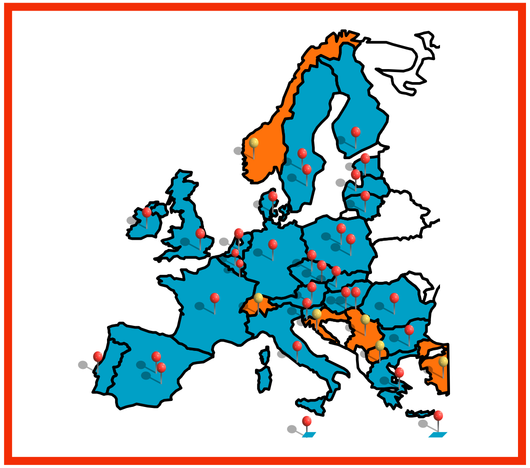
PT13

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



PT13 - OBJECTIVES

- ✓ The detection of the main VTEC/EPEC virulence genes
- ✓ The detection of the marker genes of EAggEC
- ✓ The identification of a range of relevant VTEC/EPEC serogroups
- ✓ The sub-typing of VT-coding genes
- ✓ A 3rd round of external quality assessment for PFGE, in view of the beginning of the collection, by EFSA, of molecular typing data on VTEC of food and animal origin

PT13 - DESIGN OF THE STUDY (1)

- ✓ **Detection of virulence genes:**
  - ✓ *vtx1* group and *vtx2* group for VTEC
  - ✓ *eae* for EPEC
  - ✓ *aaiC* and *aggR* for EAggEC

- ✓ **Identification of 13 target O serogroups:**
  - ✓ O26, O103, O111, O145, O157 (the so called “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 *vtx* genes:**
  - ✓ *vtx1a, vtx1c*, and *vtx1d*
  - ✓ from *vtx2a* to *vtx2g*

PT13 - DESIGN OF THE STUDY (2)

- **7 strains to be typed**

- **Methods:**
  - **PCR SOPs available in the EU-RL web site for:**
    - **Detection of virulence genes**
    - **Detection of O serogroup-associated genes**
    - **Subtyping of *vtx* genes**

- **Organized according to the requirements of ISO 17043:2010**

PT13 - Evaluation of the results

Assignement of penalty points for incorrect results

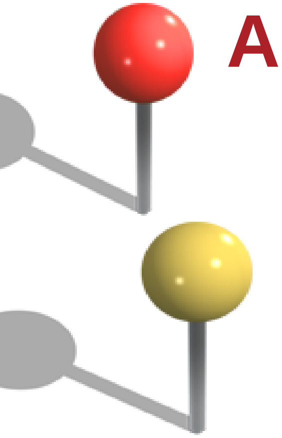
- ✓ **PCR detection of virulence genes:**
  - ✓ **4 points for each incorrect or missing result on *vtx* genes**
  - ✓ **2 points for each incorrect result on other virulence genes**
  - ✓ **1 point for missing results on other virulence genes**

- ✓ **Serogroup identification**
  - ✓ **4 points for incorrect identification of the ”top5” serogroups**
  - ✓ **2 points for incorrect identification of the other 8 serogroups**

- ✓ **Subtypes of the *vtx* genes**
  - ✓ **1 point for each incorrect or missing results**

- ✓ **A sum of 4 points considered as the threshold for under-performance in each of the sections**

PT13 – PARTICIPANTS: 40 Laboratories



34 NRLs representing
ALL the 28 EU countries

+ the NRLs of

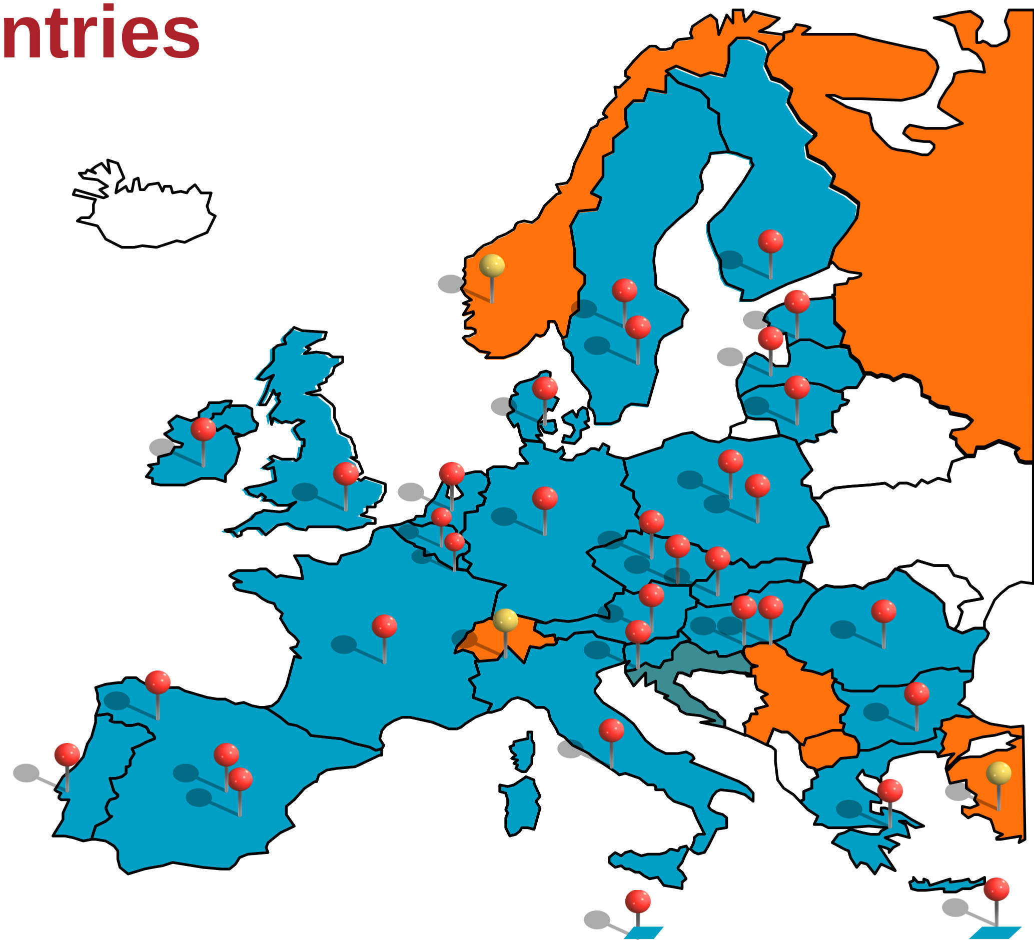
Norway

Switzerland

Turkey

Argentina (2 labs)

Russia



PT13 - SAMPLES

- ✓ 7 test strains and the needed control strains provided as cultures in soft-agar
- ✓ Samples labelled with randomly generated numerical codes
- ✓ Samples shipped on 7 April 2014
- ✓ Results submitted on-line via the web site

Characteristics of the 7 *E. coli* test strains

Strain	Serogroup	Target genes				
		eae	*vtx1*	*vtx2*	*aggR*	*aaiC*
1	O145	+	vtx1a	-	-	-
2	O91	-	-	vtx2d	-	-
3	O104	-	-	-	+	+
4	O146	-	vtx1c	vtx2a, vtx2b	-	-
5	O128	-	-	vtx2b	-	-
6	O157 (sorbitol fermenting)	+	-	2a	-	-
7	O45	+	-	-	-	-

PT13 – RESULTS: detection of virulence genes

NRL	Detection of virulence genes in the 7 test strains:				
	vtx1	vtx2	eae	aggR	aaiC
L106				7	7
L118					
L157		2			
L206					
L240					
L258			1		
L273	1				
L285	5	2	5	7	7
L313					
L354					
L367					
L402					
L433					
L463				1	
L519					
L534			1		
L544					
L551					
L553					
L566	1				
L605	1	1			
L608					
L633			1		
L674					
L679					
L687					
L691					
L692					
L699			1		
L712					
L715				7	7
L737					
L768					
L775					
L776				7	7
L905					
L947					7
L949					
L986					
L987					

PT13 - RESULTS: detection of VTEC virulence genes

- ✓ 35 NRLs (87%) identified correctly the presence/absence of *vtx* genes in the test strains
- ✓ 35 NRLs (87%) identified correctly the presence/absence of the *eae* gene in the test strains
- ✓ 36 NRLs (90%) made the test for EAggEC marker genes, and 35 (85%) identified them correctly

PT13 – RESULTS: O serogroup identification

NRL	Serogroup identification in sample:						
	1	2	3	4	5	6	7
True value	O145	O91	O104	O146	O128	O157	O45
L106		NT		NT			NT
L118				NT			
L157	O111					NT	O157
L206							
L240							
L258							
L273							NT
L285	O157		O26	O157	O104	NT	NT
L313			O125				O86
L354							
L367							NT
L402							
L433							
L463							
L519							
L534							
L544							
L551				NT	NT		
L553							NT
L566		O146		O91			
L605							
L608							
L633							O103
L674							
L679							
L687							NT
L691							
L692							
L699							
L712							
L715							
L737							
L768							
L775							
L776							
L905							
L947							
L949							
L986							
L987							

PT13 – RESULTS: O serogroup identification

Correct identification of the O serogroup:

O157: 38/40 NRLs (95%)

O145: 38/40 NRLs (95%)

O104: 38/40 NRLs (95%)

O91: 38/40 NRLs (95%)

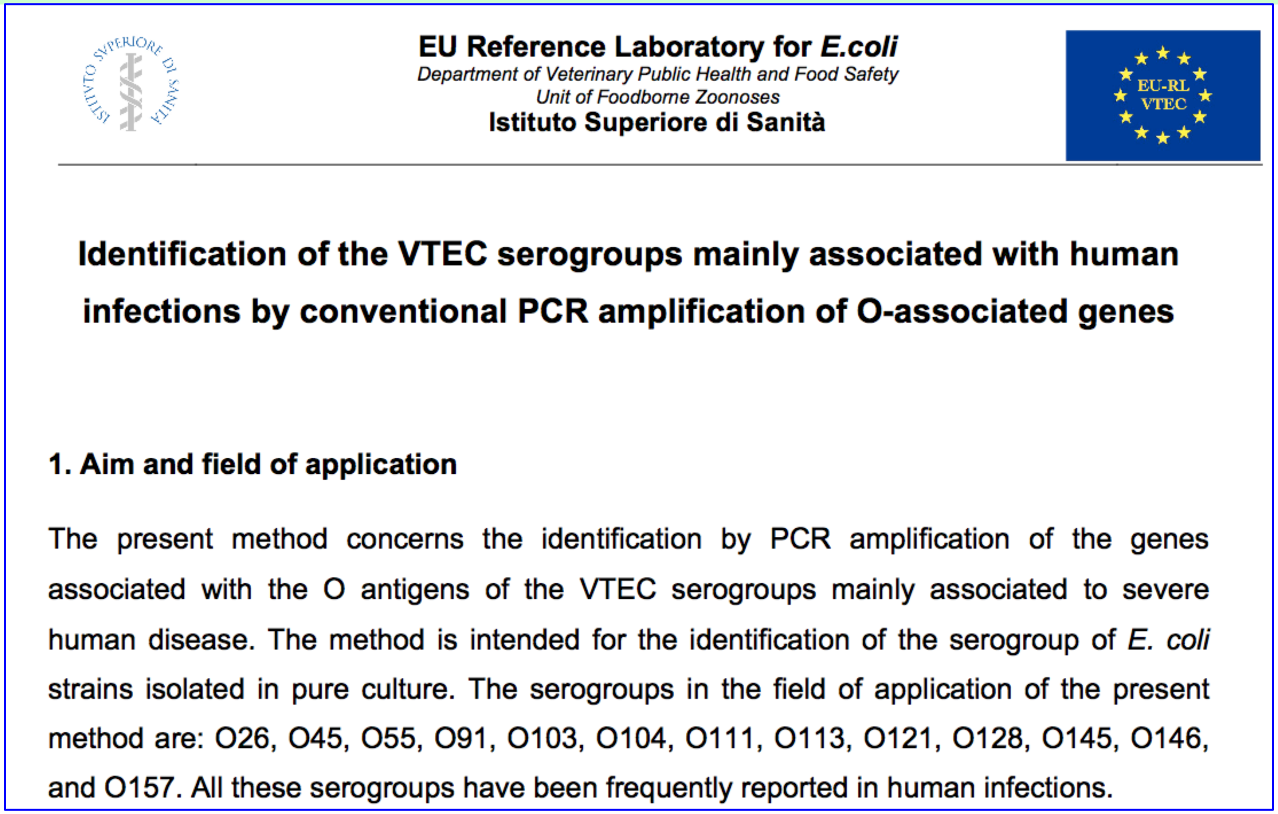
O128: 38/40 NRLs (95%)

O146: 35/40 NRLs (87%)

O45: 31/40 NRLs (77%)

PT13 – RESULTS: O serogroup identification

The SOP developed by the EU-RL for PCR detection of the genes associated with the 13 relevant VTEC/EPEC O serogroups was used by 33/40 NRLs (82%)



EU Reference Laboratory for *E.coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità

Identification of the VTEC serogroups mainly associated with human infections by conventional PCR amplification of O-associated genes

1. Aim and field of application

The present method concerns the identification by PCR amplification of the genes associated with the O antigens of the VTEC serogroups mainly associated to severe human disease. The method is intended for the identification of the serogroup of *E. coli* strains isolated in pure culture. The serogroups in the field of application of the present method are: O26, O45, O55, O91, O103, O104, O111, O113, O121, O128, O145, O146, and O157. All these serogroups have been frequently reported in human infections.

PT13 – RESULTS: *vtx1* gene subtyping

NRL	Detection of *vtx1* gene subtypes in:					
	Strain 1			Strain 4		
	vtx1a	*vtx1c*	*vtx1d*	*vtx1a*	*vtx1c*	*vtx1d*
True value	+	-	-	-	+	-
L106	+	-	-	-	+	-
L118	+	-	-	-	+	-
L157	+	+	-	-	+	-
L206	+	-	-	-	-	+
L240	+	-	-	-	+	-
L258	+	-	-		+	
L273	+	-	-	ND	ND	ND
L285	ND	ND	ND	ND	ND	ND
L313	+	-	-	-	+	-
L354	+	-	-	-	+	-
L367	-	-	-	-	+	-
L402	+	-	-	-	+	-
L433	+	-	-	-	+	-
L463	+	-	-	-	+	-
L519	+	-	-	-	+	-
L534	+	-	-	-	+	-
L544	+				+	
L551	ND	ND	ND	ND	ND	ND
L553	+	-	ND ?	-	+	ND ?
L566	+	-	-	ND	ND	ND
L605	+	-	-	-	+	-
L608	+	-	-	-	+	-
L633	-	+	-	+	-	-
L674	+	-	-	-	+	-
L679	+	-	-	-	+	-
L687	+	-	ND ?	-	+	ND ?
L691	+	-	-	-	+	-
L692	+	-	-	-	+	-
L699	+	-	-	-	+	-
L712	+	-	-	-	+	-
L715	+	-	-	-	+	-
L737	+	+	-	-	+	-
L768	+	-	-	-	+	-
L775	+	-	-	-	+	-
L776	+			-	+	-
L905	+	-	-	-	+	-
L947	ND	ND	ND	ND	ND	ND
L949	+	-	-	-	+	-
L969						
L986	+	-	-	-	+	-
L987	+	-	-	-	+	-

- ✓ **30 NRLs (75%) identified correctly the *vtx1* gene subtypes**
- ✓ **5 NRLs (12%) provided incorrect results**
- ✓ **5 NRLs (12%) dis not perform the assay**

PT13 – RESULTS: *vtx2* gene subtyping

NRL	Detection of *vtx2* gene subtypes in:													
	Strain 2							Strain 4						
	vtx2a	vtx2b	vtx2c	vtx2d	vtx2e	vtx2f	vtx2g	vtx2a	vtx2b	vtx2c	vtx2d	vtx2e	vtx2f	vtx2g
True value	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L106	-	-	+	+	-	-	-	+	+	+	-	-	-	-
L118	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L157	-	ND	+	+	ND	ND	ND	+	ND	-	+	ND	ND	ND
L206	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L240	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L258			+	+				+	+					
L273	-	-	+	+	-	-	-	+	-	-	-	-	-	-
L285	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L313	+		+	+				+	+	+	+			
L354	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L367	-	ND	+	+	-	-	-	+	ND	+	+	-	-	-
L402	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L433	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L463	-	-	+	+	-	-	-	+	+	+	-	-	-	-
L519	-	-	-	+	ND	ND	ND	+	+	-	-	-	-	-
L534	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L544	+			+				+	+					
L551	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L553	-	-	-	-	-	-	-	+	+	-	-	-	-	-
L566	+	-	+	-	-	-	-	+	+	+	-	-	-	-
L605	+	-	+	-	-	-	-	+	+	+	-	-	-	-
L608	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L633	-	-	-	+	-	-	-	+	+	-	-	-	-	
L674	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L679	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L687	-	-	-	-	-	-	-	+	+	-	-	-	-	-
L691	-	-	+	-	-	-	-	+	+	+	-	-	-	-
L692	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L699	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L712	+	-	+	+	ND	-	ND	+	+	-	-	ND	-	ND
L715	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L737	-	-	+	+	-	-	-	+	+	+	-	-	-	-
L768	+	-	+	+	-	-	-	+	+	+	+	-	-	-
L775	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L776	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L905	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L947	-	-	+	+	-	-	-	+	+	-	-	-	-	-
L949	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L986	-	-	-	+	-	-	-	+	+	-	-	-	-	-
L987	-	-	+	+	-	-	-	+	+	+	-	-	-	-

NRL	Detection of *vtx2* gene subtypes in:													
	Strain 5							Strain 6						
	vtx2a	vtx2b	vtx2c	vtx2d	vtx2e	vtx2f	vtx2g	vtx2a	vtx2b	vtx2c	vtx2d	vtx2e	vtx2f	vtx2g
True value	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L106	-	+	-	-	-	-	-	+	-	+	-	-	-	-
L118	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L157	-	-	-	ND	ND	ND	ND	-	ND	-	-	ND	ND	ND
L206	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L240	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L258	-	+	-	-	-	-	-	+						
L273	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L285	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L313		+						+		+	+			
L354	-	+	-	-	-	-	-	-	-	+	-	-	-	-
L367	-	-	-	-	ND	ND	ND	+	ND	+	+	ND	ND	ND
L402	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L433	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L463	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L519	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L534	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L544		+						+						
L551	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L553	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L566	-	+	-	-	-	-	-	+	-	-	-	+	-	-
L605	-	+	+	-	-	-	-	+	+	-	-	-	-	-
L608	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L633	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L674	+	+	-	-	-	-	-	+	-	-	-	-	-	-
L679	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L687	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L691	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L692	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L699	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L712	-	+	-	-	ND	-	ND	+	-	-	-	ND	-	ND
L715	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L737	-	+	-	-	-	-	-	+	-	+	-	-	-	-
L768	-	+	-	+	-	-	-	+	-	+	+	-	-	-
L775	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L776	-	+	-	-	-	-	-	-	-	-	+	-	-	-
L905	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L947	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L949	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L986	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L987	-	+	-	-	-	-	-	+	-	-	-	-	-	-

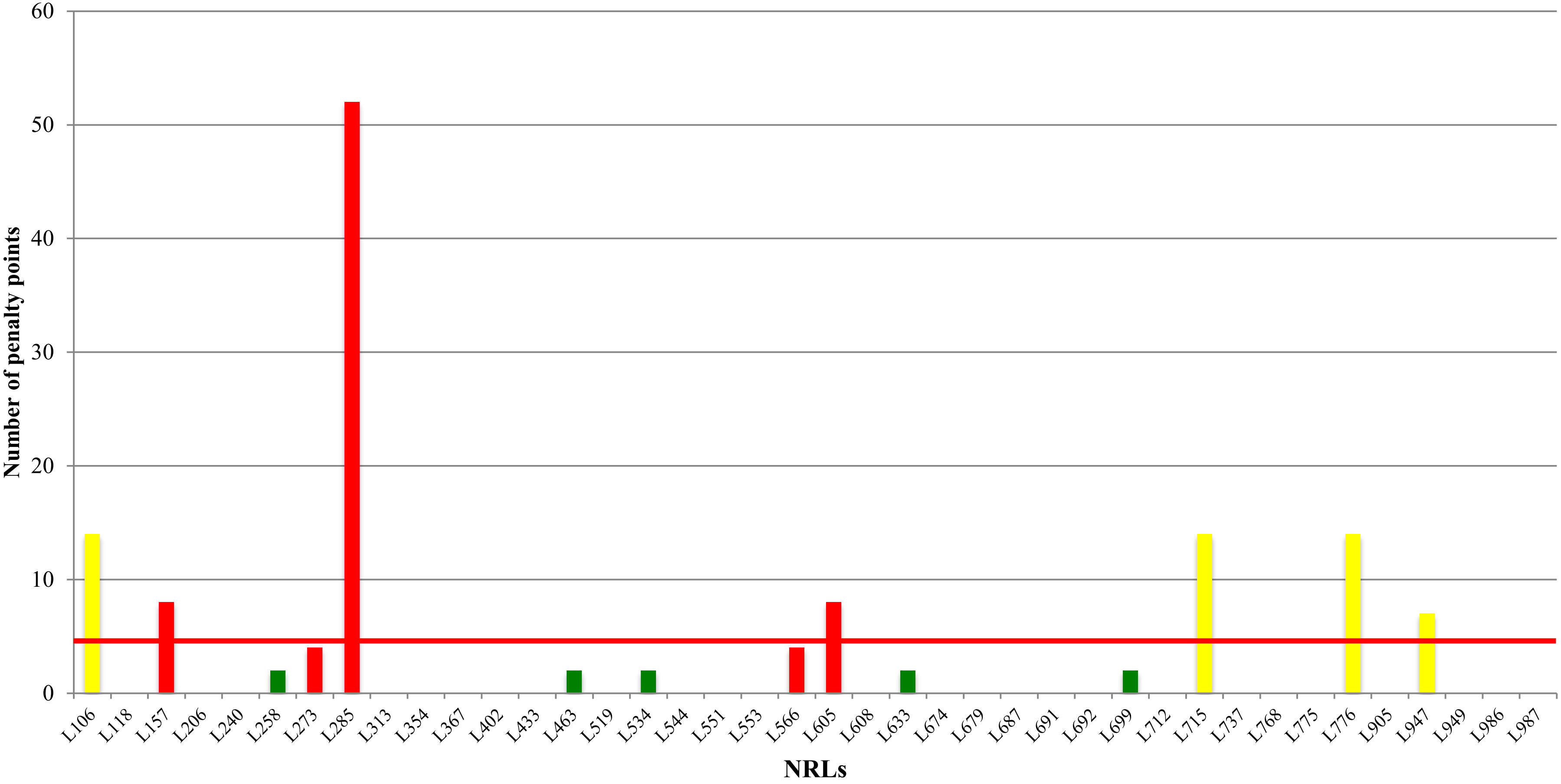
PT13 – RESULTS: *vtx2* gene subtyping

- ✓ **3 NRLs (7%) did not perform the assay**
- ✓ **10 NRLs (25%) identified correctly all the *vtx2* gene subtypes**
- ✓ **27 NRLs (67%) provided incorrect results**

- ✓ **24 NRLs provided a <u>false positive</u> results for the *vtx2c* gene in strain 2, which was positive for *vtx2d***

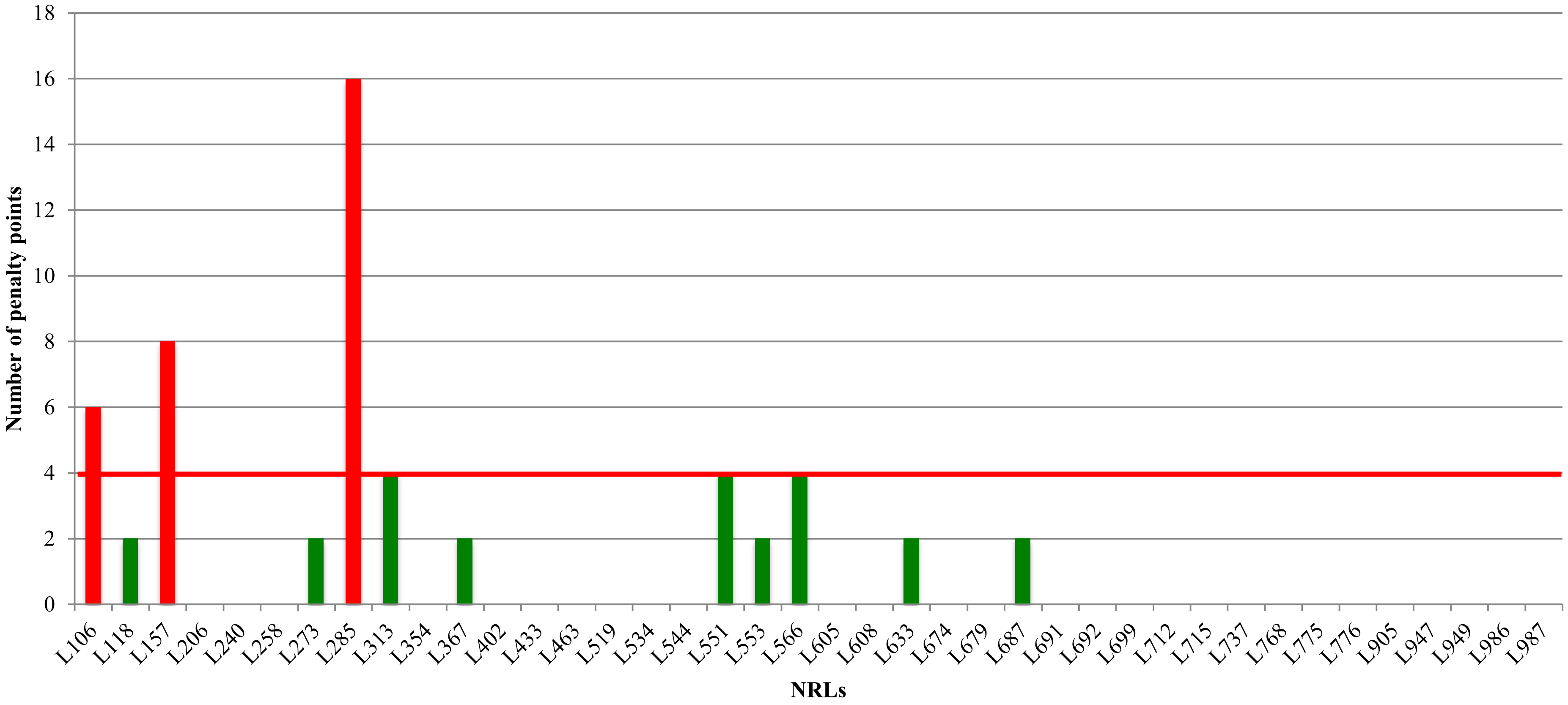
PT13 – Evaluation of the NRL performance

Detection of virulence genes (Treshold = 4 points)



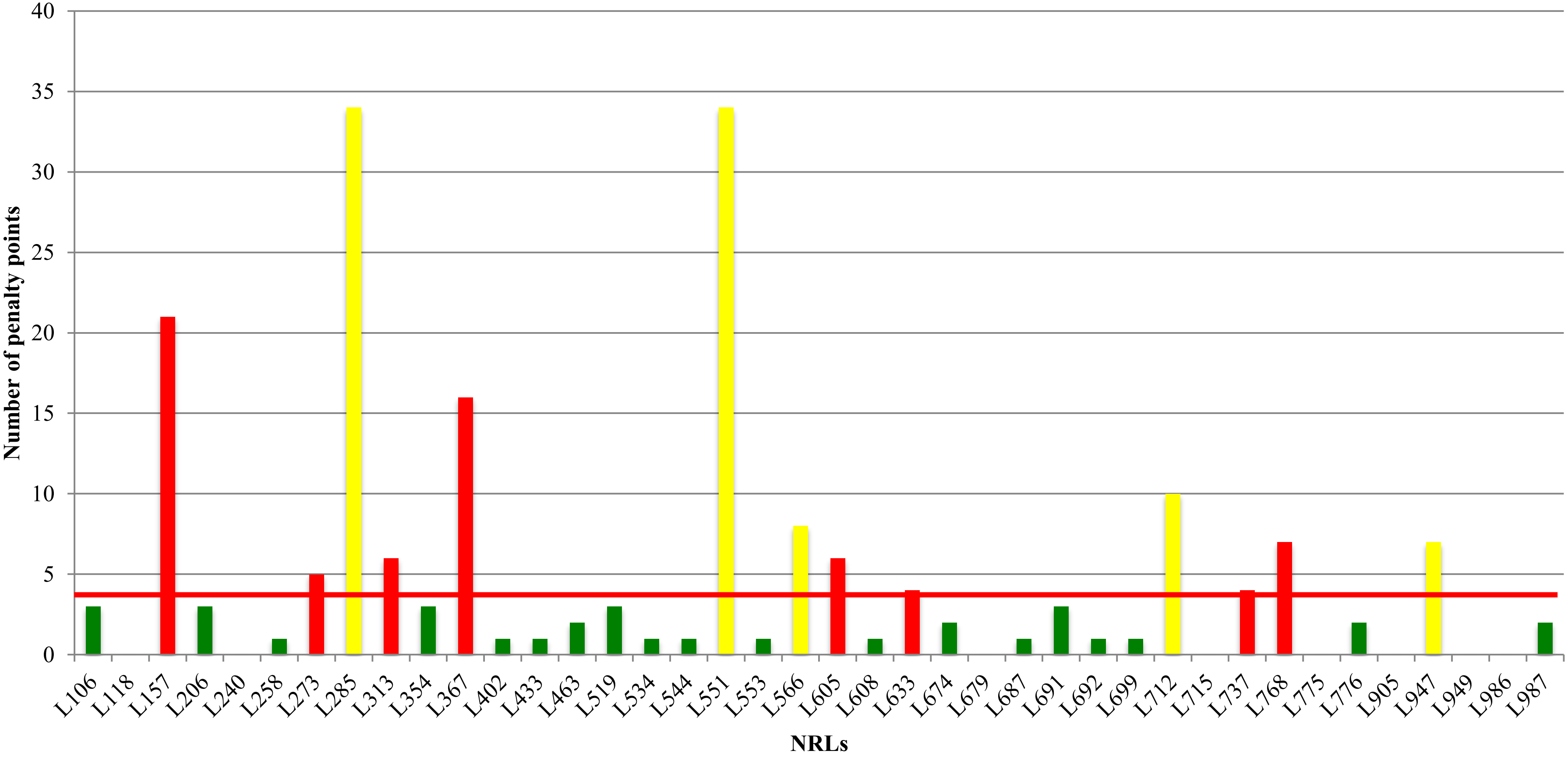
PT13 – Evaluation of the NRL performance

Identification of O serogroups (Treshold = 4 points)



PT12 – Evaluation of the NRL performance

Subtyping of *vtx* genes (Treshold = 4 points)



PT13 - REMARKS

- ✓ **40 NRLs participated**
- ✓ **Good results in the detection of virulence genes**
- ✓ **Some NRLs still do not have reagents for other pathotypes**
- ✓ **Good results for serotyping**
- ✓ **The subtyping method may still have problems in specificity for *vtx2d***