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Report of the 17th inter-laboratory study on the detection of Verocytotoxin-producing *E. coli* (STEC) in food (PT17)

Edited by:

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1. INTRODUCTION

The duties of the EU Reference Laboratory for *E. coli* (EURL-VTEC) include the organization of proficiency tests (PT) to assess the performance of the designated National Reference Laboratories (NRLs) for *E. coli* in the EU and EFTA Member States, EU Candidate Countries and certain third countries in using the methods for the detection of STEC in food and for the characterization of the isolated STEC strains.

A standard method for the detection of STEC in foodstuffs was published on November 2012 as an ISO Technical Specification: ISO TS 13136:2012 "*Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups*". This method, together with the EURL procedure "*Detection and identification of Verocytotoxin-producing Escherichia coli (VTEC) O104:H4 in food by Real Time PCR* (EU-RL VTEC_Method_04_Rev 1)", had been prescribed for the detection of STEC in sprouts by Regulation (EU) No 209/2013, which has introduced for the first time microbiological criteria for STEC in the EU legislation.

The ISO TS 13136:2012 standard was used in nine rounds of the EURL PT scheme on the detection of STEC in food matrices and animal samples: PT3, carried out on bovine carcass swabs; PT4, carried out on milk samples; PT7, carried out on vegetable samples; PT8, carried out on water samples; PT9, carried out on seeds intended for sprouting; PT12, PT14 and PT15, carried out on sprouts, and PT16 on the detection of STEC in sprout irrigation water. The reports of these PTs are available at the EURL website (www.iss.it/vtec).

This was the 10th PT (PT17) based on the ISO TS 13136:2012 standard taking into account the EURL adaptation for the detection of STEC O104:H4 (available in the EURL website) for the detection of STEC in food and water matrices. The PT was carried out on beef minced meat samples.

The choice of this matrix was due to the following reasons:

- Cattle is the major reservoir of STEC.
- Beef meat represents a food commodity traditionally associated with STEC infection.
- Minced meat has been recognized as the vehicle of STEC infections in numerous outbreaks, including the first epidemic episode occurred during the 80s when STEC O157 was identified for the first time.
- Even though microbiological criteria are not in place for this food commodity, bovine

meat samples are continuously analysed for the presence of STEC in Member States, particularly in the framework of controls at the borders concerning imports from third-countries.
- This matrix had never been proposed in previous PTs organized by the EURL-VTEC.

PT17 gives continuity to the previous studies conducted within the network of Reference Laboratories for *E. coli* on food matrices and this document represents the full evaluation report of the study.

2. DESIGN AND OBJECTIVES OF THE STUDY

The study consisted in detection and isolation of a STEC from ground beef samples spiked with different amounts of a STEC O91. Although this STEC serogroup does not belong to the top-5 STEC, it is quite commonly isolated from meat samples. Additionally, such STEC serogroup ranked sixth in the distribution of the 20 most frequent STEC serogroups in human infections in Europe in the period 2012-2014 (EU summary report on zoonoses, zoonotic agents and food-borne outbreaks 2015).

The **objectives** of the study were:
- To improve the preparedness of the NRLs towards testing food commodities for the presence of STEC, by applying to the ISO TS 13136:2012.
- To improve the preparedness of the NRLs towards the detection and isolation of STEC strains not belonging to the top-5 serogroups.
- To give further support to the NRLs and the Official Laboratories for the accreditation of the ISO TS 13136:2012.

3. PARTICIPANTS

Thirty-seven NRLs representing 26 EU Member States, Chile, Egypt, Norway, Russia and Switzerland participated in the study.

Each NRL received its own individual laboratory numerical code, which is reported in the result tables and in the individual reports.

The NRLs participating in the study were:
- Austria, AGES Austrian Agency for Health and Food Safety, Institute for Medical Microbiology and Hygiene, Graz

- Belgium, Scientific Institute of Public Health, *Direction Opérationnelle Maladies Transmissibles et Infectieuses*, Bruxelles
- Belgium, Veterinary & Agrochemical Research Centre (VAR - CODA - CERVA), Operational Directorate Bacterial Diseases, Brussels
- Bulgaria, National Diagnostic and Research Veterinary Institute, Sofia
- Chile, *Servicio Agrícola y Ganadero, Subdepartamento Laboratorios y Estación, Cuarentenaria Pecuaria*, Santiago
- Croatia, Croatian Veterinary Institute, Laboratory for Food Microbiology, Zagreb
- Cyprus, Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services, Nicosia
- Czech Republic, Veterinary Research Institute, Brno
- Denmark, FVST, *Mikrobiologisk Laboratorium*, Ringsted
- Egypt, Central Lab of Residue Analysis of Pesticides and Heavy Metals in Foods, Giza
- Estonia, Veterinary and Food Laboratory, Tartu
- Finland, Finnish Food Safety Authority Evira, Research and Laboratory Department, Food and Feed Microbiology Research Unit, Helsinki
- France, *VetAgro Sup Campus Vétérinaire de Lyon*, Marcy L'Etoile
- Germany, Federal Institute for Risk Assessment (BfR), Berlin
- Greece, National School of Public Health & Central Laboratory of Public Health, Department of Microbiology, Vari Attikis
- Hungary, National Food Safety Office, Food and Feed Safety Directorate, National Food Microbiological Reference Laboratory, Budapest
- Ireland, Central Veterinary Research Laboratory, Department of Agriculture, Food and the Marine, Celbridge
- Italy, *Istituto Superiore di Sanità*, Rome
- Latvia, Institute of Food Safety, Animal Health and Environment (BIOR), Riga
- Lithuania, National Food and Veterinary Risk Assessment Institute, Molecular biology and GMO Section, Vilnius
- Norway, Section for food bacteriology, Norwegian Veterinary Institute, Oslo
- Poland, National Institute of Public Health-National Institute of Hygiene, Department of Food Safety, Laboratory of Food Microbiology, Warsaw
- Poland, Poland National Veterinary Research Institute, Department of Hygiene of food of animal origin, Pulawy

- Portugal, *Instituto Nacional de Investigação Agrária e Veterinária, I. P., UEISTSA – Microbiologia dos Alimentos*, Vairão
- Romania, Department of Microbiology - Molecular Biology Unit, Institute for Hygiene and Veterinary Public Health, Bucharest
- Russia, International Department State Research Center for Microbiology and Biotechnology, Obolensk
- Slovakia, Department of Food Hygiene, State veterinary and food institute, Dolný Kubín
- Slovakia, NRC of Environmental Microbiology, Public Health Authority of SR, Bratislava
- Slovenia, Veterinary Faculty/ National Veterinary Institute, Ljubljana
- Spain, AESAN, *Centro Nacional de Alimentación, Servicio de Microbiología Alimentaria*, Majadahonda, Madrid
- Sweden, *Livsmedelsverket*/The National Food Agency, Uppsala
- Sweden, National Veterinary Institute (SVA), Department of Bacteriology, Uppsala
- Switzerland, Institute for food safety and hygiene, University of Zurich, Zurich
- The Netherlands, RIVM, Centre for Zoonoses and Environmental Microbiology, Bilthoven
- The Netherlands, Food and Consumer Product Safety Authority (NVWA), Wageningen
- UK, Public Health England, Royal Preston Hospital, Preston
- UK, Public Health England, Salisbury, Porton

4. MATERIALS AND METHODS

4.1. Sample preparation

Three samples (A, B and C), each consisting of 25 g of ground beef meat potentially contaminated with STEC, were sent in the blind to the NRLs.

The ground beef meat used was purchased at retail on March the 29th, 2016 and originated from a single trim, ground as the first process of the day on the 29th of March, in order to avoid contamination with other meat trims. The ground beef contained a natural background microflora (about 1.4x10^{4} CFU/g). The minced meat was portioned in 25 g samples in sterile stomacher bags and placed at -20 °C until the preparation of the PT samples. Two 25 g portions of meat were initially tested for the presence of STEC by applying the ISO TS 13136:2012 method. Both samples were negative for *stx1*, *stx2* and serogroup-associated genes, one was positive for *eae* (threshold cycle 25) at the screening. The minced meat was considered suitable for the PT purpose, being negative for STEC.

The artificial contamination of the samples was carried out on 15 April 2016, using dilutions of an exponential liquid culture (0.5 OD read at 600 nm) of the STEC O91 strain ED 76 positive for *stx1* and *stx2* genes and negative for the *eae* gene. The characteristics of the samples are reported in Table 1. Uncertainty of measurement of 0.121 log CFU/ml was associated to the standardized inoculum, using the procedure described in the ISO TS 19036:2006. The samples were spiked with three different levels of contamination: zero, low and high level. In detail, the set of samples sent to the NRLs contained 0, 5 and 50 estimated CFU per gram, respectively (Table 1). Serial dilutions of the inoculum suspensions added to the samples were plated onto MacConkey agar plates to check their actual titer. In order to define the concentration of the STEC O91 to be used, several spiking levels were tested: 10, 100, 500 and 1000 CFU/g. The artificial contamination was made in four replicates for each spiking level and the samples were tested (both PCR screening and isolation steps) in a time period of 12 days from their preparation. The data have also been used for assessing the stability of the samples. All the samples were positive in the PCR tests for *stx1* and *stx2* genes and negative for *eae* and serogroups-associated genes. Isolation of the STEC O91 was successful for all samples up to 12 days from the spiking. Based on these results, the highest level of contamination was set at 50 CFU/g and the lowest at 5 CFU/g, in order to attempt defining the limit of detection.

Table 1: Characteristics of the meat samples included in the study

Contaminant (*Genotype*)	**Contamination level in:**		
	Sample A	**Sample B**	**Sample C**
Strain ED 76, STEC O91 *(stx1+, stx2+, eae-)*	-	Low: 5 CFU/g	High: 50 CFU/g

The uncertainty of measurement associated to the ED 76 inoculum was 0.121 log CFU/ml

The test samples were labeled with randomly generated numerical codes different for each NRL, immediately refrigerated and transferred into refrigerated safety packages that were shipped on 18 April by courier. The NRLs were requested to start the analyses

immediately upon receipt and to record date of delivery and sample temperature upon reception.

4.2. Assessment of the stability and homogeneity of the samples

The stability and homogeneity of the samples were evaluated according to the requirements of ISO 17043:2010.

Samples were spiked on 31 March 2016 and tested by Real Time PCR at 0, 4, 7 and 12 days since the initial contamination. The presence of the contaminating STEC was detected and the STEC was isolated from all the samples.

When the test samples were prepared, 6 bags for each of the two levels of contamination and not contaminated samples were randomly selected for homogeneity testing and analyzed according to the PT laboratory procedures on the 17th and 18th of April 2016. All the homogeneity tests gave the expected results.

4.3. Laboratory methods

Laboratories were requested to identify the presence of STEC using the method ISO TS 13136:2012, taking into account the adaptation provided by the EURL-VTEC for the detection of STEC O104:H4 (available at the EURL website, http://www.iss.it/vtec, Laboratory Methods section).

4.4. Collection and elaboration of the NRL results

The results were submitted through an online system, using a dedicated page in the “Restricted Area” of the EURL-VTEC website.

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 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 expected results, directly from the secure page of the EURL website.

4.5. Analysis of the NRL results

4.5.1. Evaluation of the NRL performance in the real time PCR screening step

The performance of each NRL in identifying STEC target genes in the enrichment cultures was evaluated by assigning 4 penalty points to each incorrect or missing result concerning the identification of virulence genes, *stx1* and *stx2*, in the three samples.

4.5.2. Evaluation of the NRL performance in the isolation of STEC strains from the PCR-positive enrichment cultures

The performance of each NRL in the isolation and characterization of the STEC strains responsible for positive PCR screening reactions in the enrichment cultures was evaluated by assigning 4 penalty points to the lack of isolation from the positive samples. However, no penalty points have been assigned to the lack of isolation from sample B, since the calculation of the limit of detection (LOD) indicated that the level of contamination used was below the LOD_{50} (see below).

No penalty points have been assigned to the laboratories not identifying the O91 serogroup (ONT), since this is not included in the scope of the methods recommended for this PT. However, two penalties were assigned for incorrect characterization of the O-group of the isolated strain.

4.5.3. Evaluation of the NRL performance in the overall procedure

The sum of the penalty points obtained in the different steps of the procedure originated a total score, used to evaluate the overall performance of the NRLs in the PT. The performance of laboratories that obtained a score higher than 8 was considered as unsatisfactory.

4.6. Evaluation of the performance of the method

Sensitivity (Se) and Specificity (Sp) were calculated for the various STEC characters considered in the study and for the different steps of the ISO TS 13136:2012. Therefore, Se and Sp were calculated for the PCR screening for *stx1* and *stx2* genes, and for the isolation of the STEC O91 strain. The sensitivity and specificity were calculated according to the following formulas:

Sensitivity: SE = [true positives / (true positives + false negatives)] x 100

Specificity: SP = [True negatives / (true negatives + false positives)] x 100

The limit of detection (LOD) was calculated for the isolation step as described in Wilrich and Wilrich, 2009, Journal of AOAC international, 92(6):_1763-1772.

5. RESULTS

The samples were sent to 37 NRLs and 36 of them returned results (except L959).

As for the delivery conditions, 30 NRLs received the samples within 24 hours, 2 within 48 hours and 2 within 72 hours. The remaining laboratory received the samples after 7 days (L208) due to custom clearance procedures.

The reported temperature at delivery was ≤ 4 °C for 10 NRLs, between 5 °C and 8 °C for 14 NRLs, and between 10 °C and 14 °C for 11 NRLs. For the remaining NRL, L597, the temperature was 18 °C for the samples received after 48 hours.

5.1. Real-time PCR detection of STEC virulence and serogroup-associated genes in the enrichment cultures

All the 36 NRLs carried out the detection of the virulence and serogroup-associated genes and the results are reported in Table 2.

For the negative samples, one lab incorrectly reported the detection of *stx1* and *stx2* genes and another one the presence of *stx2*, *eae* and O26-associated gene.

As for sample B (low level of contamination) and sample C (high level of contamination) all labs but one correctly reported the detection of *stx1* and *stx2* genes. The NRL who reported the incorrect result was not able to detect *stx1* in both samples B and C. The *eae* gene was detected sample B by 5 labs, in four cases with the concomitant presence of wzx_{O26} gene. Six laboratories, detected *eae* gene in sample C, in three cases with the concomitant presence of wzx_{O26} gene and in one case with the presence of $ihp1_{O145}$. Four laboratories detected the presence of O104-associated gene in Sample B and two labs in sample C.

5.2. Isolation of the STEC O91 strain from PCR-positive samples.

A total of 25 laboratories isolated the STEC O91 strain in both Sample B (low level of contamination) and Sample C (high level of contamination) and correctly reported the *stx1* and *stx2* genes in the isolate. Four laboratories didn't characterize the O-group, reporting it as ONT, but no penalties were assigned to these participants, as this serogroup did not fall in the scope of the methods recommended. Finally, one laboratory reported the serogroup of the isolated STEC from samples B and C as O26. This result was considered as incorrect and 4 penalty points were assigned. One additional laboratory isolated the O91 STEC in both samples, but failed to detect the presence of *stx1* (four penalty points).

Four laboratories isolated an EPEC strain belonging to serogroup O26 that was likely part of the microflora of the ground beef and was non-homogeneously present in the test portions. This hypothesis was corroborated by a number of signals related with O26 and *eae* detection in the screening step. In the light of this result, no penalty points were assigned to labs detecting these genes in the enrichment broth.

One NRL was not successful in the isolation of the O91 *stx1 stx2* but reported the isolation of a STEC O26 with virulence profiles compatible with a non-pure culturing of the STEC

strain (*eae* from the EPEC O26 and *stx1* and *stx2* from the O91 STEC). This NRL was assigned with 4 penalty points for the lack of isolation of the STEC O91 from sample C.

One NRL isolated an EPEC O145 but not the STEC O91. This non-conformity could have been generated by the use of the IMS technique for the isolation or by following an evidence of O145-associated genes in the isolated colonies. The selection of the *E. coli* O145 has probably hindered the isolation of the STEC O91.

One laboratory reported, beside the presence of the O91 STEC, an O157 without virulence genes in sample C, and another one the isolation of an O103 possessing *stx2* but negative for *eae*. No penalty points were given to these NRLs given the correct isolation of the STEC O91.

Table 2. Real-time PCR detection of virulence and serogroup-associated genes in the enrichment cultures. 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 or not-matching the expected results.

NRL	Detection of virulence and serogroup-associated genes in:											
	Sample A				Sample B Low level contamination				Sample C High level contamination			
	vtx1	*vtx2*	*eae*	*Top-5 and O104 associated genes*	*vtx1*	*vtx2*	*eae*	*Top-5 and O104 associated genes*	*vtx1*	*vtx2*	*eae*	*Top-5 and O104 associated genes*
True value	-	-	-	-	+	+	-	-	+	+	-	-
L107												
L124												
L148												
L170											+	O26
L177												
L181												
L208								O104				O104
L280												
L328											+	O145
L343												
L356	+	+					+	O26				
L360												
L390												
L417												
L427								O104			+	O26
L435												
L444												
L524												
L597												
L614												
L630												
L653							+	O26			+	O26
L675							+	O26				
L705												
L721		+	+	O26	-		+		-		+	
L782												
L788												
L789												
L817								O104				
L838												
L844												
L873												
L886												
L907												
L912								O104				O104
L975							+	O26			+	

Table 3. Isolation and genotyping of STEC strains from the PCR-positive sprout enrichment cultures. 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 or missing results. ND indicates that the test was not done (ND). The orange boxes indicate the data not-matching the expected results, which did not produce penalties.

NRL	Sample A	Sample B								Sample C							
	-	STEC O91 Isolation	Genotype			Other E. coli Isolation	Genotype			STEC O91 Isolation	Genotype			Other E. coli Isolation	Genotype		
			vtx1	*vtx2*	*eae*		*vtx1*	*vtx2*	*eae*		*vtx1*	*vtx2*	*eae*		*vtx1*	*vtx2*	*eae*
True value	None	+	+	+	-					+	+	+	-				
L107																	
L124																	
L148																	
L170		ONT								ONT				O26	-	-	+
L177		ND								ND							
L181																	
L208		ND								ND							
L280																	
L328		ND								ND				O145	-	-	+
L343																	
L356	ONT, H4+																
L360		ND								ND							
L390																	
L417																	
L427																	
L435																	
L444														O157	-	-	-
L524		ONT								ONT							
L597																	
L614		ONT								ONT							
L630		O26								O26							
L653		ND				O26	+	+	+	ND				O26	+	+	+
L675		ND								ND							
L705																	
L721			-								-						
L782																	

L788						O103	-	+	-									
L789																		
L817		ND								ND								
L838																		
L844		ND								ND								
L873	O26, eae+	ND								ND								
L886																		
L907																		
L912		ND								ND								
L975		ONT				O26	-	-	+	ONT								

5.3. Evaluation of the NRL performance in the PT procedures

For each NRL, the number of penalty points was determined using the criteria described in section 4.5.

Figure 1 shows the score achieved by each NRL.

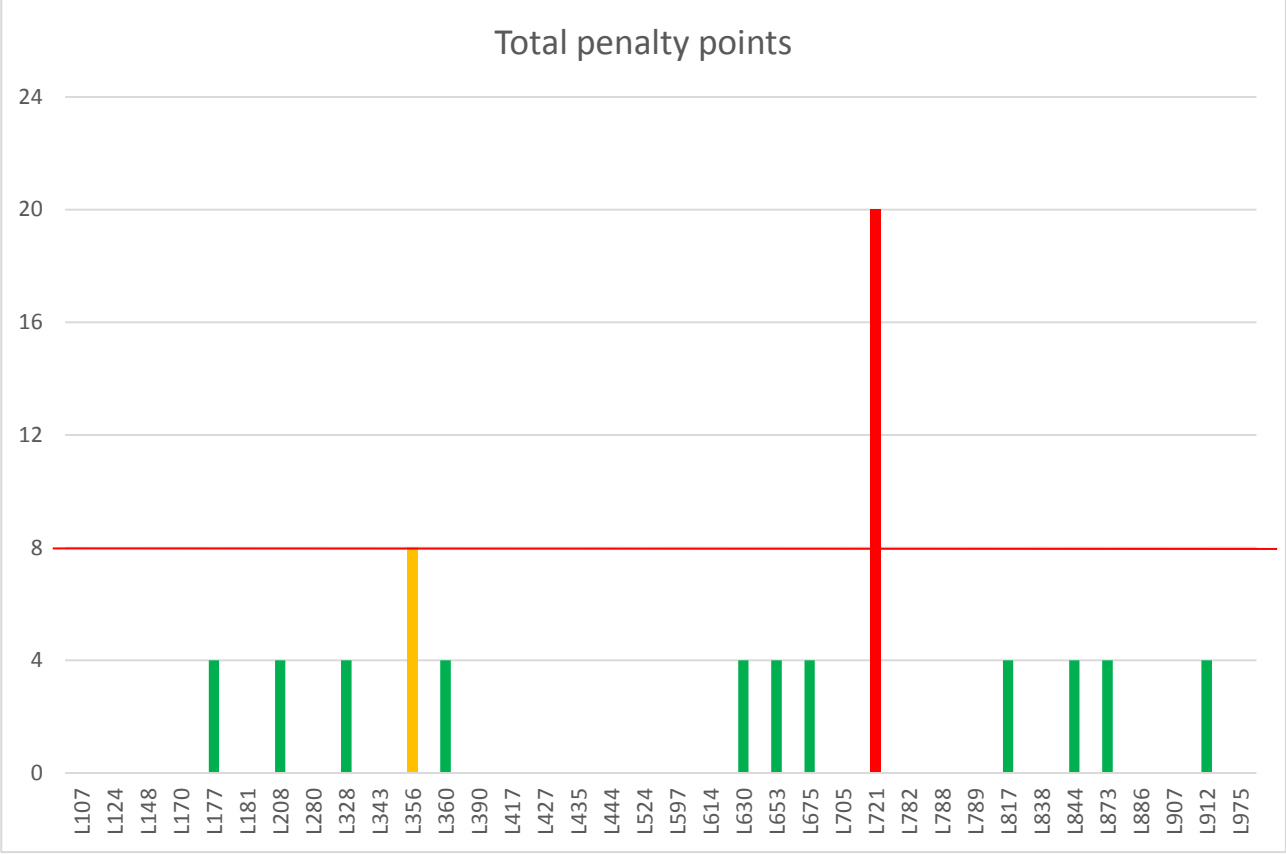

Figure 1. Evaluation of the NRL performance in the PT procedures. The score was calculated according to the criteria described in section 4.5. The orange bars indicate a light underperformance, the red bars indicate a severe underperformance. The performance of the NRLs that obtained a score higher than 8 was not considered as satisfactory.

Figure 2 shows the number of NRLs grouped according to their score. Two NRLs obtained a score higher than 8 and their performance was not considered as satisfactory.

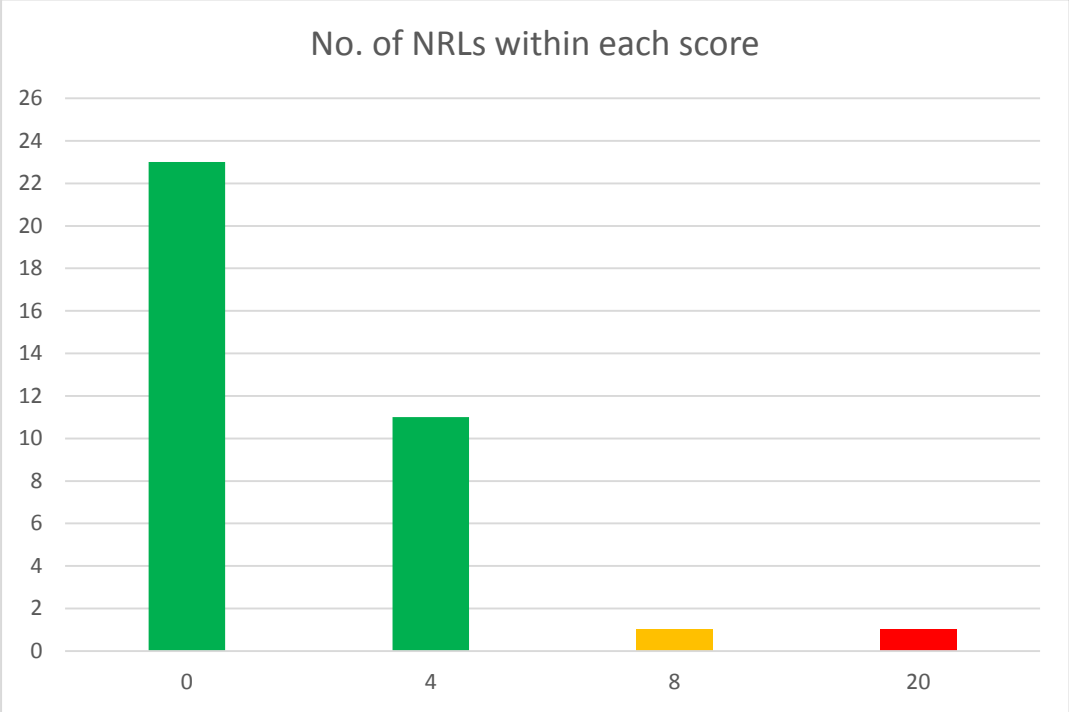

Figure 2. Number of NRLs within each penalty score. The score was calculated according to the criteria described in section 4.5. The orange bars indicate a light underperformance, the red bars indicate the NRLs that obtained a score higher than 8 and whose performance was not considered as satisfactory.

5.4. Evaluation of the performance of the methods

5.4.1. PCR screening step

The Sensitivity (Se) and Specificity (Sp) of the method was calculated for the detection of the *stx1* and *stx2* genes. The results provided by 35 of the 36 NRLs were considered for the performances of the *stx1*-PCR, excluding those of L721, which was considered as outlier. As a matter of fact, the analysis of the results provided by this NRL, clearly showed a general problem with the *stx1*-PCR. The results provided by 36 of the 36 NRLs were considered for the performances of the *stx2*-PCR.

The analysis of the results returned the following values:

- *stx1* PCR Se: 100 % (high level) and 100 % (low level).
- *stx2* PCR Se: 100 % (high level) and 100 % (low level).
- *stx1* PCR Sp: 97.2 %.
- *stx2* PCR Sp: 94.7 %.

5.4.2. STEC O91 isolation step

The Sensitivity of the isolation procedure was the following:

- Se: 78.3 % for both high and low levels of contamination level.

The limit of detection (LOD) calculated for the isolation step returned the following values:

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1		0,002	0,190	13,463	9,213	19,672	58,184	39,818	85,020	0,000

6. CONCLUDING REMARKS

The consolidation of the use of the ISO TS 13136 standard is crucial to ensure a harmonized approach to food testing for STEC throughout the EU. At the same time, the definition of the performance parameters of the standard itself is necessary to grant the NRLs and OLs an easier access to accreditation, which in turn ensures the comparability of the results produced by different laboratories in the different Member States. The EURLs play a central role in developing methods, in producing data on their performances as well as in assessing the proficiency of the NRLs in using them through the delivery of PT schemes.

This PT aimed at extending the scopes of the EURL-VTEC PT schemes concerning the couples food matrix/contaminating STEC with respect to both assessing the NRLs network proficiency and method's performances.

The PT17 was based on the analysis of ground beef contaminated with a STEC O91, bringing to eight the number of matrices analyzed so far and to seven the number of different STEC serogroups, including the "top five", plus O104 and O91. These serogroups together accounted for more than 70 % of the STEC human infections reported to ECDC in the 2013 and 2014 (EU summary report on zoonoses, zoonotic agents and food-borne outbreaks 2015). At the same time, the spectrum of food matrices analyzed so far, covers most of the epidemiologically relevant food commodities such as milk, vegetables including sprouts, water and beef meat.

The analysis of the results provided by the laboratories participating in the PT17 induces the following remarks:

1. Thirty-seven NRLs representing 26 EU Member States plus Chile, Egypt, Norway, Russia and Switzerland participated in the study, confirming the consolidation of the network of national laboratories for *E. coli*.
2. The participation of Chile for the first time indicates that the interest towards the EURL-VTEC activities is increasing outside the EU.
3. The results confirmed the suitability and fit for purpose of the method ISO TS 13136:2012. The presence of the STEC O91 virulence genes was identified correctly by 35 NRLs (97.2 %) in both samples B (low level of contamination) and C (high level

of contamination). The contaminating STEC O91 strain was isolated by 26 NRLs (72.2 %) both samples B (low level of contamination) and C (high level of contamination).

4. To evaluate the NRL performance, penalty points were assigned for all the incorrect results provided, with the exception of those related with the detection of *eae* and the lack of identification of the O91 serogroup, since this serogroup is *eae*-negative and this O-group falls outside the scope of the ISO TS 13136 standard. The detection of other serogroups in the enrichement cultures was not considered as an error, given the presence of natural microflora in the samples, which in some cases produced isolated strains as in the case of EPEC O26 and O145. Since the contamination level of sample B was identified as being below the LOD_{50} no penalties were assigned to the lack of isolation from this samples. As a whole, using these metrics, one out of the 36 NRLs that contributed results had an unsatisfactory performance, confirming that nearly all the European NRLs are able to detect STEC O91 contamination in beef minced meat.
5. The results of the study confirmed that the ISO TS 13136:2012 method represents a suitable tool for the detection of all the STEC sergroups analysed so far in the food commodity most regarded as vehicles of human infections.
6. One NRL isolated an EPEC O145, but failed to isolate the STEC present in the sample. This was probably due to the identification of this serogroup in the enrichment that has driven the strategy towards the isolation, by using an O145-specific IMS procedure or by following this trait in the screening of the suspected colonies. This observation suggests that a broad non-biased strategy should be adopted when testing food for STEC by following the *stx* genes.
7. The results of this PT allowed to determine the performance parameters of the method for the concerned couple matrix/STEC type, which sum to the other already determined in the previous PT rounds and will be made available through publication in the EURL-VTEC website in order to support the NRLs and Official Laboratories, which can refer to such parameters to have the method correctly accredited.