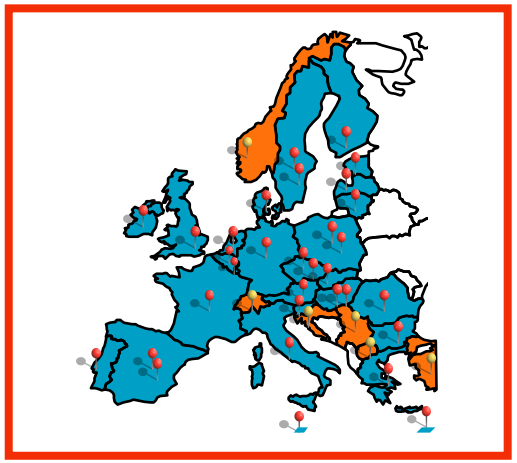
PT14 and PT15

Detection of VTEC in sprouts



PT14 and 15 – 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 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
- ✓ The aims of these PTs were:
  - ✓ To improve the preparedness of the NRLs towards testing such matrix for the presence of VTEC, according to the EU rules
  - ✓ To expand the range of serogroups for which the analytical performance parameters of the detection method have been determined
  - ✓ To give further support to the NRLs and the Official Laboratories for the accreditation of the ISO/TS 13136:2012

PT14 and 15 - DESIGN OF THE STUDIES

- ✓ Three 25 g portions of sprouts potentially contaminated with VTEC were sent to the participating laboratories
- ✓ The samples were spiked with three different levels of contamination of the same VTEC strain (High level, Low level, 0)
- ✓ Organized according to the requirements of ISO 17043:2010

PT14 and PT15 - Evaluation of the results

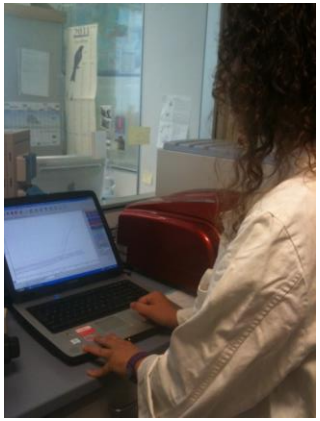
Assignement of penalty points for incorrect results

- ✓ **RT-PCR screening step: the virulence genes that were identified incorrectly**
  - ✓ **4 points for each incorrect or missing result on virulence and serogroup-associated genes**
- ✓ **Isolation step**
  - ✓ **4 points for the lack of isolation of VTEC strain in sample A**
- ✓ **Performance in the overall procedure**
  - ✓ **Sum of penalty points obtained in the two steps of the PT**
  - ✓ **A sum of 8 points considered as the threshold for under-performance**
- ✓ **Evaluation of the performances of the method**
  - ✓ **Sensitivity (Se) and Specificity (Sp) were differentially calculated for the various VTEC characters considered in the studies**
  - ✓ **LOD of the isolation step was calculated**

PT14 and PT15 – Methodology

Upon reception, test samples added with 225 ml BPW and homogenized

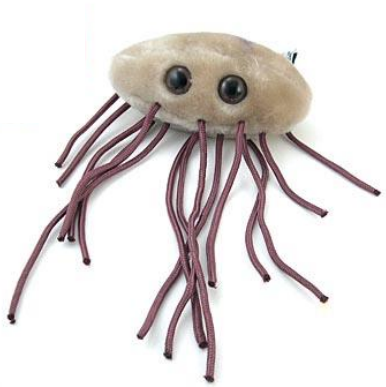
Extract DNA from 1 ml of enrichment cultures



Real Time PCR for VTEC
ISO/TS 13136 and adaptation for *E. coli* O104:H4

Negative → STOP

Positive → Go for isolation



PT14 – Autumn 2014

41 NRLs representing
28 EU countries

+ the NRLs of

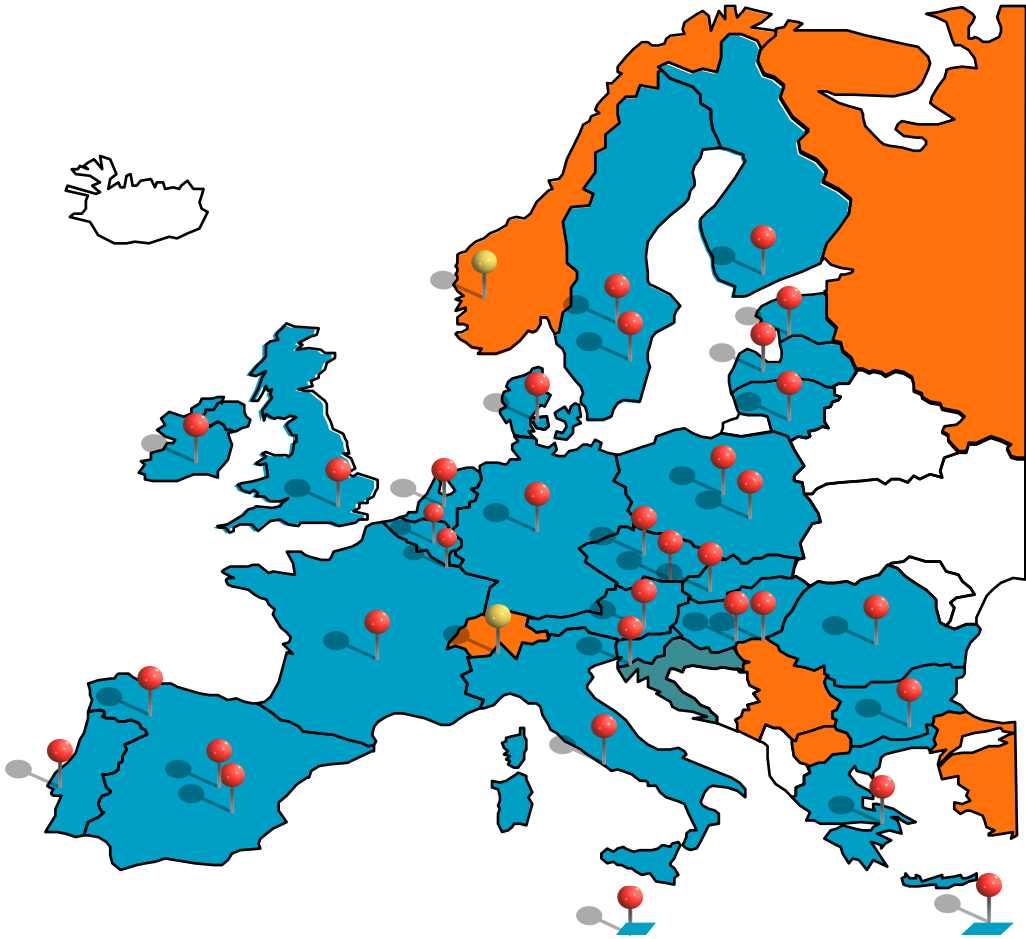
Norway

Switzerland

Turkey

Russia

Egypt



PT14 – Characteristics of the sprout samples

alpha-alpha (90 %) and watercress (10 %) sprouts

Contaminant (*Genotype*)	Contamination level in:		
	Sample A	**Sample B**	**Sample C**
VTEC O104 (*vtx1+, vtx2-, eae-, fliC$_{H4}$-, aggR-, aaiC-*)	High: 1000 CFU/g	Low: 100 CFU/g	-

UoM: 0,125 log CFU/ml

Test samples were immediately refrigerated and sent on November the 24th into refrigerated safety boxes

PT14 – assessment of stability and homogeneity

Stability: *ad hoc* spiked samples were tested in a total 11 days time span (T=0, 4, 6 and 11 days)

Homogeneity: When the test samples were prepared, 10 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

PT14 Results: Detection of virulence and serogroup-associated genes

40 Labs

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample A High level contamination				Sample B Low level co		Sample C					
	vtx1	*vtx2*	*eae*	wzx_{O104}	*vtx1*	*vtx2*						
True value	+	-	-	+	+	-						
L106												
L118		+	+			+						
L157		+				+						
L206												
L240												
L258												
L273												
L307								-				
L313												
L354												
L367												
L401					-							
L402												
L433												
L463												
L519												
L534												
L544												
L551												
L566												
L605					-							
L608												
L633												
L674	-	+			-	+						
L679												
L687												
L691												
L692												
L699												
L712												
L718												
L737												
L775												
L776												
L804												
L905												
L949												
L969				ND								
L986												
L987								-				

Virulence genes - Sample A: 37 (92%) correct results, the remaining 3 labs provided 5 uncorrect results

Virulence genes - Sample B: 35 (87%) correct results, the remaining 5 labs provided 7 uncorrect results

O104-associated gene
Sample A: 38 labs (95%)
Sample B: 33 labs (82%)

PT 14 Results: isolation of the VTEC O104 strain

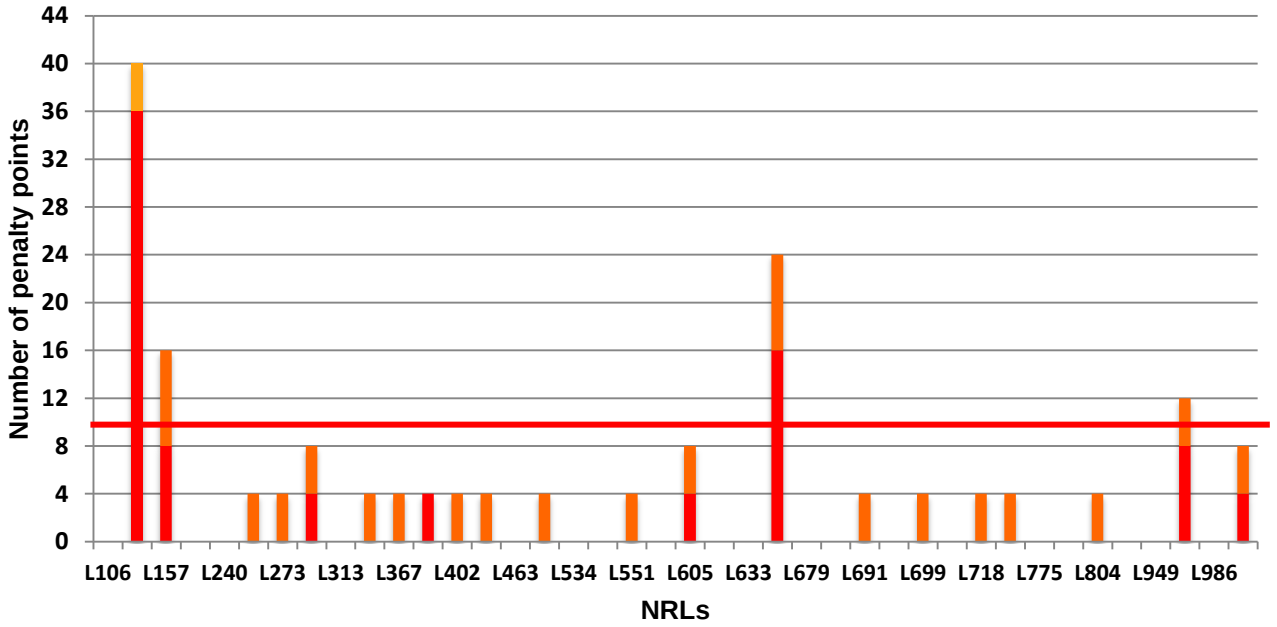
NRL	VTEC strain isolation and genotyping from:										
	Sample A					Sample B					Sample C
	VTEC O104 Isolation	Genotype				VTEC O104 Isolation	Genotype				
		vtx1	*vtx2*	*fliCH4* (H4)	*aaiC* and/or *aggR*		*vtx1*	*vtx2*	*fliCH4* (H4)	*aaiC* and/or *aggR*	
True value	+	+	-	-	-	+	+	-	-	-	None
L106											
L118											
L157			+	ND				+	ND		
L206											
L240						-					
L258				ND					ND		
L273				ND					ND		
L307				+		-					
L313											
L354				ND					ND		
L367				ND					ND		
L401											
L402				ND					ND		
L433				ND					ND		
L463											
L519				ND					ND		
L534						-					
L544											
L551	-					-					
L566											
L605	ND										
L608											
L633											
L674		-	+				-	+			
L679											
L687						-					
L691				+							
L692						-					
L699				ND					ND		
L712											
L718				ND		-					
L737	-					-					
L775											
L776											
L804				ND					ND		
L905											
L949											
L969											
L986											
L987				ND							

Sample A:
35 (87%) isolated O104 strain

Sample B:
27 (67%) isolated O104 strain

However,11 labs reported uncorrect/missing results for the characterization of the isolated strain

Evaluation of the NRL performance in the PT14 procedure



Real Time PCR screening: red
isolation step: orange

Performance higher than 8 was considered as unsatisfactory – 4 labs

Evaluation of the performance of the method

PCR screening
(evaluated for 39 labs)

Sensitivity

vtx1	*wzx*$_{O104}$
HL 97.4%	HL 100%
LL 92.3%	LL 94.4%

Specificity

vtx1	*vtx2*	*eae*
100%	94.8%	100%

Isolation step
(evaluated for 37 labs for HL and 35 labs for the LL)

Se: 94.6% (high level) and 77.1% (low level)

- $LOD_{50\%}$: 182.58 CFU per gram (c.i. 6.4-16.1)
- $LOD_{95\%}$: 789.1 CFU per gram (c.i. 27.6-70)

PT 14: CONCLUDING REMARKS (I)

- ✓ **41 NRLs joined the study, results sent by 40**
- ✓ **Penalty points were assigned and only 4 laboratories out of the 40 NRLs that contributed results showed an unsatisfactory performance**
- ✓ **Almost all the European NRLs are able to detect VTEC contamination in sprouts, according to the prescriptions of Reg. (EU) 209/2013**

PT 14: CONCLUDING REMARKS (II)

- ✓ **Testing for *fliC*$_{H4}$ is prescribed by Reg. (EU) 209/2013, and therefore penalty points were assigned to missing result**

- ✓ **The results of the PT enabled us to evaluate the performance parameters of the method indicated in the Reg. (EU) 209/2013 for the detection of VTEC in sprouts, labs can refer to these parameters for the accreditation of the method**

PT15 – Spring 2015

36 NRLs representing
26 EU countries

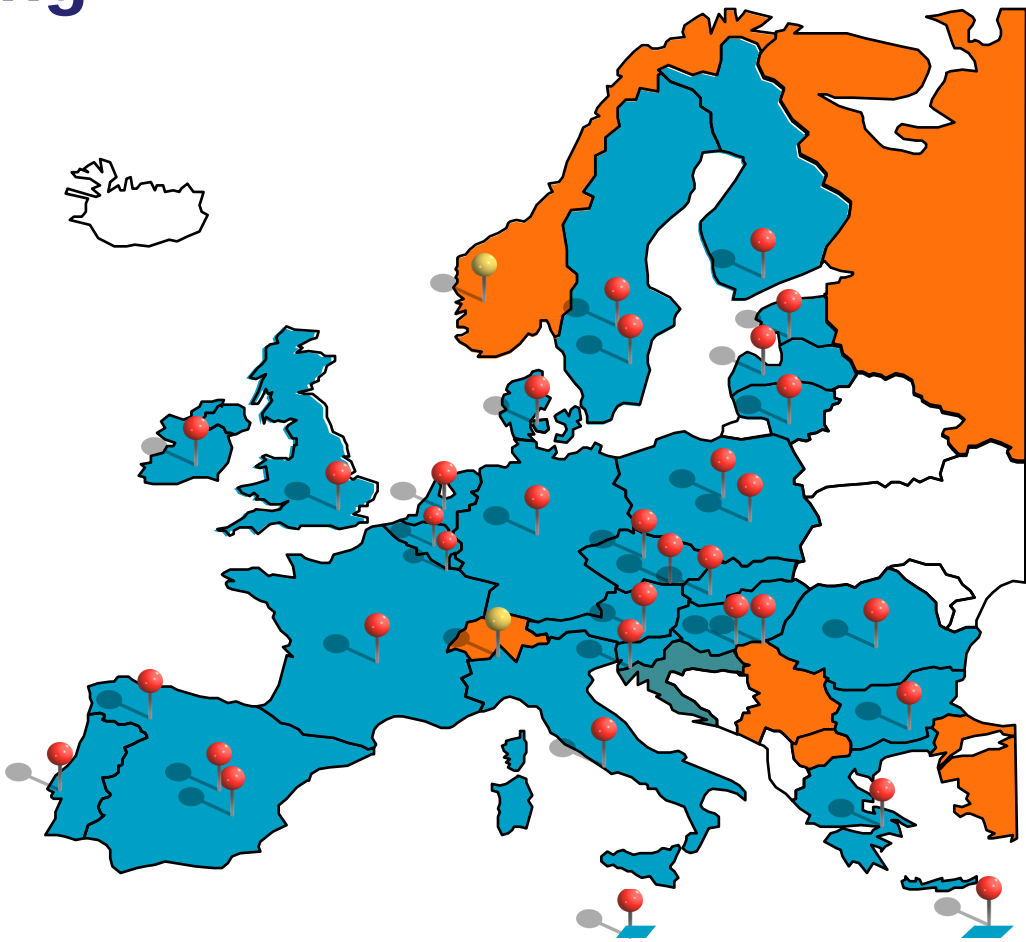
+ the NRLs of

Norway

Switzerland

Russia

Egypt



PT15 – Characteristics of the sprout samples

alpha-alpha (90%) and watercress (10%) sprouts
Background microflora around 10^8 CFU/g

Contaminant (*Genotype*)	Contamination level in:		
	Sample A	**Sample B**	**Sample C**
VTEC O111 (*vtx1*+, *vtx2*+, *eae*+)	High: 1000 CFU/g	Low: 100 CFU/g	-

UoM: 0,138 log CFU/ml

Test samples were immediately refrigerated and sent on April the 20^{th} into refrigerated safety boxes

PT15 – assessment of stability and homogeneity

Stability: *ad hoc* spiked samples were tested in a total 13 days time span (T=0, 3, 6, 10 and 13 days)

Homogeneity: When the test samples were prepared, 10 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

PT15 Results: Detection of virulence and serogroup-associated genes

36 Labs

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample A High level contamination				Sample B Low level contamination				Sample C			
	vtx1	*vtx2*	*eae*	*wzx*$_{O111}$	*vtx1*	*vtx2*	*eae*	*wzx*$_{O111}$				
True value	+	+	+	+	+	+	+	+				
L130												
L140						-						
L180												
L190												
L213												
L244												
L257												
L261		-				-						
L285												
L317	-	-			-	-						
L324					-							
L327												
L400												
L415												
L469				O157	-	-						
L470												
L498												
L528												
L545												
L546												
L547								O157/O111				
L568												
L574												
L583												
L615												
L627												
L658												
L714												
L782				ONT				ONT				
L831	-				-							
L849												
L887												
L936												
L957												
L968												
L997												

Virulence genes - Sample B: 30 labs (83%) correct results, the remaining 4 labs provided 8 uncorrect results

O-group associated genes - Sample B: 32 (89%) correct results

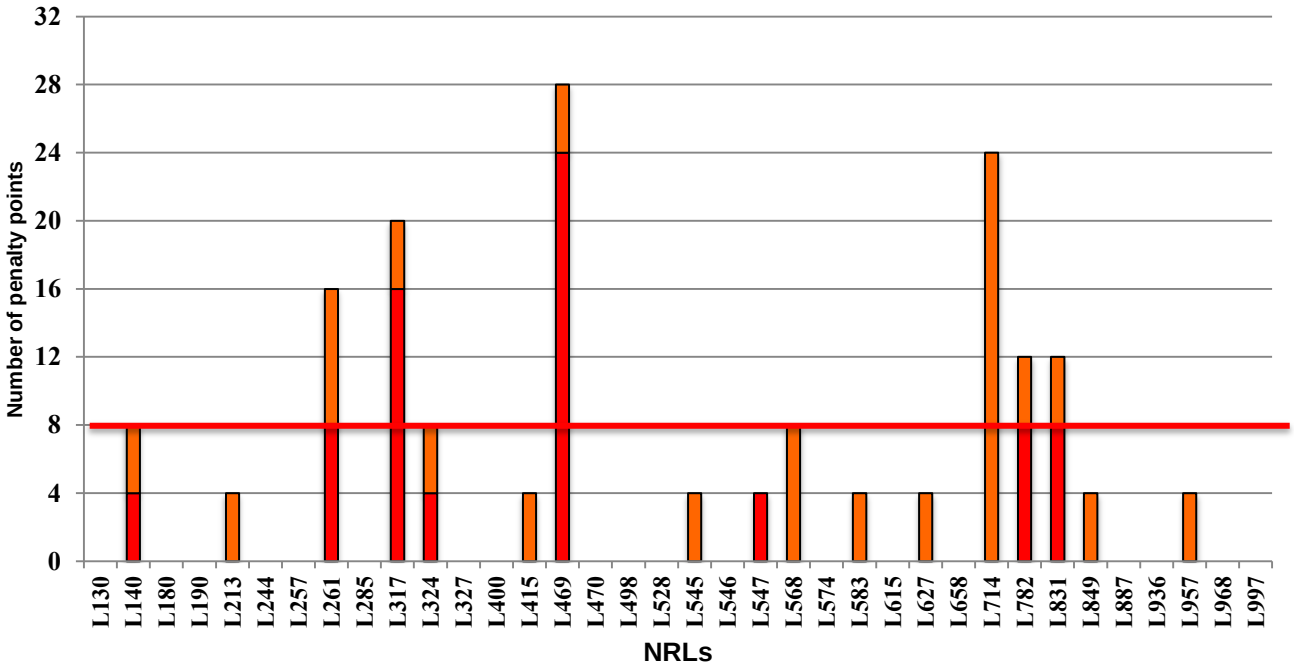
PT 15 Results: isolation of the VTEC O111 strain

VTEC strain isolation and genotyping from:									
NRL	Sample A				Sample B				Sample C
	VTEC O111 Isolation	Genotype			VTEC O111 Isolation	Genotype			-
		vtx1	*vtx2*	*eae*		*vtx1*	*vtx2*	*eae*	
True value	+	+	+	+	+	+	+	+	None
L130									
L140									
L180									
L190									
L213									
L244									
L257									
L261			-				-		
L285									
L317									
L324						-			
L327									
L400									
L415									
L469									
L470									
L498									
L528									
L545									
L546									
L547									
L568				-				-	
L574									
L583									
L615									
L627									
L658									
L714		-	-	-		-	-	-	
L782									
L831									
L849									
L887									
L936									
L957									
L968									
L997									

Sample B:
16 labs (44%) isolated O111 strain

However, 4 labs reported uncorrect/missing results for the characterization of the isolated strain

Evaluation of the NRL performance in the PT15 procedure



Real Time PCR screening: red
isolation step: orange

Performance higher than 8 was considered as unsatisfactory – 6 Labs

Evaluation of the performance of the method

PCR screening
(evaluated for 33 labs)

Sensitivity

vtx1	*vtx2*	*eae*	wzx_{O111}
HL 97.0%	HL 97.0%	HL 100%	HL 100%
LL 94.0%	LL 94.0%	LL 100%	LL 94.4%

Specificity

vtx1	*vtx2*
100%	100%

Isolation step
(evaluated for 33 labs)

Se: 69.7% (high level) and 45.5% (low level)

- $LOD_{50\%}$: 377.4 CFU per gram (c.i. 256.5-556)
- $LOD_{95\%}$: 1,631.1 CFU per gram (c.i. 1,106.9-2,403.3)

PT15 – FINAL REMARKS (I)

- ✓ **36 NRLs participated, all of them submitted the results**

- ✓ **The presence of the VTEC O111 genes was identified correctly by 31 NRLs (86 %) in sample A (high level of contamination) and by 28 NRLs (78 %) in sample B (low level of contamination)**

- ✓ **Isolation was achieved by 23 NRLs (64 %) from sample A and by 17 NRLs (47 %) from sample B .**

PT15 – FINAL REMARKS (II)

- ✓ **No penalty points were attributed to the lack of isolation for sample B, and only 6 labs obtained unsatisfactory results**

- ✓ **Unsuccessful attempts of isolation of O111 strain from PCR-positive enrichment cultures reflects the lack of selective/differential media**

- ✓ **The results of the PT allowed the evaluation of the performance parameters of the method indicated in the Reg. (EU) 209/2013 for the detection of VTEC in sprouts, labs can refer to these parameters for the accreditation of the method**

More to come…..

Forthcoming PT16: detection of VTEC in irrigation water, according to Reg. (EU) 209/2013

Aims: evaluate the performance of an ad hoc method for testing such matrix for the presence of VTEC and improve the preparedness of the NRLs towards testing such matrix according to the EU rules

Get ready to receive the samples

at the beginning of December

Sneak Peek to PT16 procedure

Samples will consist in 200 ml irrigation water

Centrifuge at 5000 rpm for 30-40 minutes at + 4° C

Decant Supernatant

Resuspend the pellet in 10X BPW of its volume

Enrichment is carried out over night

Extract DNA from 1 ml and test for the presence of VTEC