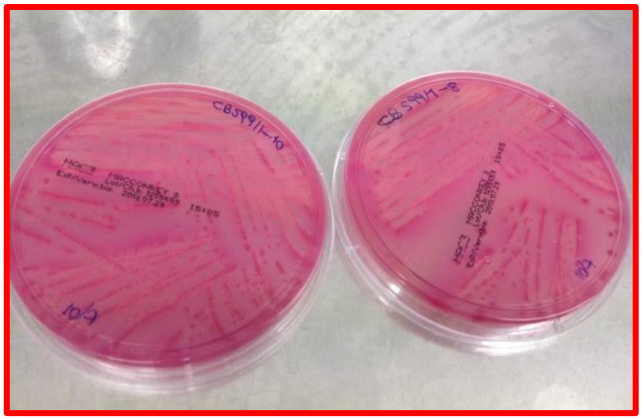
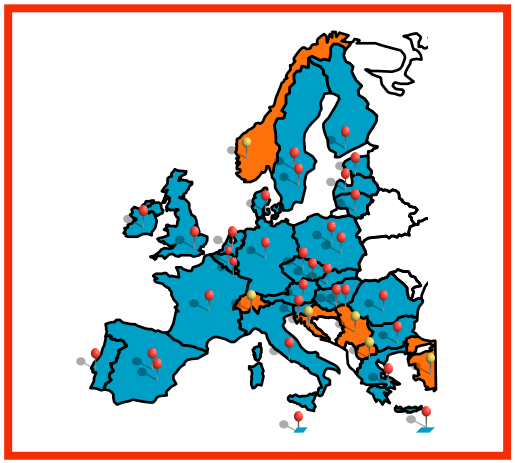
PT18

Identification and typing of STEC and other pathogenic *E. coli*





Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Department
European Reference Laboratory for *Escherichia coli*

The objectives of the study were:

- ✓ The detection of the main STEC/EPEC virulence genes.
- ✓ The detection of the EAEC marker genes.
- ✓ The identification of virulence genes of ETEC and EIEC
- ✓ The identification of a range of relevant STEC serogroups.
- ✓ The subtyping of Shiga Toxins (Stx)-coding genes.
- ✓ The 5th round of external quality assessment (EQA) for PFGE

PT18: Design of the study (I)

- ✓ identification of the *E. coli* pathotypes by PCR amplification of the target virulence genes:
  - *stx1* group, *stx2* group and the intimin-coding *eae* gene for STEC
  - the *eae* gene for EPEC
  - the *aaiC* and *aggR* genes for EAEC
  - *lt*, st_h and st_p for ETEC
  - *ipaH* for EIEC

- ✓ Identification of 13 target O serogroups:
  - O26, O103, O111, O145, O157 ("top 5")
  - O45 and O121 (considered as adulterants in beef in the USA)
  - O104 (relevant after the 2011 outbreak)
  - O55, O91, O113, O128, O146 (prevalent in human infections in Europe according to the ECDC data)

- ✓ Subtyping of stx genes:
  - *stx1a*, *stx1c* and *stx1d*
  - from *stx2a* to *stx2g*

PT18: Design of the study (II)

Ten *E. coli* strains to be typed

Sample/ Strain	Pathogroup	Serogroup	Target virulence genes (*stx* subtypes)						
			stx1	***stx2***	***eae***	***aggR***	***aaiC***	***lt***	***st_h***
1	**STEC**	O111	*stx1a*	-	+	-	-	-	-
2	**STEC**	O26	*stx1a*	-	+	-	-	-	-
3	**STEC**	O103	*stx1a*	-	+	-	-	-	-
4	**EAEC**	O78	-	-	-	+	+	-	-
5	**EAEC**	O104	-	-	-	+	+	-	-
6	**ETEC**	O6	-	-	-	-	-	+	+
7	**EPEC**	O128	-	-	+	-	-	-	-
8	**STEC**	O157	-	*stx2a*	+	-	-	-	-
9	**STEC**	O113	*stx1c*	*stx2b*	-	-	-	-	-
10	**STEC**	O91	-	*stx2a* *stx2d*	-	-	-	-	-

PT18: Participants

36 NRLs representing ALL the 28 EU countries

+ the NRLs of

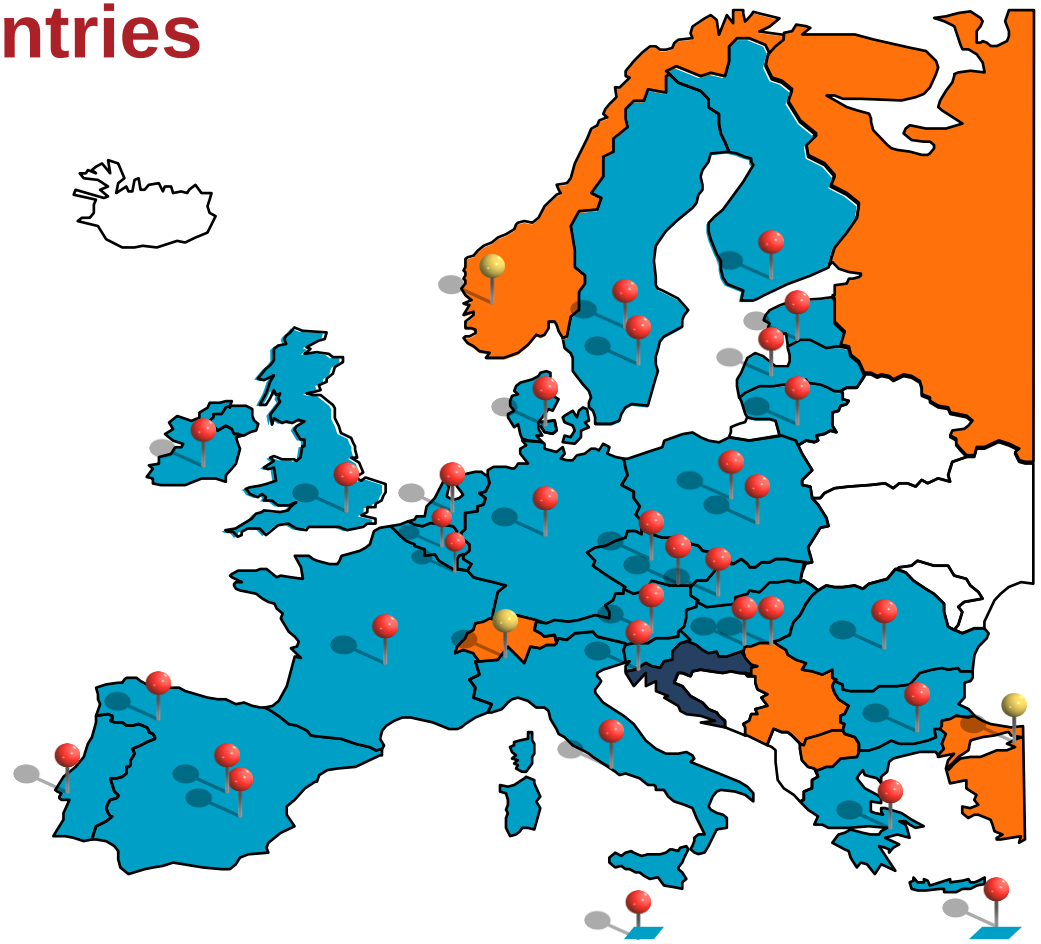
Egypt

Norway

Russia

Switzerland

Turkey



PT18 – Samples

- ✓ 10 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on the 20th of October 2016
- ✓ 25th of October 2016, the homogeneity test was performed on a set of 10 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on the 2nd of November 2016
- ✓ Results submitted on-line via the web site from all the 36 NRLs

PT18 – Results: Detection of virulence genes

NRL	Detection of virulence genes in the 10 test strains:						
	stx1	*stx2*	*eae*	*aggR*	*aaiC*	*lt*	*st$_h$*
L148							
L170							
L181							
L208	1	1		5	5	5	5
L280							
L328	1			1	1	5	5
L343	1						
L360							
L405							
L417	1		1				
L427							
L435							
L444						1	1
L492							
L524						1	1
L597							
L630							
L647				5	5	5	5
L653							
L675							
L705			1				
L721						1	1
L731							1
L782							
L788						5	5
L789							
L817							
L826						1	
L838							
L844							
L879							
L886							5
L907							
L912							
L959				1 1	1 1	4	4
L973						5	5

Penalty Points for the detection of virulence genes

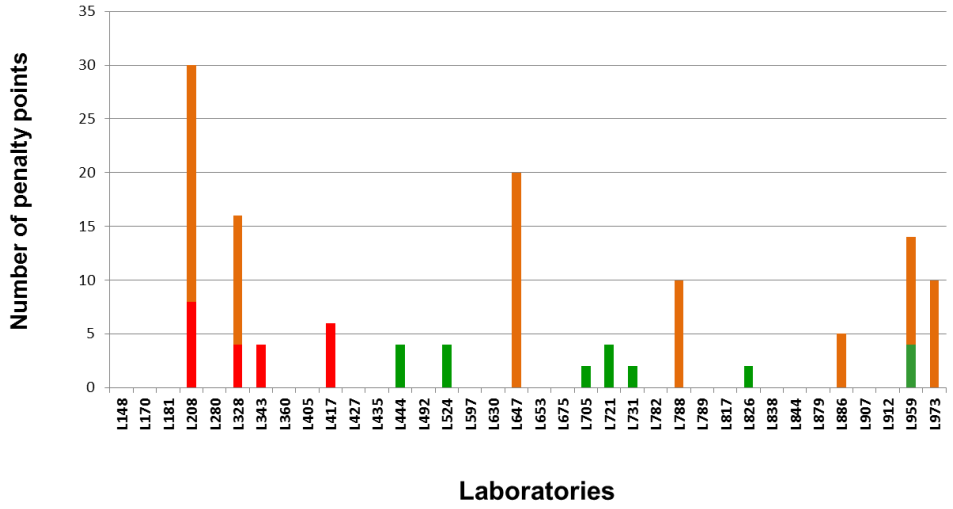
- ✓ **4 penalty points** to each incorrect result concerning the identification of the *stx* genes

- ✓ **2 penalty points** to each incorrect result concerning the identification of *eae*, *aggR*, *aaiC, lt* and st_h.

- ✓ **1 penalty point** was assigned when reporting the detection of a certain virulence gene as "Not Done". If the "Not Done" concerned the genes *aggR*, *aaiC, lt and* st_h referred to *eae* positive strains, no penalty point was assigned.

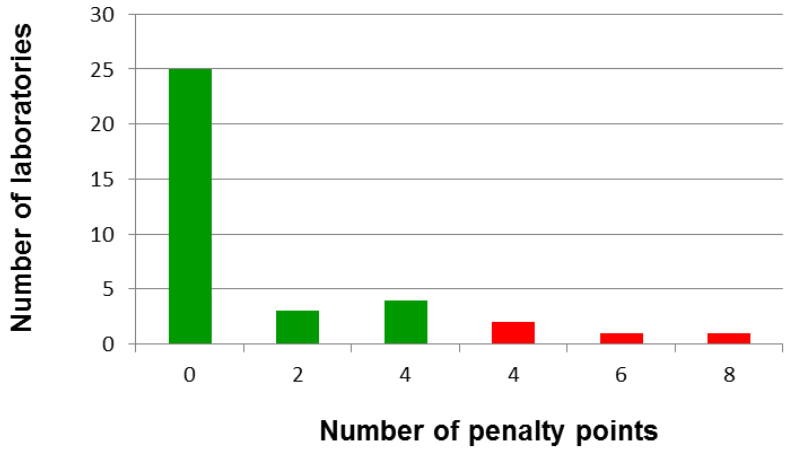
A threshold of 4 penalty points was set in order to identify the laboratories not performing adequately for this part of the PT

Evaluation of the PCR results for the detection of virulence genes, by NRL



Orange bars: test not done

Evaluation of the PCR results for the detection of virulence genes: number of NRLs within each penalty score (only incorrect results)



PT18 – Results: Identification of the serogroups (35/36 NRLs)

NRL	Serogroup identification in sample:									
	1	2	3	4	5	6	7	8	9	10
True value	O111	O26	O103	O78	O104	O6	O128	O157	O113	O91
L148				O137						
L170				NT		NT				
L181				NT		NT				
L208			NT	NT		NT	NT		NT	NT
L280										
L328				NT						
L343				NT		NT	O128ab			
L360				NT		NT				
L405				NT						
L417				NT		NT				
L427				NT		NT				
L435				NT		NT				
L444										
L492						NT				
L524				NT		NT	NT		NT	
L597		NT		NT		NT	NT			
L630				NT		NT				
L653				NT	NT	NT				
L675				NT		NT	NT		NT	NT
L705				NT		NT				
L721				NT		NT				
L731										
L782										
L788				O55		NT				
L789				NT		NT				
L817				NT		NT				
L826										
L838				NT		NT				
L844				NT		NT				
L879							O128ab			
L886				O91	NT	NT				
L907						NT	NT			
L912						NT				
L959	NT		NT		NT	NT	NT		NT	NT
L973				O138		NT	O128ab		NT	

Red boxes: incorrect results for STEC

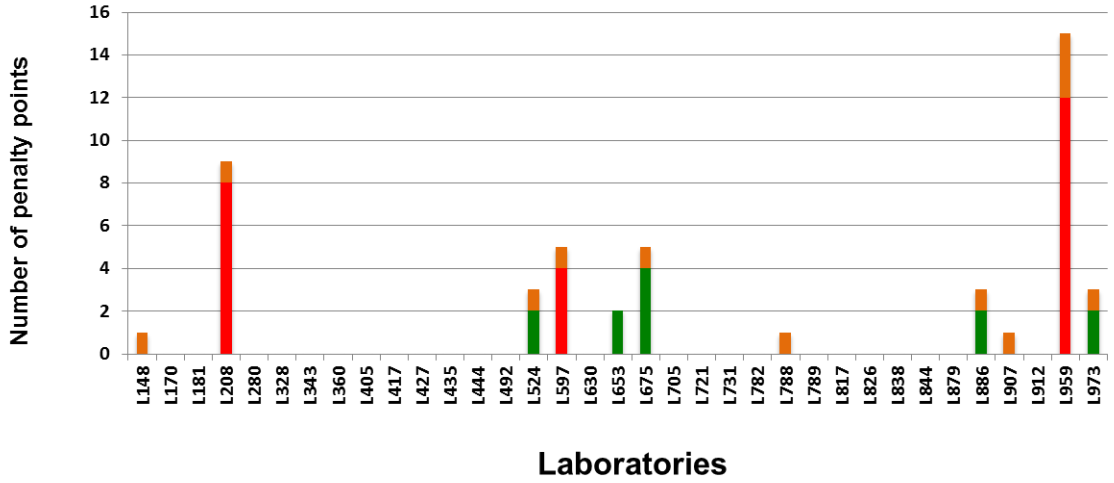
Orange boxes: incorrect results for non-STEC

Penalty Points for the serogroup determination

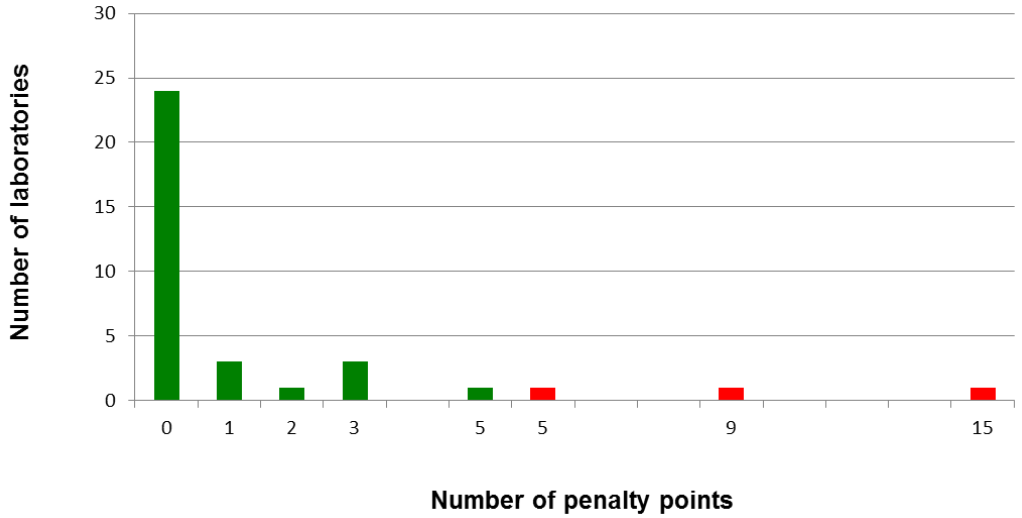
- ✓ **4 penalty points:** incorrect result concerning the typing of the strains belonging to the “top 5” serogroups : O26, O103, O111, O145, O157
- ✓ **2 penalty points:** incorrect result concerning the typing of the strains belonging to : O45, O55, O91, O104, O113, O121, O128, O146
- ✓ **1 penalty point:** incorrect result concerning the typing of the strains belonging to pathotype other than STEC, with the exception for serogroup O104 (2 penalty points, even if the strain was an EAEC)

A threshold of 4 penalty points was set in order to identify the laboratories not performing adequately for this part of the PT

Evaluation of the results on serogroup identification, by NRL



Evaluation of the results on serogroup identification: number of NRLs within each penalty score



PT 18 - Results: *stx* genes subtyping (33/36 NRLs)

stx1

NRL	Strain 1			Strain 2			Strain 3			Strain 9		
	stx1a	*stx1c*	*stx1d*	*stx1a*	*stx1c*	*stx1d*	*stx1a*	*stx1c*	*stx1d*	*stx1a*	*stx1c*	*stx1d*
True value	+	-	-	+	-	-	+	-	-	-	+	-
L148												
L170												
L181												
L280												
L328			ND			ND			ND	+		
L343										ND	ND	ND
L360												
L405												
L417												
L427												
L435												
L444												
L492												
L524												
L597												
L630												
L653												
L675												
L705												
L721												
L731												
L782												
L788												
L789												
L817												
L826												
L838										+	-	
L844												
L879												
L886												
L907												
L912												
L973												

PT 18 - Results: *stx* genes subtyping (33/36 NRLs)

stx2

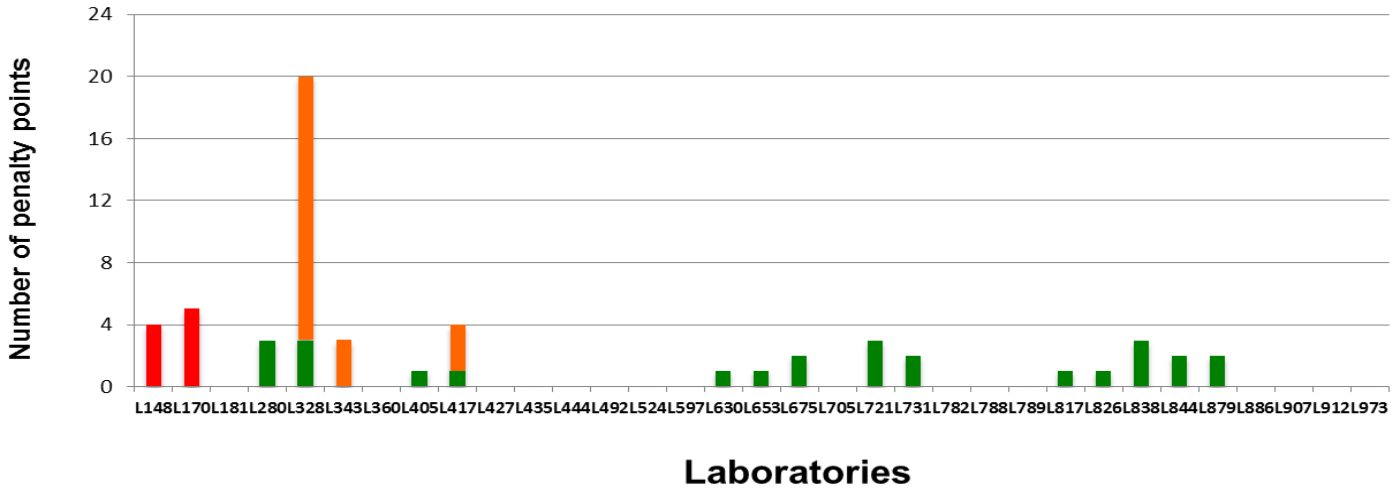
NRL	Strain 8							Strain 9							Strain 10						
	stx2a	*stx2b*	*stx2c*	*stx2d*	*stx2e*	*stx2f*	*stx2g*	*stx2a*	*stx2b*	*stx2c*	*stx2d*	*stx2e*	*stx2f*	*stx2g*	*stx2a*	*stx2b*	*stx2c*	*stx2d*	*stx2e*	*stx2f*	*stx2g*
True value	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-
L148			+	+										+			+				
L170	-	+						+	-									-			
L181																					
L280															-		+	-			
L328		+						+							ND	ND	ND	ND	ND	ND	ND
L343																					
L360																					
L405																		-			
L417				ND							ND						+	ND			
L427																					
L435																	+				
L444																					
L492																					
L524																					
L597																					
L630															-						
L653																	+				
L675			+														+				
L705																					
L721															-		+	-			
L731																	+	-			
L782																					
L788																					
L789																					
L817																	+				
L826																	+				
L838															-						
L844														+			+				
L879																	+	-			
L886																					
L907																					
L912																					
L973																					

Evaluation of the NRL performance in the identification of the *stx* gene subtypes

- ✓ **one penalty point** for *stx* genes that were typed incorrectly or for results reported as “Not Done”
- ✓ Results that were not uploaded (“null” field) were also considered as “Not Done”.

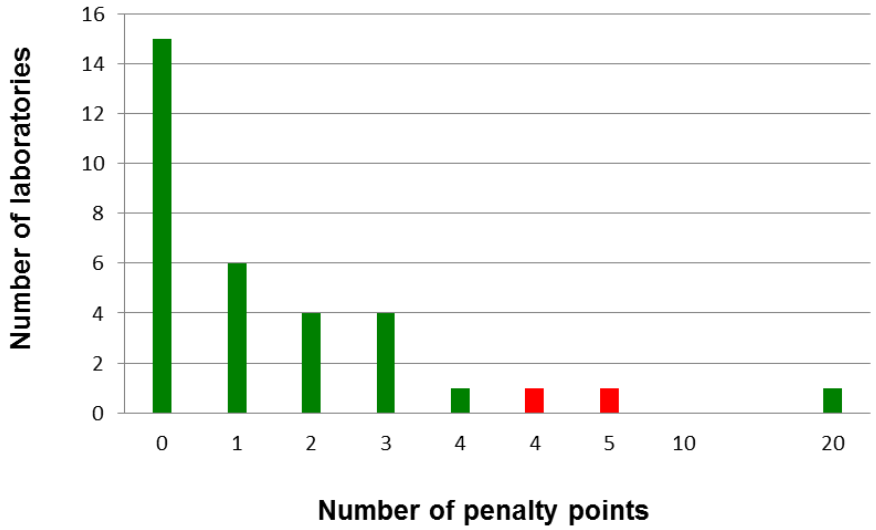
A threshold of 4 penalty points was set in order to identify the laboratories not performing adequately for this part of the PT

Evaluation of *stx* gene subtyping results, by NRL



Orange bars: test not done

Evaluation of *stx* gene subtyping results: number of NRLs within each penalty score



PT18: Concluding remarks

- ✓ 36 NRLs representing ALL the 28 EU countries and 5 non-EU countries participated in the study
- ✓ Good results in the detection of the STEC target genes and serogroup identification (89 % for *stx* and 94% for *eae* - improvement from the last PT on strain typing)
- ✓ Good results for the detection of the target genes of ETEC (67%) and EAEC (89%)
- ✓ *stx* genes subtyping: most of the incorrect results concerned a false positivity of the *stx2c* subtype
- ✓ 8 NRLs were evaluated as underperforming
- ✓ Some areas of improvement have been identified and will be managed
- ✓ Excellent preparedness has been built in the EU towards the ability to identify the main virulence genes of STEC, while the capacity to detect other *E. coli* pathotypes and their most represented serogroups is also present with a good performance