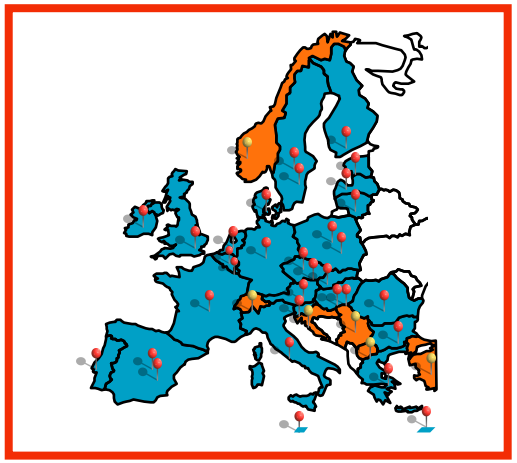
PT12

Detection of VTEC and other pathogenic *E. coli* in sprouts



PT12 – OBJECTIVES

- ✓ **To further train the NRLs in the use of the international standard method CEN/ISO/TS 13136 for the detection of VTEC (Reg. 209/2013)**

- ✓ **To introduce reliable methods for the detection of pathogenic *E. coli* other than VTEC in the diagnostic panel available in the *E. coli* NRL network**

PT12 - DESIGN OF THE STUDY

- ✓ **Three 25 gr portions of sprouts potentially contaminated with VTEC, EAggEC, ETEC and EIEC**
- ✓ **Methods:**
  - ✓ **CEN/ISO/TS 13136 for the detection of VTEC**
  - ✓ **Real Time PCR SOPs for the target viulence genes:**
    - ✓ ***aaiC* and *aggR* for EAggEC**
    - ✓ ***lt, sth*, and *stp* for ETEC**
    - ✓ ***ipaH* for EIEC**
- ✓ **Organized according to the requirements of ISO 17043:2010**
- ✓ **Samples shipped on 18 November 2013**

PT12 – PARTICIPANTS: 38 NRLs

34 NRLs representing
28 EU countries

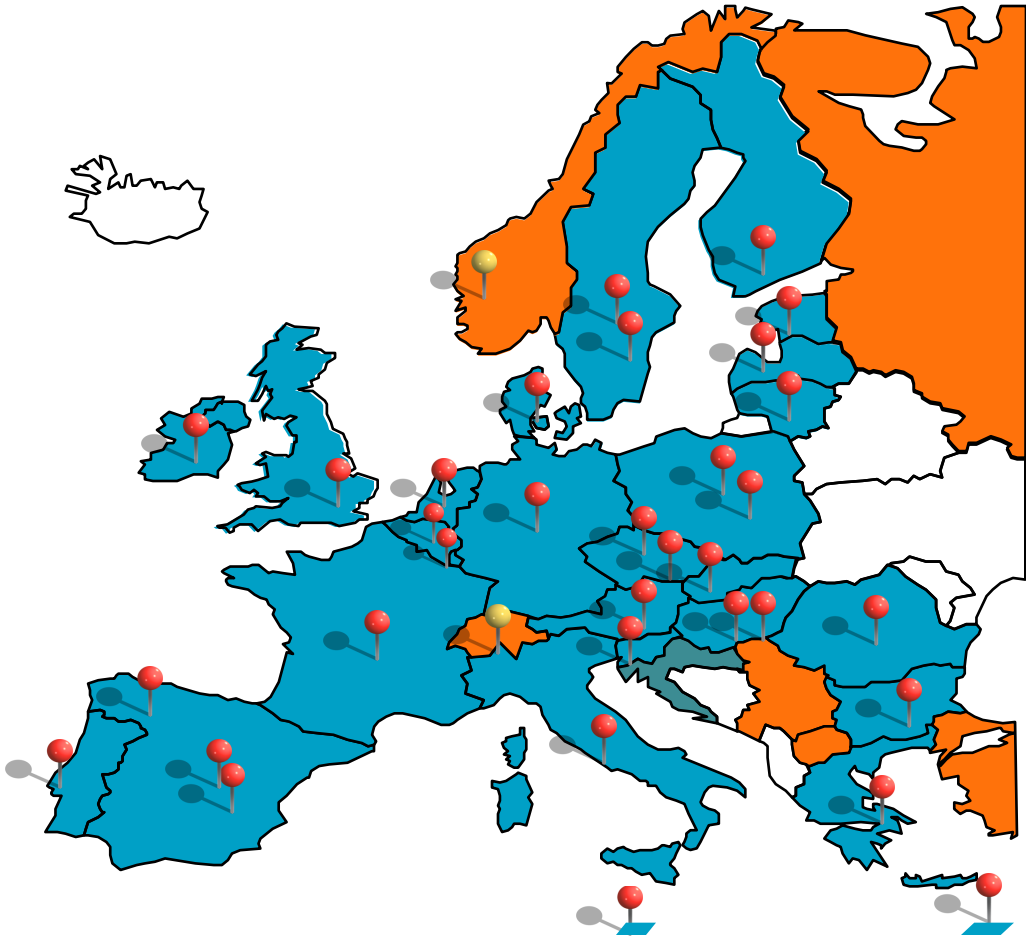
+ the NRLs of

Norway

Switzerland

Argentina

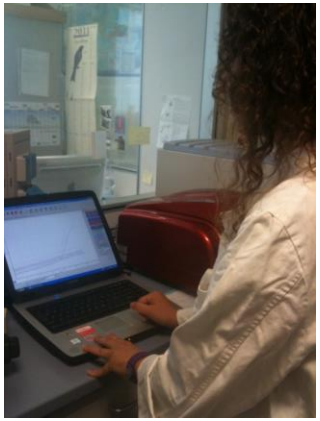
Egypt



PT12 - Laboratory procedure

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

Positive → Go for isolation

Negative → **Real Time PCR for *E. coli* pathogroups proposed in PT11**

Test for EIEC, ETEC, EAggEC
Isolation from the positive samples

PT12 - 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 *vtx* genes**
  - ✓ **2 points for each incorrect result on other virulence genes**
  - ✓ **1 point for missing results on other virulence genes**

- ✓ **Isolation step**
  - ✓ **4 points for the lack of isolation of VTEC strains**
  - ✓ **2 points for the lack of isolation of other patho-groups**

- ✓ **Performance in the overall procedure**
  - ✓ **A sum of 8 points considered as the threshold for under-performance**
  - ✓ **Score higher than 8 without errors on *vtx* genes or VTEC isolation still considered as satisfactory**

Characteristics of the sprout samples included in the study

Contaminant (*Genotype*)	Sample A	Sample B	Sample C
VTEC O157 (*vtx1, vtx2, eae*)	1.5 x 10^3 CFU/g	-	-
EIEC (*ipaH*)	-	1.8 x 10^2 CFU/g	-

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

Table 2 - Detection of virulence genes in:

NRL	Sample A									Sample B									Sample C								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*lt*	*sth*	*stp*	*ipah*	*vtx1*	*vtx2*	*eae*	*aggR*	*aaiC*	*lt*	*sth*	*stp*	*ipah*	*vtx1*	*vtx2*	*eae*	*aggR*	*aaiC*	*lt*	*sth*	*stp*	*ipah*
True value	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
L105																											
L151																											
L162													ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L163																											
L166													ND	ND	ND	ND	ND										
L172																											
L187																											
L209														ND		ND	ND	-					ND		ND	ND	
L227																		-									+
L232													ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L268																											
L318					+		+				+	+	+					-									
L376	-																										
L384															ND	ND	ND	ND						ND	ND	ND	ND
L416																											ND
L430																											
L460																											
L518																											
L542																											
L549																											
L566																											
L607																			+	+	+						
L614													ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L623																											
L677													ND	ND	ND	ND	ND					ND	ND	ND	ND	ND	
L694																											
L706															ND	ND	ND										
L728																											
L732																											
L751																											
L758																											
L920																											
L936													ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L950			-																								
L962																											
L975													ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L988																											

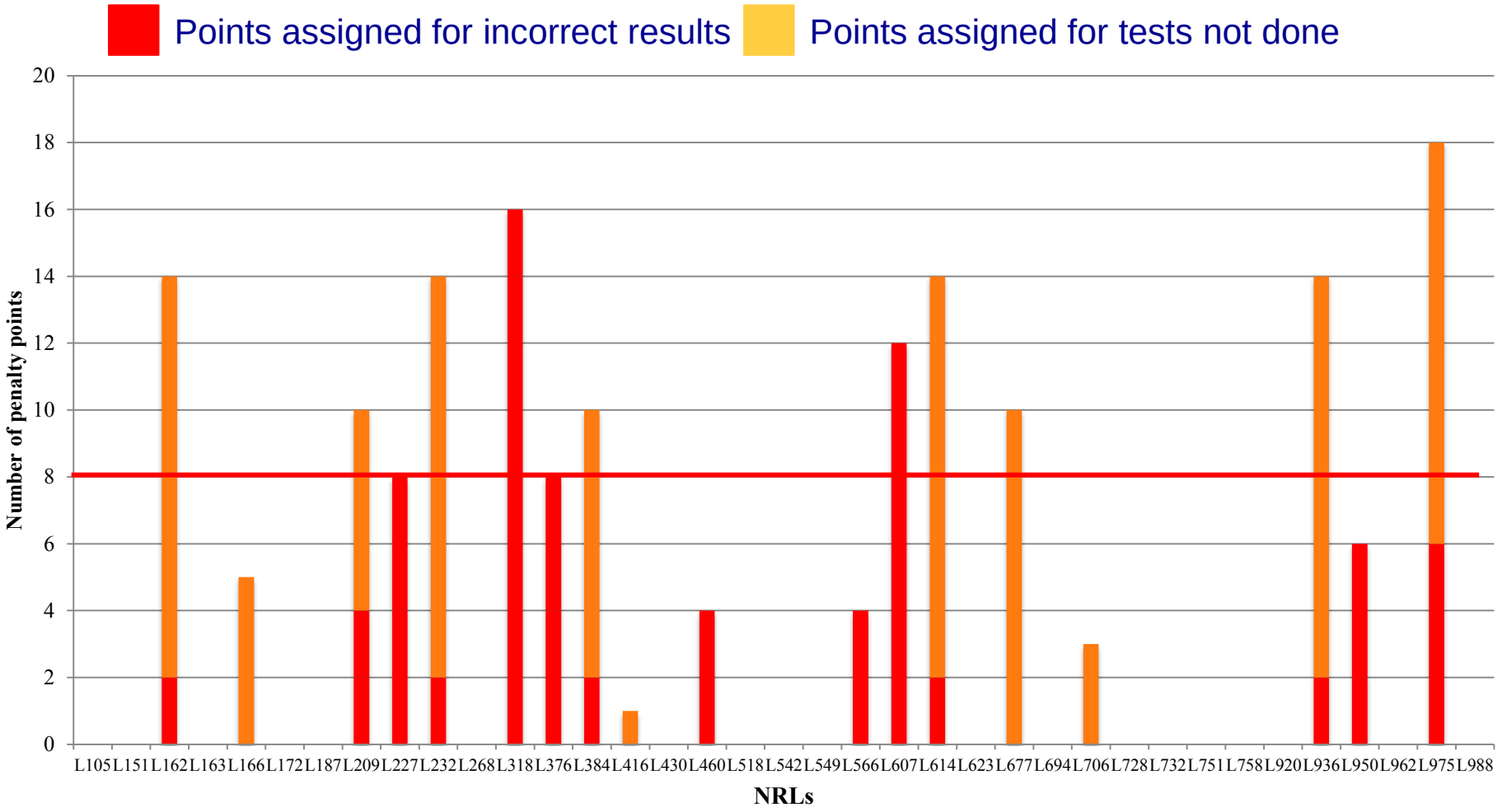
PT12 - Real-time PCR detection of O-associated genes in the enrichment cultures

NRL	Detection of virulence and serogroup-associated genes in sample A								
	vtx1	*vtx2*	*eae*	*O157*	*O26*	*O103*	*O111*	*O145*	*O104*
True value	+	+	+	+	-	-	-	-	-
L105									
L151									
L162									
L163									
L166									
L172									
L187									
L209									
L227									
L232									
L268									
L318									
L376	-								
L384									
L416									
L430									
L460									
L518									
L542									
L549									
L566									
L607									
L614									
L623									
L677									
L694									
L706									
L728									
L732									
L751									
L758									
L920									
L936									
L950			-						
L962									
L975									
L988									

PT12 – Isolation of *E. coli* strains from the PCR-positive enrichment cultures

NRL	*E. coli* strain isolation and genotyping from:							
	Sample A					Sample B		Sample C
	Pathogroup	Genotype				Pathogroup	Genotype	Pathogroup
		Serogroup	*vtx1*	*vtx2*	*eae*		*ipah*	
True value	VTEC Isolation	O157	+	+	+	EIEC Isolation	+	None
L105								
L151								
L162						NO		
L163								
L166								
L172								
L187								
L209						NO		
L227						NO		EIEC
L232						NO		
L268								
L318						NO		
L376			-					
L384						NO		
L416								
L430								
L460	NO							
L518								
L542								
L549								
L566	NO							
L607								VTEC
L614						NO		
L623								
L677								
L694								
L706								
L728								
L732								
L751								
L758								
L920								
L936						NO		
L950	NO							
L962								
L975	NO					NO		
L988								

PT12 – Evaluation of the NRL performance



PT12 - REMARKS

- ✓ 38 NRLs participated
- ✓ Results sent by 37
- ✓ Nearly all the European NRLs are now able to perform the standard method ISO/TS 13136:2012 for VTEC
- ✓ 10 NRLs did not perform the detection of all the other *E.coli* pathogroups (NRL specificity or budget constraints)
- ✓ 28 of the 31 NRLs (90%) that carried out the specific test identified correctly the presence of the EIEC strain in sample B