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Report of the 27th inter-laboratory study on the detection of Shiga toxin-producing *E. coli* (STEC) in herbs (PT27) - 2020

Edited by:

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1. OBJECTIVES AND DESIGN OF THE STUDY

The objective of Proficiency Test 27 (PT27) consisted in the examination of artificially contaminated basil samples, in order to enhance the preparedness of NRLs in testing herbs for the presence of STEC. The PT was organized according to the International Standard ISO/IEC 17043:2010 “Conformity assessment – General requirements for proficiency testing”.

The present document represents the full evaluation report of PT27.

2. PARTICIPANTS

NRLs were invited to take part to the inter-laboratory study and the 29 Laboratories who agreed were:

EU-NRLs

1. Austria, *Österreichische Agentur für Gesundheit und Ernährungssicherheit* GmbH
2. Belgium, Foodborne Pathogens/Unit Toxins and toxi-infections, Scientific Directorate Infectious Diseases in Humans (Sciensano)
3. Bulgaria, National Diagnostic and Research Veterinary Institute
4. Cyprus, Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services
5. Denmark, Section for Microbiology, The Danish Veterinary and Food Administration, Ringsted
6. Estonia, Veterinary and Food Laboratory
7. Finland, Finnish Food Safety Authority Evira
8. France, VetAgro Sup, LMAP/LNR/Equipe BPOE de l’UMR 5557 Ecologie Microbienne, Marcy L'Etoile
9. Germany, Bundesinstitut für Risikobewertung, German Federal Institute for Risk Assessment, Unit Food Microbiology, Host-Pathogen-Interaction, Department Biological Safety, Berlin
10. Hungary, National Food Microbiological Reference Laboratory
11. Italy, *Istituto Superiore di Sanità*
12. Latvia, Institute of Food Safety, Animal Health and Environment (BIOR)
13. Poland, National Institute of Public Health-National Institute of Hygiene, Warsaw
14. Poland, National Veterinary Research Institute (NVRI), Dept. Hygiene of Food of Animal Origin, Pulawy
15. Portugal, *Instituto Nacional de Investigação Agrária e Veterinária*, Vairão
16. Slovakia, Department of Food Hygiene, State veterinary and food institute, Dolný Kubín
17. Slovakia, NRC of Environmental Microbiology, Public Health Authority, Bratislava
18. Slovenia, Institute for food, feed and environment, Unit for food safety, University of Ljubljana, Veterinary Faculty
19. Spain, Microbiology Food Department, Agencia Española de Seguridad Alimentaria y Nutrición-Centro Nacional de Alimentación (CNA), Majadahonda (Madrid)
20. Spain, Bacteriology Department -2, Central Veterinary Laboratory-Animal health, Ministry of Agriculture, Fisheries and Food, Algete (Madrid)

21. Sweden, National Veterinary Institute (SVA)
22. Sweden, The National Food Agency
23. The Netherlands, National Institute for Public Health and the Environment (RIVM)

Non-EU NRLs

1. Norway, Section for food safety and antimicrobial resistance, Norwegian Veterinary Institute
2. Russia, State Research Center for Microbiology and Biotechnology, Obolensk
3. Switzerland, Agroscope
4. UK, Public Health England, FWEM Laboratory, London
5. UK, Public Health England, FWE Laboratory, Porton
6. UK, Public Health England, FWEM Laboratory, York

3. MATERIALS AND METHODS

3.1. Sample preparation

The basil used in the study was purchased from a local retailer.

The presence of a natural background microflora has been evaluated by plating on TSA and MacConkey agar serial dilutions of 25 g of basil homogenized in Buffered Peptone Water (BPW). No growth was observed on both media. Two samples consisting of 25 g of basil have been assayed for the presence of STEC according to the ISO TS 13136:2012 and both were negative.

Stability tests were conducted in September 2020 and the results obtained are reported in Table 1.

Table 1. Results obtained in the stability testing assays.

STEC O88 Concentration	T0 Replicate 1		T1 (3 days) Replicate 1		T2 (7 days) Replicate 1		T3 (10 days) Replicate 1		T4 (14 days) Replicate 1	
Test	RT PCR	Isolation	RT PCR	Isolation	RT PCR	Isolation	RT PCR	Isolation	RT PCR	Isolation
5 CFU/g	+	+	+	+	+	+	+	+	-	-
50 CFU/g	+	+	+	+	+	+	+	+	-	-

Three specimens, each consisting of 25 g of basil in sterile stomacher bags, potentially contaminated with STEC, were sent in the blind to the laboratories. The characteristics of the samples are reported in Table 2 and were considered as the gold standard for the analysis of the results.

Table 2: Characteristics of the samples included in the study

Contaminant (*Genotype*)	Contamination level in:		
	Sample 1	Sample 2 (interval)	Sample 3 (interval)
ED049 STEC O88 *(stx1+, stx2+)*	0 CFU	5 CFU/g (4.7-8.3)	50 CFU/g (47-83)

The contamination of the samples was carried out on 6th November 2020, using dilutions of an exponential liquid culture (0.5 OD read at 600 nm) of the STEC O88 strain ED049. An uncertainty of measurement of 0.22 log CFU/ml was associated to the standardized inoculum, using the procedure described in the ISO/TS 19036:2006.

When the test samples were prepared, two negative samples were tested, both giving the expected results. Six samples of the three contamination levels (n=18) were randomly selected for homogeneity testing and analyzed: all the samples examined gave the expected results.

The test samples were eventually labeled with randomly generated numerical codes different for each participating laboratory and shipped refrigerated on 9th November 2020 by courier. The NRLs were requested to start the analyses immediately upon receipt and to record the date of delivery and sample temperature upon reception.

3.4. Collection and elaboration of the results

The results were submitted through an on-line form prearranged by the EURL for *E. coli*. The instruction on how to report the results and the link to access the form was sent by E-mail to all the participants laboratories.

3.4.1. Evaluation of the NRL performance in the Real Time PCR screening step

The performance of each NRL in identifying the STEC target genes in the enrichment cultures was evaluated by assigning four penalty points to each incorrect or missing result concerning the identification of the *stx1* and *stx2* genes. Two penalty points have been

assigned to the laboratories identifying the presence of *eae* gene. The identification of one of the top five serogroups in the screening step also generated two penalties.

3.4.2. 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 from the enrichment cultures of the positive samples was evaluated by assigning two penalty points to the lack of isolation from sample 2 and 3 or to the isolation from sample 1.

As for strain characterization, four penalty points were assigned to each incorrect or missing result concerning the identification of the *stx1* and *stx2* genes, while two penalty points were assigned to each result indicating the presence of *eae* gene in the isolate. Finally, two penalty points were assigned to laboratories that reported the identification of a serogroup different from that of the strain used to contaminate the samples (O88). No penalty points were instead assigned to the laboratories reporting the serogroup of the STEC isolated strain as not typeable (ONT).

3.4.3. 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 laboratories that obtained a score higher than eight were considered as under-performant.

3.4.4. Evaluation of the performance parameters of the method

Sensitivity (*Se*) and Specificity (*Sp*) were calculated for the screening and isolation steps, respectively.

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

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

4. RESULTS

The test samples were sent to 27 laboratories and all of them returned the results.

One laboratory recoded the samples but failed in connecting the codes newly assigned to the original ones provided by the EURL for *E. coli* (L175). The results reported by this participant laboratory were thus excluded from the analysis.

The parcels containing the specimens were sent on the 9th November 2020 and were received in 24 hours by all the participating laboratories, with the exception of one laboratory receiving the parcel after 48 hours.

As far as the shipment conditions were concerned, the temperature at delivery ranged between 4 °C and 18 °C for most of the laboratories. Only one participant recorded the temperature of the parcel as room temperature and one didn't report this information.

The analysis of the results submitted by the participating laboratories are reported in Figures 1-3. Figures 1 represents graphically the proportion of laboratories correctly identifying the presence of STEC in samples.

Figure 1. Screening step: Proportion of Laboratories correctly detecting the presence or absence of STEC in the samples (green: correct result; red: incorrect result)**.** L776 has been excluded from the analysis

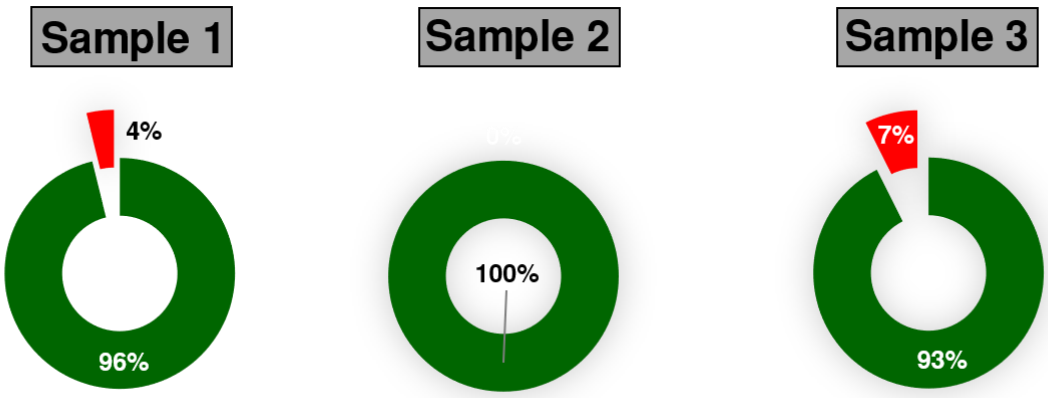


Figure 2. Real-time PCR detection of virulence and serogroup-associated genes in the enrichment cultures. Green boxes: correct results, red boxes: incorrect results. The true values are reported in yellow.

Figure 3. **Isolation and genotyping of STEC strains from basil samples**. Green boxes: correct results, ONT: the serogroup O88 was not identified in the isolated STEC strain, red boxes: incorrect results. The true values are reported in yellow.

	Sample 1
True Value	None
L136	
L157	
L187	
L203	
L229	
L240	
L258	
L286	
L295	
L337	
L376	
L421	
L424	
L519	
L537	
L543	
L556	
L676	
L695	
L737	
L810	
L825	
L840	
L843	
L896	
L967	

	Sample 2 - Lower level of contamination
True Value	O88 stx1+ stx2+ eae-
L136	ONT
L157	ONT
L187	ONT
L203	ONT
L229	ONT
L240	ONT
L258	ONT
L286	ONT
L295	ONT
L337	O91
L376	ONT
L421	ONT
L424	
L519	
L537	ONT
L543	ONT
L556	Not achieved
L676	
L695	ONT
L737	ONT
L810	
L825	ONT
L840	ONT
L843	ONT
L896	ONT
L967	

	Sample 3 - Higher level of contamination
True Value	O88 stx1+ stx2+ eae-
L136	ONT
L157	ONT
L187	ONT
L203	
L229	ONT
L240	ONT
L258	ONT
L286	ONT
L295	ONT
L337	O91
L376	ONT
L421	ONT
L424	
L519	
L537	ONT
L543	ONT
L556	O45
L676	
L695	ONT
L737	ONT
L810	
L825	ONT
L840	ONT
L843	ONT
L896	Isolation not achieved
L967	

The ***Se* and *Sp* parameters of the screening step calculated on the basis of the PT27 results** were as follows:

	***Se* (Lower level)**	***Se* (Higher level)**	***Sp***
stx1	100%	96.3%	96.3%
stx2	100%	96.3%	96.3%
eae	N. A.	N. A.	98.1%

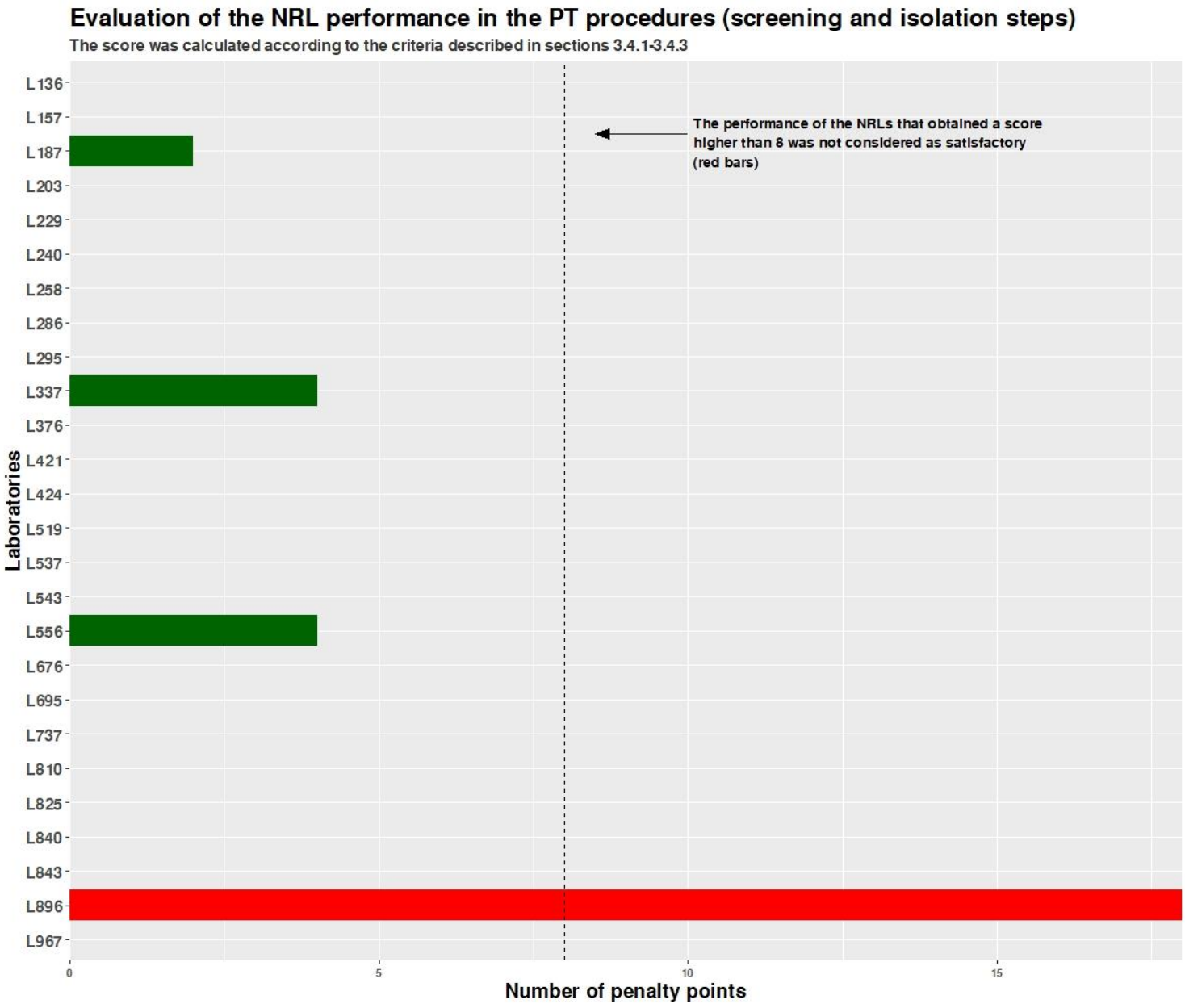
The ***Se* of the isolation step** has been calculated as **96.1%,** evaluated on the basis of the results provided by 25 laboratories for sample 2 and **96.3%** on the basis of the results provided by 26 participants for sample 3.

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 sections *3.4.1-3.4.3*.

Figure 4 shows the score achieved by each NRL. With the exception of one laboratory (L896), all the Laboratories complied the definition of satisfactory proficiency.

Figure 4



6. CONCLUDING REMARKS

1. A lower participation was recorded for PT27 compared with the previous rounds of PTs organized by the EURL for *E. coli*. However, considering the COVID-19 pandemic, the level of participation observed confirmed the eagerness of the network to collaborate in the EURL initiatives.
2. Only one participant was considered under-performant and will be contacted for follow up actions.
3. The identifcation of the O88 serogroup was problematic and only achieved by laboratories performing WGS confirming the usefulness of this approach. On the other hand this serogroup was not comprised in the field of application of the method ISO TS 13136 and thus has not been used to assess the proficiency of the laboratories in the determination of this feature.