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Report of the 33rd inter-laboratory study on the detection of Diarrhoeagenic *E. coli*, including STEC, in cheese (PT33) - 2022

Edited by:

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1. OBJECTIVES AND DESIGN OF THE STUDY

Proficiency Test 33 (PT33) consisted in the examination of artificially contaminated cheese samples, in order to enhance the preparedness of NRLs in testing cheese for the presence of diarrhoeagenic *E. coli*, including 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 PT33.

2. PARTICIPANTS

NRLs were invited to take part to the inter-laboratory study and 27 European Laboratories agreed:

1. Austria, *Österreichische Agentur für Gesundheit und Ernährungssicherheit* GmbH (AGES)
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. Ireland, Department of Agriculture, Food and the Marine
12. Italy, Istituto Superiore di Sanità
13. Latvia, Institute of Food Safety, Animal Health and Environment (BIOR)
14. Lithuania, National food and Veterinary Risk Assessment Institute
15. Netherlands, National Instutute for Public Health and the Environment (RIVM)
16. Netherlands,Wageningen Food Safety Research
17. Poland, National Veterinary Research Institute (NVRI), Dept. Hygiene of Food of Animal Origin, Pulawy
18. Portugal, *Instituto Nacional de Investigação Agrária e Veterinária*, Vairão
19. Romania, Institute for Hygiene and Veterinary Public Health
20. Slovakia, Department of Food Hygiene, State veterinary and food institute, Dolný Kubín
21. Slovakia, NRC of Environmental Microbiology, Public Health Authority, Bratislava
22. Slovenia, Institute for food, feed and environment, Unit for food safety, University of Ljubljana, Veterinary Faculty

23. Spain, Microbiology Food Department, Agencia Española de Seguridad Alimentaria y Nutrición-Centro Nacional de Alimentación (CNA), Majadahonda (Madrid)
24. Spain, Bacteriology Department -2, Central Veterinary Laboratory-Animal health, Ministry of Agriculture, Fisheries and Food, Algete (Madrid)
25. Sweden, National Veterinary Institute (SVA)
26. Sweden, The National Food Agency
27. The Netherlands, National Institute for Public Health and the Environment (RIVM)

3. MATERIALS AND METHODS

3.1. Samples preparation

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

The presence of natural background microflora was evaluated by plating on TSA and MacConkey agar serial dilutions of 25 gr of cheese homogenized in Buffered Peptone Water (BPW). No growth was observed on both media. Two samples consisting of 25 g of cheese have been assayed for the presence of STEC and Enteroaggregative *E. coli* (EAEC) and both were negative for the presence of these pathogens. Stability tests were conducted on May 2022 and the results obtained are reported in **Table 1**.

Table 1. Results obtained in the stability testing assays.

EAEC O104 Concentration	**T0**		**T1 (3 days)**		**T2 (7 days)**		**T3 (10 days)**	
Test	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**
2 CFU/g	+	+	+	+	+	+	+	+
STEC O78 Concentration	**T0**		**T1 (3 days)**		**T2 (7 days)**		**T3 (10 days)**	
Test	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**	**RT-PCR**	**Isolation**
2 CFU/g	+	+	+	+	+	+	+	+

Three specimens, each consisting of 25 g of cheese in sterile stomacher bags were sent in the blind to the participating 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

Cheese Samples	Contamination
Sample 1	Negative
Sample 2	2 CFU/g EAEC O104 *aggR, aaiC**
Sample 3	2 CFU/g STEC O78 *stx1***

*Uncertainty of measurement: 0.25 log CFU/ml
**Uncertainty of measurement: 0.4 log CFU/ml

The test samples were spiked on 24th of June 2022, using dilutions of an exponential liquid culture (0.5 OD read at 600 nm). The uncertainty of measurement associated to the standardized inoculum was evaluated using the procedure described in the ISO/TS 19036:2006.

The test samples were labeled with randomly generated numerical codes different for each participating laboratory and shipped refrigerated on 27th of June 2022 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 were sent by E-mail to all the participant laboratories.

3.4.1. Evaluation of the NRL performance

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* gene. Two penalty points have been assigned to the laboratories incorrectly reporting the presence of *eae*. In addition, two penalty points were assigned to each incorrect or missing result concerning the identification of the *aggR* and *aaiC* genes.

Two penalty points were assigned in case of lack of isolation of EAEC from sample 2 and of STEC from sample 3. As for EAEC and STEC strains' characterization, the same evaluation was used.

No penalty points were assigned to the laboratories reporting the serogroup of the STEC isolated strain as not typeable (ONT) as the serogroup of the isolate was not in the list of

the top 14. However, two penalty points were assigned to laboratories that reported the identification of a serogroup different from that of the STEC strain used to contaminate the samples (O78) when the serogroup identified fell in that list.
The serogroup of the EAEC strain was not the subject of the performance assessment.

3.4.2. 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.

4. RESULTS

The test samples were sent to 27 laboratories and 26 of them returned the results.
The parcels containing the specimens were sent on June 27th 2022 and were received in 24 hours by all the participating laboratories, with the exception of one laboratory receiving the parcel after 48 hours and one laboratory for which the shipment was repeated on July 4th 2022, because of a technical problem, and received in 24 hours.
As far as the shipment conditions were concerned, the temperature at delivery ranged between 4 °C and 25 °C for most of the laboratories. Four participants recorded the temperature of the parcel as room temperature.
The analysis of the results submitted by the participating laboratories are reported in **Figures 1-2**.

Figure 1. Real-time PCR detection of virulence genes in the enrichment cultures. Green boxes: correct results, red boxes: incorrect results. The true values are reported in yellow.

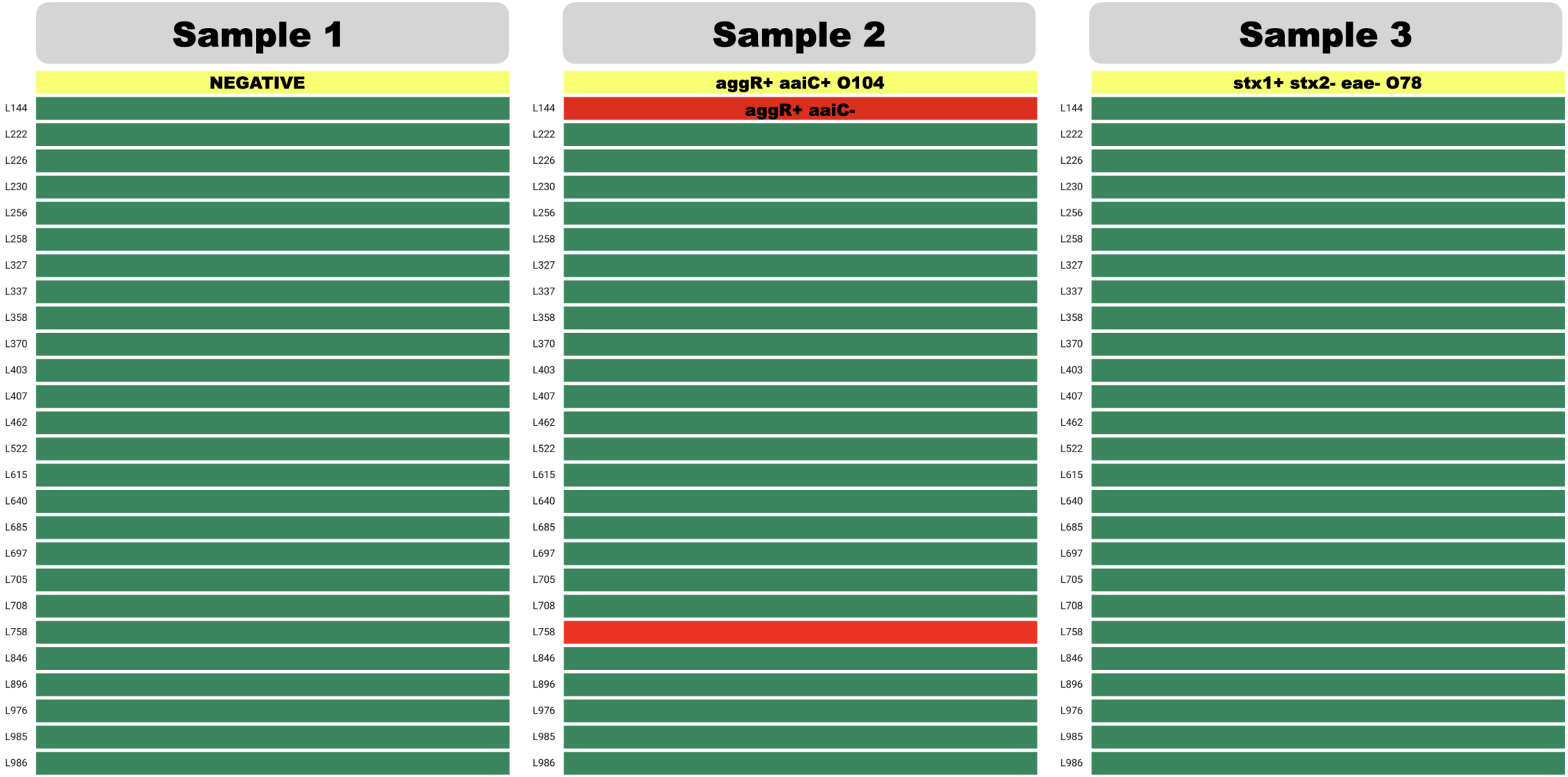
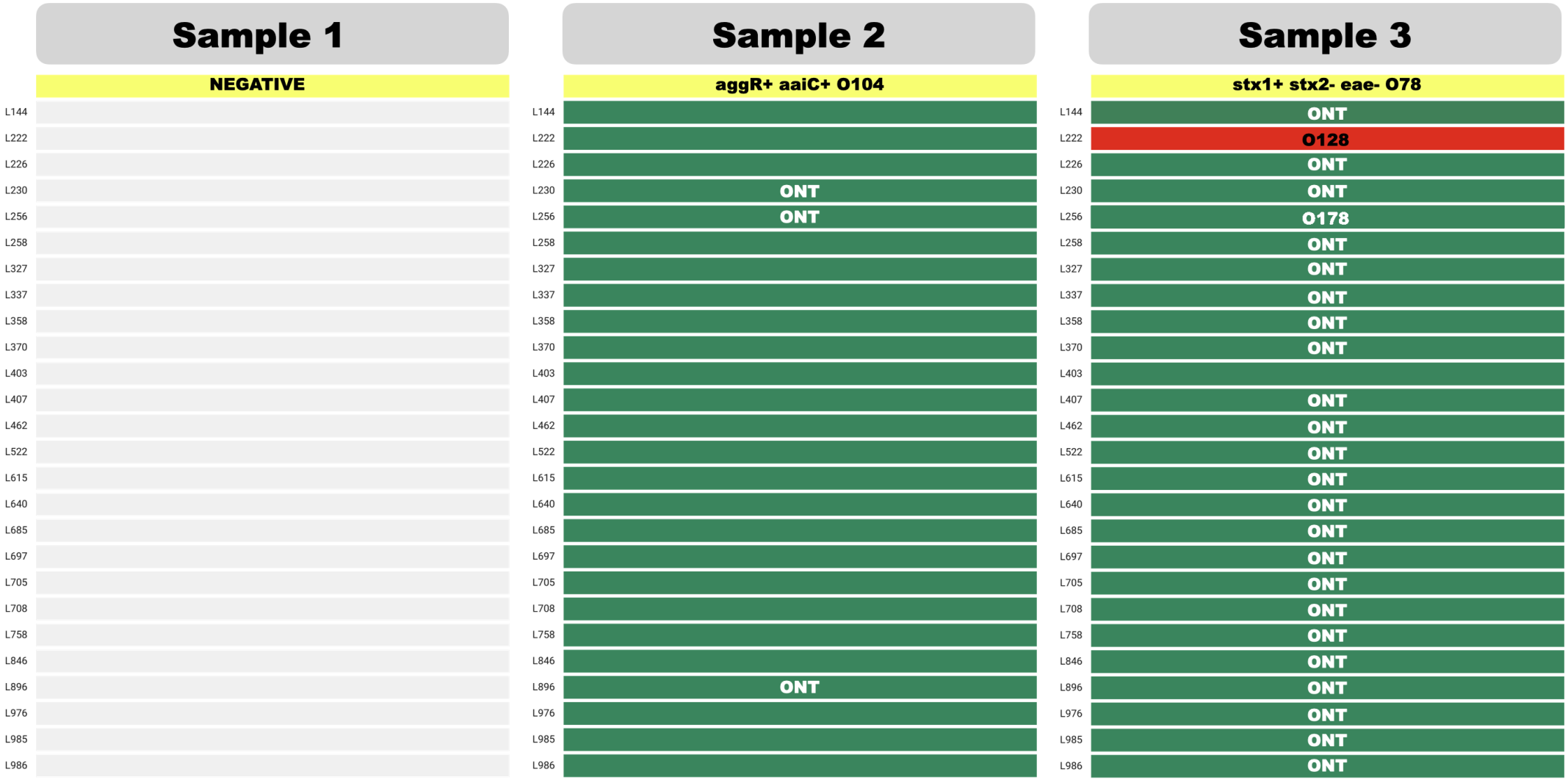


Figure 2. **Isolation and genotyping of STEC strains from cheese samples**. Green boxes: correct results, ONT: the serogroups O104 and O78 were not identified in the isolated strains, red boxes: incorrect results. The true values are reported in yellow.

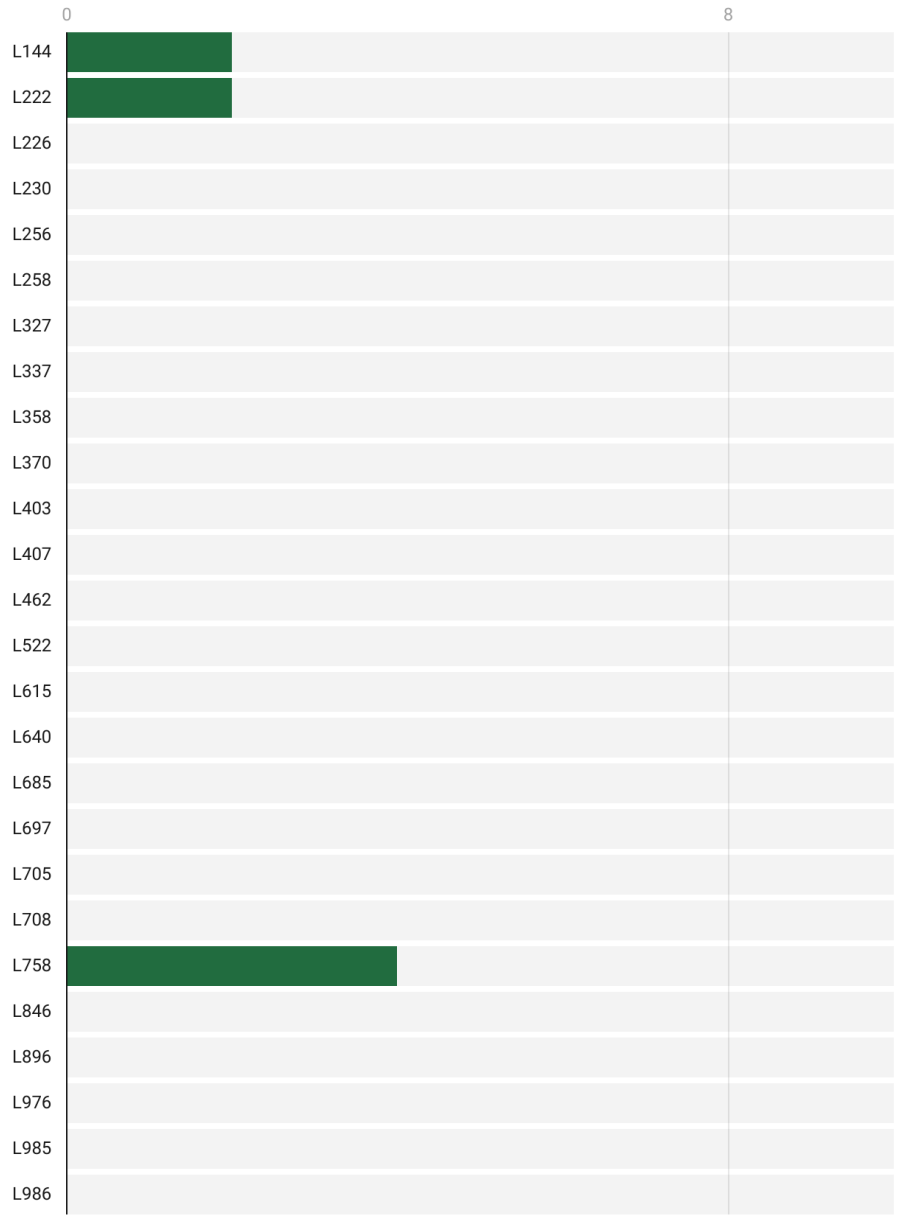


5.3. Evaluation of the NRL performance in the PT procedures

For each NRL, the number of penalty points was determined using the criteria described in sections *3.4.1-3.4.3*.

Figure 3 shows the score achieved by each NRL. All the Laboratories complied the definition of satisfactory proficiency.

Figure 3. **Evaluation of the NRLs performance in the PT procedures (screening and isolation steps).** The score was calculated according to the criteria described in sections 3.4.1-3.4.3. All the NRLs showed satisfactory performance, as none of them was assigned with 8 penalty points or more



6. CONCLUDING REMARKS

1. A high participation was recorded for PT33, which saw the participation of 26 NRLs.
2. No NRL was considered under-performant. This results indicates that the capability of the NRLs in identifying the STEC and other pathogenic *E. coli* virulence genes is higly satisfactory.
3. The identifcation of the O78 serogroup was problematic and achieved only by one participant. On the other hand this serogroup was not comprised in the top-14 serogroups and thus has not been used to assess the proficiency of the laboratories in the determination of this feature.