EU Reference Laboratory for *E. coli*

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Report of the 11th inter-laboratory study (PT11) on the identification and typing of Verocytotoxin-producing *E. coli* (VTEC) and other pathogenic *E. coli* strains - 2013

Edited by:

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1. INTRODUCTION

The duties of the EU Reference Laboratory for *E. coli* (EU-RL VTEC) include the organization of proficiency tests (PT) on detection and typing methods for the designated National Reference Laboratories (NRLs) for *E. coli* in the EU Member States. The NRLs of the European Economic Area (EEA) countries, EU Candidate Countries, and third countries participate in these tests on a voluntary basis.

In the past years, the EU-RL VTEC organized five PT rounds (PT1, PT2, PT5, PT6, and PT10) on the identification and typing of VTEC strains. These PTs aimed at evaluating and improving the capability of the NRLs to identify an *E. coli* strain as a VTEC, to identify the VTEC serogroups most involved in severe human infections, and to determine the types and subtypes of VT-coding genes, which may have an influence on the severity of human infections. The reports of these PTs are available at the EU-RL VTEC www.iss.it/vtec, in the Proficiency Tests section.

The present study (PT11) was dedicated to the identification and typing of VTEC strains, as well as to the identification of *E. coli* strains belonging to pathogenic groups (patho-groups) other than VTEC, namely: Enteroaggregative *E. coli* (EAggEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), and Enteroinvasive *E. coli* (EIEC). These *E. coli* patho-groups are well known causes of traveler diarrhea, but have been also reported in sporadic cases and foodborne outbreaks in industrialized countries.

Therefore, the objectives of the study were:

1. To assess the capacity of the NRLs to identify and type the main virulence genes of VTEC, to determine their serogroups and the subtypes of *vtx* genes;
2. To assess the capacity of the NRLs to identify the different groups of pathogenic *E. coli*, using Real Time PCR procedures that can also be applied to detect their presence in contaminated foods.
3. To carry out a second PT round for PFGE typing, in view of the start of the program for the collection of molecular typing data on VTEC strains of food and animal origin by the European Food Safety Authority (EFSA), to improve the EU preparedness to face foodborne outbreaks.

This document represents the evaluation report of the study, as far as the results on the identification of the *E. coli* pathogroups and the typing of VTEC virulence genes and O antigens are concerned. The PFGE results will be presented in a separate report.

2. DESIGN OF THE STUDY

The study was conducted according to the International Standard ISO/IEC 17043:2010 "Conformity assessment – General requirements for proficiency testing" and consisted of three parts:

1. Identification of the *E. coli* patho-groups by Real Time PCR amplification of the following target virulence genes:

- *vtx1* group, *vtx2* group and the intimin-coding *eae* for VTEC
- the *eae* gene for EPEC
- the *aaiC* and *aggR* genes for for EAggEC
- the *lt*, *sth,* and *stp* enterotoxin-coding genes for ETEC
- the *ipaH* gene for EIEC

2. Determination of the serogroups of the VTEC and EPEC strains identified. Participants were requested to identify the strains belonging to any of the following 12 serogroups, selected on the basis of their prevalence in human infections in Europe: O26, O55, O91, O103, O104, O111, O113, O121, O128, O145, O146, O157.

3. Subtyping of the *vtx* genes harbored by the VTEC strains identified. Participants were requested to identify the subtypes of the *vtx1* group (*vtx1a, vtx1c and vtx1d*) and *vtx2* group (from *vtx2a* to *vtx2g*) genes detected.

3. PARTECIPANTS

Thirty-six NRLs, representing 28 EU Member States, Norway, Switzerland and Turkey participated in the study. Each NRL received its own individual laboratory numerical code, which was reported in the result tables.

The NRLs participating in the study were:

- Austria, Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH
- Belgium, Scientific Institute of Public Health (also representing Luxembourg)
- Bulgaria, National Diagnostic and Research Veterinary Institute
- Croatia, Croatian Veterinary Institute, Laboratory for Food Microbiology
- Cyprus, Laboratory for the Control of Food of Animal Origin (LCFAO)
- Czech Republic, Veterinary Research Institute
- Denmark, National Food Institute, Technical University of Denmark
- Estonia, Veterinary and Food Laboratory
- Finland, Finnish Food Safety Authority Evira, Kuopio
- France, VetAgro Sup Campus Vétérinaire de Lyon

- Germany, Federal Institute for Risk Assessment (BfR)
- Greece, National School of Public Health & Central Laboratory of Public Health
- Hungary, National Food Safety Office, National Food Microbiological Reference Laboratory
- Hungary, Central Agricultural Office, Feed Investigation National Reference Laboratory
- Ireland, Central Veterinary Research Laboratory
- Italy, Istituto Superiore di Sanità
- Latvia, Institute of Food Safety, Animal Health and Environment BIOR
- Lithuania, National Food and Veterinary Risk Assessment Institute
- Netherlands, RIVM, Centre for Zoonoses and Environmental Microbiology
- Netherlands, Food and Consumer Product Safety Authority (NVWA)
- Norway, Norwegian Veterinary Institute
- Poland, National Institute of Public Health-National Institute of Hygiene, Warsaw
- Poland, National Veterinary Research Institute, Pulawy
- Portugal, Instituto Nacional de Investigação Agrária e Veterinária (INIAV)
- Romania Institute for Hygiene and Veterinary Public Health
- Slovakia, State veterinary and food institute, Dolný Kubín
- Slovakia, Public Health Authority, Bratislava
- Slovenia, National Veterinary Institute
- Spain, Laboratorio Central de Veterinaria de Algete (MAGRAMA)
- Spain, Centro Nacional de Alimentación, Servicio de Microbiología Alimentaria (AESAN)
- Spain, University of Santiago de Compostela, Lugo
- Sweden, National Food Agency (SLV)
- Sweden, National Veterinary Institute (SVA)
- Switzerland, Institute for food safety and hygiene, University of Zurich
- Turkey, Public Health Institution, Ankara
- United Kingdom (UK), Public Health England (also representing Malta)

4. MATERIALS AND METHODS

4.1. Sample preparation

The test materials sent to the NRLs were constituted by 6 strains of *E. coli* (samples 1 to 6), selected among those present in the EU-RL VTEC reference collection and checked for the presence of all the required genetic and phenotypic features. The characteristics of the strains are reported in Table 1 and were considered as the gold standard.

Table 1: Characteristics of the *E. coli* strains included in the study

Strain	Patho group	Sero group	Virulence genes (subtype)								
			vtx1	***vtx2***	***eae***	***aggR***	***aaiC***	***ipah***	***sth***	***stp***	***lt***
1	**ETEC**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**
2	**VTEC**	**O113**	**+ (*vtx1c*)**	**+ (*vtx2b*)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
3	**EIEC**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**
4	**VTEC**	**O121**	**-**	**+ (*vtx2a*)**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
5	**EPEC**	**O26**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
6	**EAggEC**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**-**	**-**	**-**

As for the stability of the samples, previous experiences supported the assumption that the time range between the preparation of the specimens and the deadline for submission of results by NRLs was short enough to assure the detection of all the strain characteristics.
The test samples were prepared on 14 June. They consisted of freshly prepared bacterial cultures inoculated into microbank bacterial preservation system vials. The cultures were incubated 18 hours at 37 °C ± 1 °C and labelled with randomly generated numerical codes (3 or 4 digits), different for each NRL. The test samples were stored at room temperature until 17 June, when the samples were sent by courier to the participating laboratories.

4.2. Laboratory methods

The identification of the *E. coli* patho-groups was carried out by Real Time PCR amplification of their specific target virulence genes using the methods available in the EU-RL VTEC web site (http://www.iss.it/vtec), Laboratory Methods section.

The determination of the serogroup of the VTEC and EPEC strains identified could be performed by participants using any serological or molecular method currently in use in their laboratories. However, a PCR (end point) procedure for detecting the serogroup-associated genes was available in the EU-RL web site, Laboratory Methods section.

The subtypes of the *vtx* genes of the VTEC strains identified were determined by an end point PCR method based on the paper of Scheutz et al. (J. Clin. Microbiol. 2012; 50: 2951-63). The method was available in the EU-RL web site, Laboratory Methods section.

4.3. Collection and elaboration of the NRL results

The results were submitted directly through an on-line system, using a dedicated page in the “Restricted Area” of the EU-RL VTEC web site. The NRLs received their own user ID and password for the log-in procedure and a step-by-step procedure for the submission of the results. After the log-in, they had access to a dedicated section for submitting the test results. This section also contained a *Shipment form* with the list of the samples to be analyzed and the fields to collect the information on the arrival date, temperature and quality of the sample, and the possibility to write notes and to specify any problem with the sample delivery/packaging. At the end of the study, the participants could print their own instant-generated individual reports, containing the submitted and the expected results, directly from the secure page of the EU-RL web site.

4.4. Analysis of the NRL results

4.4.1. Evaluation of the NRL performance in the identification of the different groups of pathogenic E. coli

The performance of each NRL in identifying the patho-groups of *E. coli* was evaluated by assigning penalty points for the virulence genes that were identified incorrectly. The penalty points were assigned as follows, according to the public health relevance of the different patho-groups:

- **4 penalty points** were assigned to each incorrect result concerning the identification of the *vtx* genes, that represent the main virulence determinants of VTEC and the main target of the ISO/TS 13136:2012, the international standard aiming at the detection of these pathogenic *E. coli* in food. The detection of these genes was the object of

several previous PTs. Moreover, there is a microbiological criterion for the presence of VTEC in a food commodity in a EU regulation (Reg. EU 209/2013).

- **2 penalty points** were assigned to each incorrect result concerning the identification of the other virulence genes considered in the PT (*eae, AggR, aaiC, lt, st, ipaH*).
- **1 penalty point** was assigned to each result reported as "Not Done".

The sum of the penalty points obtained originated a total score used to evaluate the performance of NRLs. In particular, a threshold of 4 penalty points was set in order to identify the laboratories not performing adequately. The NRLs that summed up a score of 4 without making errors in the identification of *vtx* genes represented an exception and their performance was still considered as satisfactory.

4.4.2. Evaluation of the NRL performance in the identification of the O serogroups

The performance of each NRL in identifying the serogroup of the VTEC and EPEC strains was evaluated by assigning penalty points for strains that were typed incorrectly.

The following distinction was made, according to the public health importance of the serogroups, based on the data on human STEC infections published yearly by the ECD-FWD surveillance program in the EU Summary Report on Trends and Sources of Zoonoses.

- **4 penalty points:** assigned to each incorrect result concerning the typing of the strains belonging to the 5 serogroups most frequently isolated from cases of hemolytic uremic syndrome in Europe: O26, O103, O111, O145, O157 (the so called "top 5").
- **2 penalty points:** assigned to each incorrect result concerning the typing of the strains belonging to the other 7 serogroups of public health relevance that were in the scope of this study: O55, O91, O104, O113, O121, O128, O146.

The sum of the penalty points originated a score used to evaluate the performance of the NRLs. In particular, a threshold of 4 penalty points was set in order to identify the laboratories not performing adequately. The NRLs that summed up a score of 4 without making errors in the typing of the strains belonging to the "top 5" serogroups represented an exception and their performance was still considered as satisfactory.

4.4.3. Evaluation of the NRL performance in the identification of the vtx gene subtypes

The performance of each NRL in identifying the subtypes of the *vtx* genes in the two VTEC strains included in the test materials was evaluated by assigning penalty points for *vtx* genes that were typed incorrectly, according to the following criteria:

- **2 penalty points** were assigned to each incorrect result concerning the typing of *vtx2a* and *vtx2c* genes, the two variants that have been strongly associated with strains causing HUS.
- **1 penalty point** was assigned to each incorrect result concerning the typing of all the other *vtx1 a*nd *vtx2* gene subtypes, and to each result reported as "Not Done".

In particular, a threshold of 4 penalty points was set in order to identify the laboratories not performing adequately. The NRLs that summed up a score of 4 without making errors in the typing of *vtx2a* and *vtx2c* genes, represented an exception and their performance was still considered as satisfactory.

5. RESULTS

Results were submitted by 35 of the 36 NRLs that received the samples.

5.1. Identification of the *E. coli* patho-groups by amplification of their target virulence genes and evaluation of the NRL performance for these tests

The results of the Real Time PCR tests performed for the detection of the target virulence genes of the different *E. coli* patho-groups are reported in Table 2 (1-3), while Table 3 summarizes the results of the Real Time PCR tests performed for all the six test strains for the detection of their virulence genes.

All the NRLs, carried out the tests for the detection of *vtx* genes, while the detection of the EPEC, EAggEC, ETEC and EIEC target virulence genes was not performed by four NRLs for all or part of the strains.

Thirty NRLs (86 %) identified correctly the presence/absence of all the target genes in all the test strains, while five NRLs provided a total of six incorrect results.

As for the detection of *vtx* genes, 33 NRLs (94 %) identified correctly their presence/absence in all the test strains, while two NRLs provided a total of two incorrect results: one false positive for *vtx1* and one false positive for *vtx2.*

As for the detection of the other virulence genes, 28 NRLs (80 %) identified correctly the presence/absence of the target genes in all the test strains. They represented the 90 % of the 31 NRLs that carried out the tests. Four of those NRLs provided a total of 4 incorrect results: one false positive for the *aggR* and for *lt* genes, and one false negative for the *aggR*, and *aaic* genes.

Table 2 (1). Identification of the different groups of pathogenic *E. coli* by Real Time PCR amplification of their virulence genes (strains 1 and 2). The green boxes indicate the correct results, in agreement with the true value reported on the top of each column. The red boxes indicate the wrong results. ND indicates that the test was not done.

	Detection of virulence genes in:																	
	Sample 1 (ETEC strain)									**Sample 2 (VTEC strain)**								
	vtx1	***vtx2***	***eae***	***aggR***	***aaiC***	***ipah***	***sth***	***stp***	***lt***	***vtx1***	***vtx2***	***eae***	***aggR***	***aaiC***	***ipah***	***sth***	***stp***	***lt***
True value	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
L105																		
L151																		
L162				**ND**	**ND**	**ND**	**ND**	**ND**	**ND**				**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
L163																		
L166																		
L172																		
L187																		
L209																		
L227																		
L268																		
L273			**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**			**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
L318																		
L376																		
L416																		
L430																		
L460																		
L518																		
L542																		
L549																		
L550																		
L553				**ND**	**ND**	**ND**							**ND**	**ND**	**ND**			
L566																		
L607																		
L614																		
L623																		
L706													**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
L709																		
L728																		
L732																		
L751																		
L758																		
L920																		
L950																		
L962																		
L975																		

Table 2 (2). Identification of the different groups of pathogenic *E. coli* by Real Time PCR amplification of the virulence genes (strains 3 and 4). The green boxes indicate the correct results, in agreement with the true value reported on the top of each column. The red boxes indicate the wrong results. ND indicates that the test was not done.

	Detection of virulence genes in:																	
	Sample 3 (EIEC strain)									Sample 4 (VTEC strain)								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*	*vtx1*	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-
L105																		
L151																		
L162				ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L163																		
L166																		
L172																		
L187																		
L209																		
L227																		
L268																		
L273			ND	ND	ND	ND	ND	ND	ND			ND	ND	ND	ND	ND	ND	ND
L318																		
L376																		
L416																		
L430																		
L460																		
L518																		
L542																		
L549																		
L550																		
L553				ND	ND	ND							ND	ND	ND			
L566																		
L607																		
L614																		
L623																		
L706							ND	ND	ND				ND	ND	ND	ND	ND	ND
L709																		
L728										+								
L732																		
L751																		
L758																		
L920																		
L950																		
L962																		
L975		+																

Table 2 (3). Identification of the different groups of pathogenic *E. coli* by Real Time PCR amplification of the virulence genes (strains 5 and 6). The green boxes indicate the correct results, in agreement with the true value reported on the top of each column. The red boxes indicate the wrong results. ND indicates that the test was not done.

NRL	Detection of virulence genes in:																	
	Sample 5 (EPEC strain)									Sample 6 (EAggEC strain)								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*	*vtx1*	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
L105																		
L151																		
L162				ND	ND	ND	ND	ND	ND				ND	ND	ND	ND	ND	ND
L163																		
L166																		
L172																		
L187																		
L209														ND				
L227																		
L268																		
L273				ND	ND	ND	ND	ND	ND			ND	ND	ND	ND	ND	ND	ND
L318																		
L376																		
L416																		
L430																		
L460													-	-				
L518																		
L542																		
L549																		
L550																		
L553				ND	ND	ND			+				ND	ND	ND			
L566																		
L607																		
L614																		
L623																		
L706				ND	ND	ND	ND	ND	ND						ND	ND	ND	ND
L709																		
L728																		
L732																		
L751																		
L758																		
L920				+														
L950																		
L962																		
L975																		

Table 3. Summary of the PCR results obtained for the detection of the virulence genes. The green boxes indicate that all the genes were identified correctly in the given strain. The red boxes indicate that incorrect results were reported for the given strain. The numbers in the box indicate the number of incorrect results.

NRL	Detection of virulence genes in all the 6 test strains:								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
L105									
L151									
L162				ND	ND	ND	ND	ND	ND
L163									
L166									
L172									
L187									
L209									
L227									
L268									
L273			*	ND	ND	ND	ND	ND	ND
L318									
L376									
L416									
L430									
L460				1	1				
L518									
L542									
L549									
L550									
L553				ND	ND	ND			1
L566									
L607									
L614									
L623									
L706				*	*	*	*	*	*
L709									
L728	1								
L732									
L751									
L758									
L920				1					
L950									
L962									
L975		1							

* The gene was not tested in all the samples

For each NRL, the number of penalty points was determined using the criteria described in section 4.4.1. Figure 1 shows the score achieved by each NRL, and Figure 2 the number of NRLs grouped according to their score. Four NRLs obtained a high score, since they did not perform the detection of all or part of the virulence genes of the *E. coli* patho-groups other than VTEC.

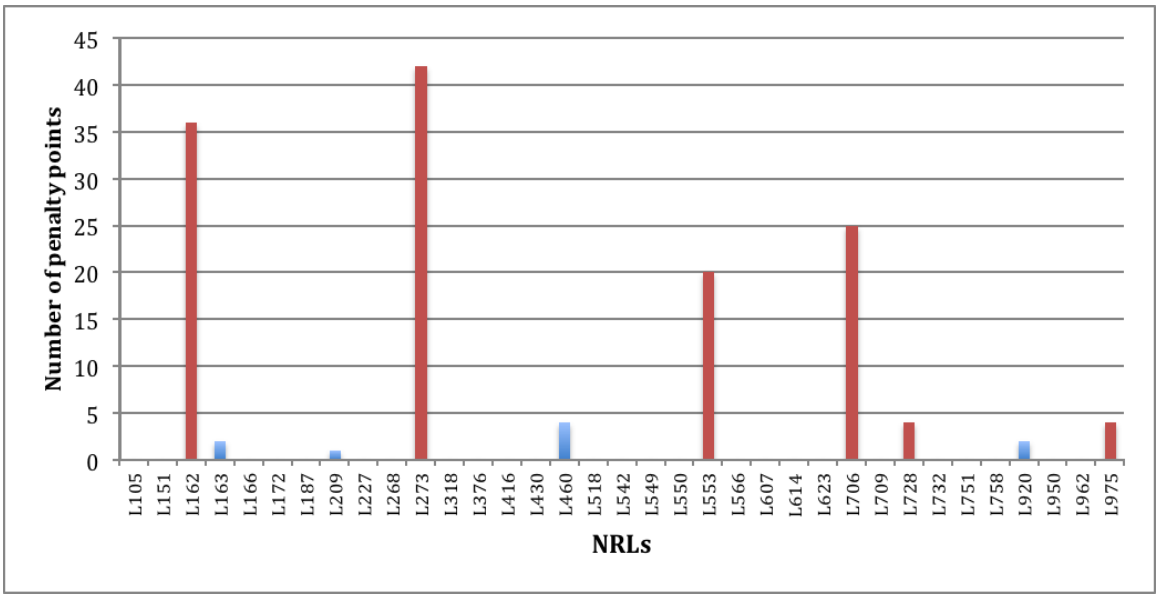

Figure 1. Evaluation of the PCR results for the detection of virulence genes by NRL.

The score was calculated according to the criteria described in section 4.4.1. The red bars indicate the NRLs whose performance was not considered as satisfactory.

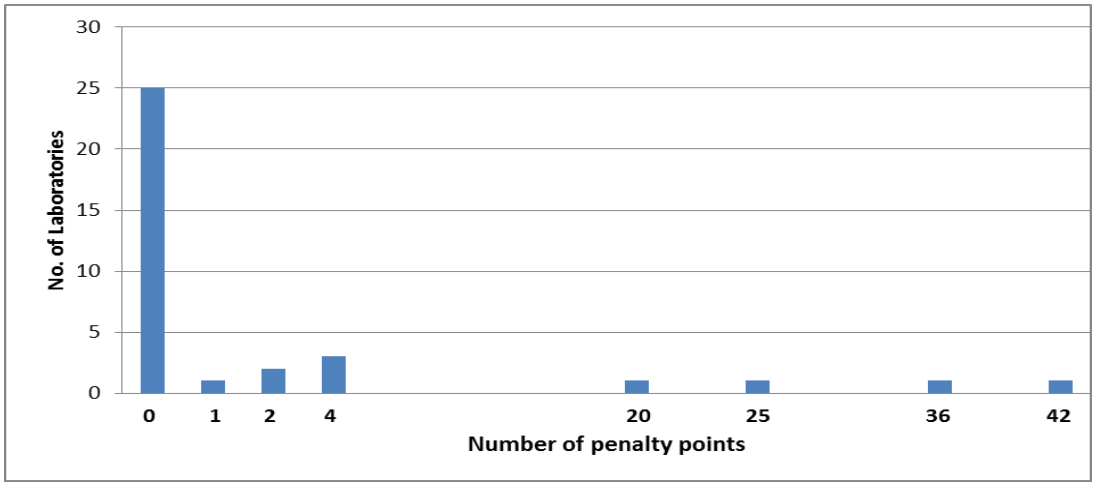

Figure 2. Evaluation of the PCR results for the detection of virulence genes: number of NRLs within each penalty score.

6.2. Identification of the O serogroups and evaluation of the NRL performance for this test

The identification of the O serogroups of the VTEC and EPEC strains included in the PT was carried out by all the 35 NRLs and the results are shown in Table 4.

Table 4. Identification of the serogroup of the test strains. The green boxes indicate the correct results, in agreement with the true value reported on the top of each column. The red boxes indicate the incorrect results, and display the results provided by the NRL.

NRL	Serogroup identification in sample:		
	2	4	5
True Value	O113	O121	O26
L105	-		
L151	O125ac		
L162			
L163			
L166			
L172			
L187			
L209	-	-	
L227			
L268			
L273			
L318			
L376			
L416			
L430			
L460			
L518			
L542			
L549			
L550			
L553			
L566			
L607			
L614			
L623			
L706			
L709			
L728			
L732			

L751			
L758			
L920			
L950			
L962			
L975			

Thirty-two NRLs (91 %) identified correctly the serogroup of all the 3 VTEC/EPEC strains. The other 3 NRLs provided a total of 4 incorrect results. In particular, NRL L209 did not identify the O113 and O121 strains, and L105 and L151 failed to identify the VTEC strain O113.

For these NRLs, the number of penalty points was determined using the criteria described in section 4.4.2. Therefore, L209 was assigned the score 4, and L105 and L151 the score 2. Figure 3 shows the number of NRLs grouped according to their score.

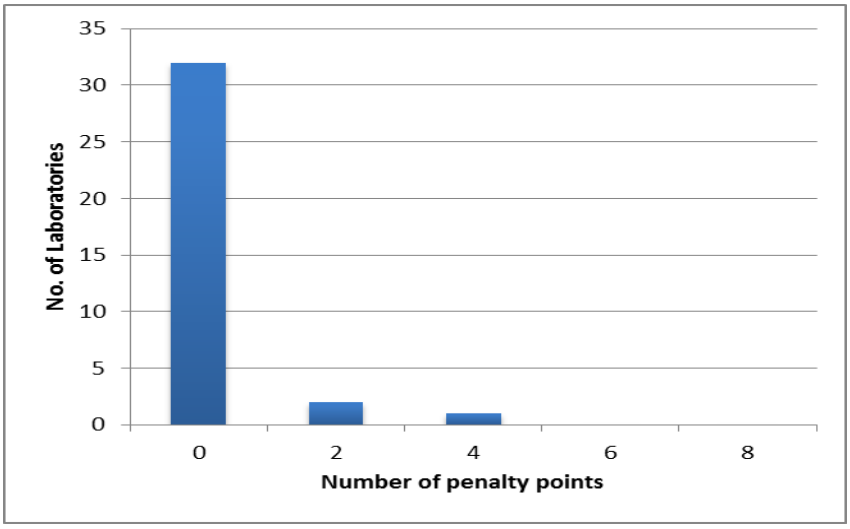

Figure 3. Evaluation of serogrouping results: number of NRLs within each penalty score.

6.3. Identification of the *vtx* gene subtypes by conventional PCR

The identification of the subtypes of the *vtx* genes harbored by the two VTEC strains was performed by 32 of the 35 NRLs that participated in the study (91 %). The results are reported in Table 5.

Table 5. Subtyping of the *vtx* gene by PCR in samples 2 and 4. The green boxes indicate the correct results, in agreement with the true value reported on the top of each column. The red boxes indicate the wrong results. The white boxes indicate that the test was not done (ND).

	Detection of the *vtx* gene subtype in:																
	Sample 2 (*vtx1+,vtx2+*)										**Sample 4 (*vtx2+*)**						
NRL	***vtx1a***	***vtx1c***	***vtx1d***	***vtx2a***	***vtx2b***	***vtx2c***	***vtx2d***	***vtx2e***	***vtx2f***	***vtx2g***	***vtx2a***	***vtx2b***	***vtx2c***	***vtx2d***	***vtx2e***	***vtx2f***	***vtx2g***
True value	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
L105																	
L151																	
L162					-												
L163																	
L166																	
L172																	
L187																	
L227						+											
L268																	
L318																	
L376																	
L416																	
L430																	
L460				+	-									+			
L518													+				
L542																	
L549																	
L550																	
L553																	
L566	+	-			-					+			+		+		
L607																	
L614																	
L623																	
L706																	
L709																	
L728																	
L732																	
L751																	
L920																	
L950		-			-				+		-					+	
L962																	
L975																	

The presence/absence of the *vtx* gene subtypes was identified correctly in the two *vtx*-positive strains by 26 of the 32 NRLs (81 %) that carried out the subtyping, while six NRLs reported a total of 17 incorrect results: 7 false negative results (4 for *vtx2b,* 1 for *vtx2a, and* 2 for *vtx1c*) and 10 false positive results (1 for *vtx2a,* 3 for *vtx2c,* 1 for *vtx2d,* 1 for *vtx2e,* 2

for *vtx2f,* 1 for *vtx2g,* and 1 for *vtx1a*). For each NRL, the number of penalty points was determined using the criteria described in section 4.4.3. Figure 1 shows the score achieved by each NRL, and Figure 2 the number of NRLs grouped according to their score. Three NRLs obtained a score higher or equal to 4.

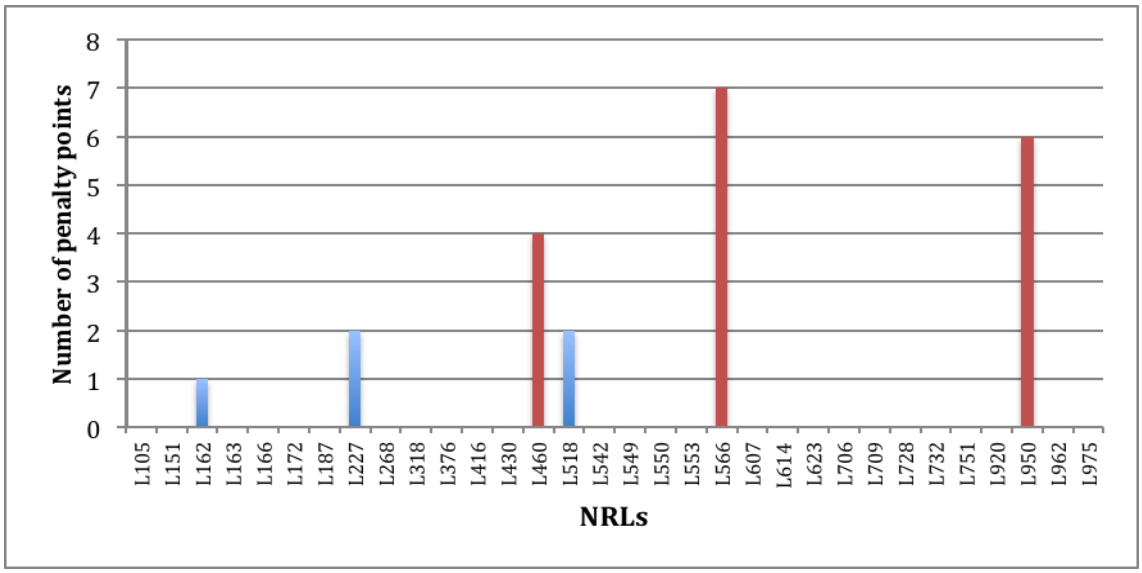

Figure 4. Evaluation of the PCR subtyping of the *vtx* gene by NRL. The score was calculated according to the criteria described in section 4.4.3. The red bars indicate the NRLs whose performance was not considered as satisfactory.

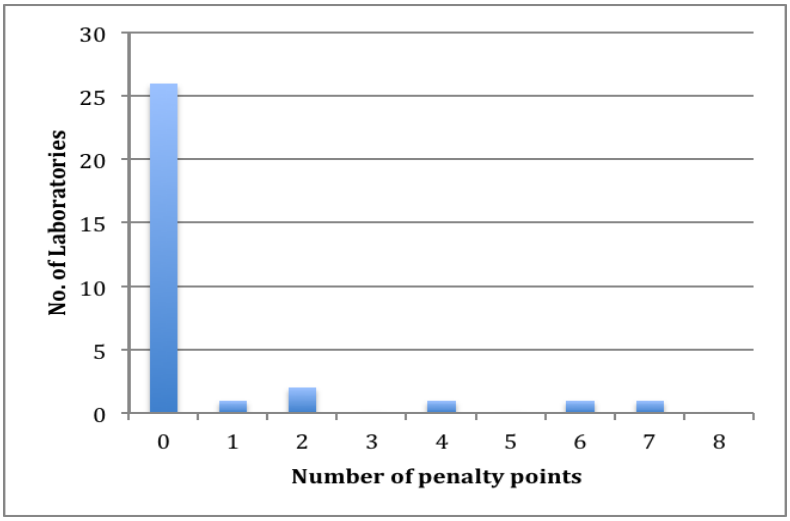

Figure 5. Evaluation of *vtx* subtyping results: number of NRLs within each penalty score.

7. CONCLUDING REMARKS

PT 11 was the first study on the identification of *E. coli* strains belonging to patho-groups other than VTEC, namely: EAggEC, ETEC, EPEC, and EIEC. These *E. coli* patho-groups are well known causes of traveler diarrhea, but have been also reported in sporadic cases and foodborne outbreaks in industrialized countries.

The following remarks can be drawn.

1. Thirty-three NRLs, representing all the 28 EU Member States, and the NRLs of Norway, Switzerland and Turkey participated in the study. Thirty-five NRLs submitted results.
2. Thirty-three NRLs (94 %) identified correctly the presence/absence of *vtx* target genes in the two VTEC test strains.
3. Thirty-two NRLs (91 %) identified correctly the serogroup of the 3 VTEC/EPEC test strains included in the PT.
4. The identification of the *vtx* gene subtypes was carried out by 32 NRLs, 26 of which (81 %) performed correctly all the tests. Both the number of participants and the rate of correct results represented a remarkable improvement with respect to the previous PTs on *vtx* gene subtyping (19 participants and 47 % submitting correct results in PT10).
5. Twenty-eight NRLs (80 %) identified correctly the presence/absence of the target genes of the test strains belonging to patho-groups other than VTEC. These results can be considered as satisfactory, since this was the first PT including the other *E. coli* patho-groups.
6. In conclusion, most NRLs performed satisfactorily in the PCR identification of the main virulence genes of VTEC, indicating that a very good preparedness has been established in the EU towards these foodborne pathogens, now included as a food safety microbiological criterion for sprouts in Reg. (EC) 209/2013. Moreover, most NRLs are now able to identify the other groups of pathogenic *E. coli* in the case of foodborne outbreaks due to these pathogens.