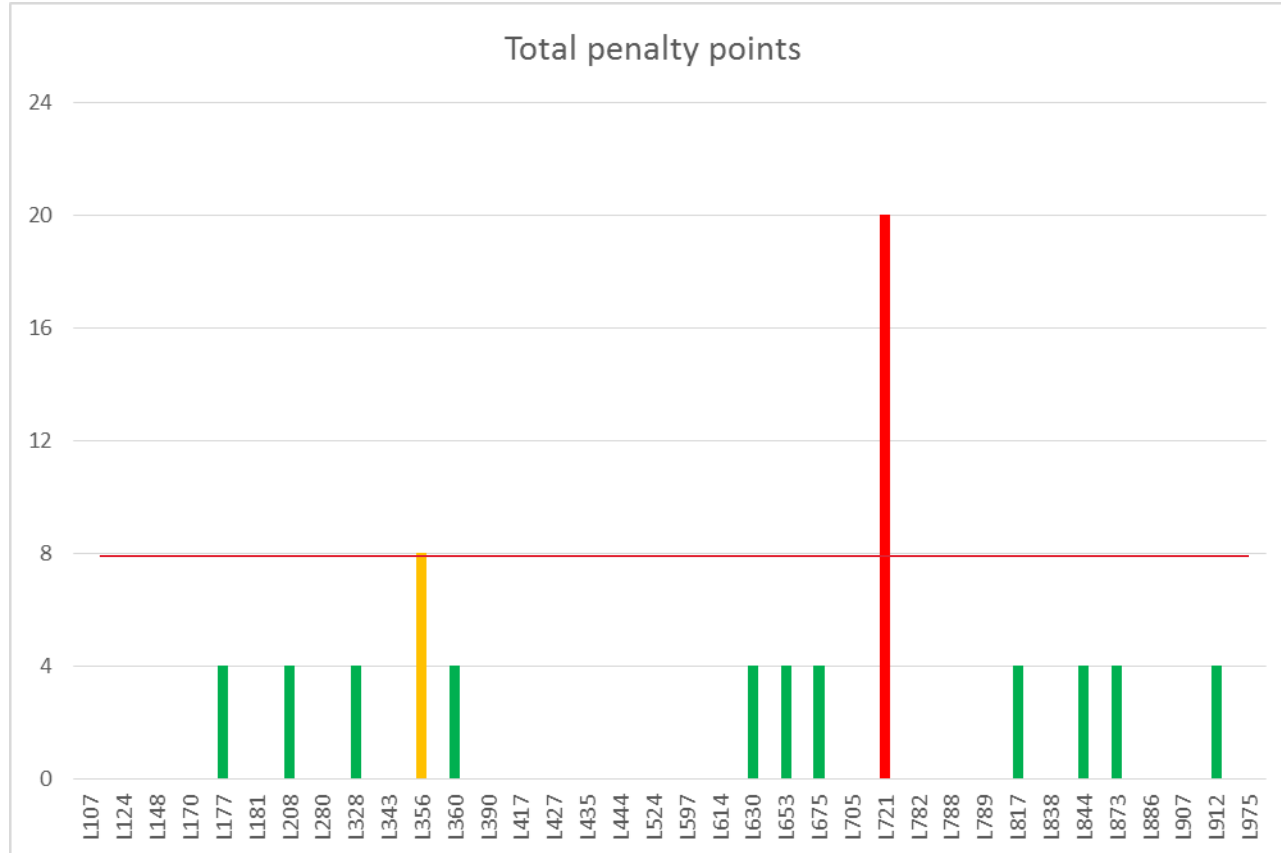
4/25 labs didn't determine the O91 serogroup (ONT)

One lab reported the STEC isolated strain as O26

3 lab isolated an EPEC O26 and one isolated an EPEC O145

One lab reported results compatible with a mixed culture of the STEC O91 and the EPEC O26

Evaluation of the NRL performance in the PT17 procedure



Uncorrect detection of *stx* genes in both screening and isolation: 4 penalty points

Lack of isolation in sample C: 4 penalty points

Lack of determination of O91 serogroup (ONT): no penalty points

Uncorrect serogroup of the isolated strain: 2 penalty points

Performance higher than 8 was considered as unsatisfactory – 1

Evaluation of the performance of the method

PCR screening

(evaluated for 35 labs for *stx1* and 36 for *stx2*)

Sensitivity

<i>stx1</i>	<i>stx2</i>
HL 100.0%	HL 100.0%
LL 100.0%	LL 100.0%

Specificity

<i>stx1</i>	<i>stx2</i>
97,2 %	94,7%

Isolation step

Se: 78.3% (both high and low levels)

- LOD_{50%}: 13.5 CFU per gram (c.i. 9.2-19.7)
- LOD_{95%}: 58.2 CFU per gram (c.i. 39.8-85.0)



- ✓ 37 NRLs participated, 36 submitted the results
- ✓ The presence of the STEC O91 virulence genes was identified correctly by 35 NRLs (97.2 %) in both samples B (low level of contamination) and C (high level of contamination).
- ✓ Isolation was achieved by 25 NRLs (78.3 %) in both samples and C
- ✓ ISO TS 13136:2012 method represents a suitable tool for the detection of all the STEC serogroups analysed so far in the food commodity most regarded as vehicles of human infections.

THANKS FOR YOUR ATTENTION!