# Report of the 41st inter-laboratory study (PT41) on the identification and typing of pathogenic *E. coli* - 2024

**Edited by:**

*Valeria Michelacci, Arnold Knijn, Paola Chiani, Guendalina Fornari Luswergh, Federica Gigliucci, Theodora Socheri, Federica Melone, Fabio Minelli, Margherita Montalbano Di Filippo, Rosangela Tozzoli, Stefano Morabito*

## 1. OBJECTIVES OF THE STUDY

The objectives of PT41 were to assess the proficiency of the NRLs for *E. coli* in:

1. The detection of the main STEC virulence genes (*eae* and *stx* genes).
2. The identification of a range of relevant STEC serogroups (at least the 14 serogroups indicated in the EURL-VTEC_Method_003_rev2).
3. The detection of virulence genes of pathogenic *E. coli* other than STEC (*ipaH* for EIEC, *sth, stp* and *lt* for ETEC, *aggR* and *aaiC* for EAEC, methods available at the EURL for *E. coli* website).
4. Subtyping of Shiga Toxins (Stx)-coding genes.
5. The characterisation of a set of samples provided as genomic sequences and the identification of clusters of isolates based on genomic analysis (voluntary exercise).

This document represents the evaluation report of this study.

## 2. DESIGN OF THE STUDY

The study was designed according to the International Standard ISO/IEC 17043:2010 “Conformity assessment – General requirements for proficiency testing”.

PT41 was conducted on a set of six STEC strains and consisted of the following four mandatory sections:

1. The identification of the Shiga toxin-producing *E. coli* main virulence genes by PCR amplification. Participants were requested to detect the following targets:

- *stx1*, *stx2* and the intimin-coding *eae* gene.

2. The identification of virulence genes associated to other Diarrhoeagenic *E. coli* (DEC) pathotypes, and in particular: *ipaH* for EIEC, *sth, stp* and *lt* for ETEC, *aggR* and *aaiC* for EAEC

3. The determination of a range of relevant STEC serogroups. Participants were requested to identify the serogroup of the test strains assaying at least the following 14 serogroups, selected because of their epidemiologic or regulatory importance:

- O26, O103, O111, O145 and O157: the top 5 STEC serogroups, causing severe human infections worldwide.
- O45 and O121: epidemiologically relevant and regarded as adulterants in beef in the USA legislation.
- O104: relevant after the 2011 German outbreak.

- O55, O80, O91, O113, O128, O146: selected based on their prevalence in human infections in Europe in the last years, according to the data collected by the European Centre for Disease Prevention and Control (ECDC).

4. Subtyping of the s*tx* genes present in the STEC strains. Participants were requested to identify three subtypes of the s*tx1* gene (s*tx1a, stx1c* and *stx1d*) and seven of s*tx2* gene (from s*tx2a* to s*tx2g*).

In addition, a voluntary exercise on WGS data analysis was carried out in PT41. A set of genomic sequences from pathogenic *E. coli* strains other than those sent for PT41 were provided. Participants were asked to report on the quality of the eight sequences provided, on their characterisation in terms of serotype and virulence genes. In addition participants were asked to perform a correlation analysis including both the set of sequences provided and those of the six PT41 test strains, if produced by the participants. Either SNPs or cgMLST cluster analysis were accepted.

## 3. PARTICIPANTS

Thirty NRLs applied to participate in the study. Each NRL received its own individual laboratory numerical code, used to identify the laboratories in the results' tables.

The NRLs participating in the study were the following:

1. Austria, Austrian Agency for Health and Food Safety (AGES), Graz
2. Belgium, NRL STEC, institute of Public Health Sciensano, Brussels
3. Bulgaria, National Diagnostic and Research Veterinary Medical Institute/NDRMVI/, NRL"Listeria and Escherichia coli", Sofia
4. Croatia, Croatian Veterinary Institute, Laboratory for Food Microbiology, Zagreb
5. Cyprus, Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services
6. Denmark, Danish Veterinary and Food Administration, Ringsted
7. Estonia, National Centre for Laboratory Research and Risk Assessment (LABRIS)
8. Finland, Finnish Food Authority, Kuopio
9. France, VetAgroSup, LMAP/LNR E. coli STEC, Marcy-l'Etoile
10. Germany, Federal Institute for Risk Assessment (BfR), Berlin
11. Germany, Friedrich-Loeffer-Institut, Jena

12. Hungary, National Food Chain Safety Office, Food Chain Safety Directorate, Microbiological NRL, Budapest
13. Iceland, Matís ohf/Icelandic Food and Biotech R&D, Reykjavík
14. Ireland, Food Microbiology Division, Department of Agriculture, Food and the Marine, Celbridge Co. Kildare
15. Italy, Istituto Superiore di Sanità, Rome
16. Latvia, Institute of Food Safety, Animal Health and Environment "BIOR", Riga
17. Norway, Norwegian Veterinary Institute (NVI), Ås
18. Poland, National Institute of Public Health (NIH) - National Research Institute, Warsaw
19. Poland, National Veterinary Research Institute (NVRI), Department of Food Safety (previously Department of Hygiene of Food of Animal Origin), Pulawy
20. Romania, Institute for Hygiene and Veterinary Public Health, Bucharest
21. Slovakia, State Veterinary and Food Institute, Dolný Kubín
22. Slovakia, Public Health Authority of the Slovak Republic, Bratislava
23. Slovenia, University of Ljubljana, Veterinary Faculty, National Veterinary Institute
24. Spain, National Plant Health and Hygiene Laboratory, Lugo
25. Spain, Laboratorio Central de Veterinaria, Algete
26. Spain, Centro Nacional de Alimentación, Agencia Española de Seguridad Alimentaria y Nutrición (AESAN), Madrid
27. Sweden, Swedish Veterinary Agency (SVA), Uppsala
28. Switzerland, AGROSCOPE, Research Group of Bacteriological Food Safety, Bern
29. The Netherlands, National Institute for Public Health and the Environment (RIVM), Bilthoven
30. The Netherlands, Wageningen Food Safety Research, Wageningen

## 4. MATERIALS AND METHODS

### 4.1. Sample preparation

Six *E. coli* test strains (reported as strains 1 to 6 in Table 1a), selected among those present in the EURL-VTEC reference collections and checked for the presence of all the required genetic and/or phenotypic features, were sent to the NRLs.

The characteristics of the strains reported in Table 1a were considered as the gold standard. Table 1b reports the virulence genes detected by the WGS-based virulotyping performed at the EURL-VTEC.

The test strains were prepared on the 26$^{th}$ of September 2024, as fresh bacterial cultures seeded into soft (0.3 %) nutrient agar in borosilicate vials. The cultures were incubated 18 hours at 37°C ± 1°C and labelled with randomly generated numerical codes (3 digits), different for each set of strains sent to the NRLs. Previous data produced by the EURL-VTEC indicate that bacterial cultures prepared in this way are stable at least up to five weeks. On October the 2$^{nd}$, 2024, a homogeneity test was performed on six randomly selected sets of test strains. The

remaining test samples were stored at room temperature until October the 14th 2024, when the parcels were shipped to the participating laboratories by courier.

Additionally, as part of a voluntary exercise on WGS analysis, instructions to download a set of eight genomic sequences of as many *E. coli* strains were provided to the laboratories *via* email including the link to a shared folder containing raw WGS data in *fastq* format and a text file listing the MD5 checksum for the provided files, to allow confirmation of correct file transfer. The characteristics of the provided genomes are reported in Tables 2a and 2b.

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

| ID PT41 | Serotype | MLST | Virulence genes profile | *stx1* subtype | *stx2* subtype | Cluster |
|---|---|---|---|---|---|---|
| **Strain 1** | O146:H21 | ST442 | *stx1* | *stx1c* | - | No |
| **Strain 2** | O55:H9 | ST301 | *eae stx2* | - | *stx2f* | No |
| **Strain 3** | O145:H28 | ST137 | *eae stx1* | *stx1a* | - | No |
| **Strain 4** | O26:H11 | ST29 | *eae stx2* | - | *stx2a* | Yes |
| **Strain 5** | O86:H2 | ST10 | *aggR aaiC* | - | - | No |
| **Strain 6** | O124:H30 | ST6 | *ipaH* | - | - | No |

**Table 1b: Virulence genes detected in the test strains by WGS-based virulotyping**

| ID PT41 | Additional virulence genes |
|---|---|
| **Strain 1** | *celb, cia, ehxa, espi, gad, iha, irea, iss, iucc, iuta, kpse, lpfa, mchb, mchc, mchf, ompt, senb, suba, terc, tia, trat* |
| **Strain 2** | *cba, cia, cma, cvac, eae, efa1, ehxa, espa, espb, espf, espp, gad, hlyf, hra, iha, iron, iss, mchb, mchc, mchf, nlea, nleb, nlec, ompt, sita, terc, tir, trat* |
| **Strain 3** | *asta, cba, celb, chua, cif, efa1, ehxa, espa, espb, espf, espj, gad, iha, iucc, iuta, neuc, nlea, nleb, nlec, tccp, terc, tir, trat* |
| **Strain 4** | *asta, cba, cif, cma, eae, efa1, ehxa, espa, espb, espf, espj, etpd, fyua, gad, iha, iss, iucc, iuta, lpfa, nlea, nleb, ompt, tccp, terc, tir, trat* |
| **Strain 5** | *aaic, aap, aar, aata, afad, agg3b, agg3c, agg3d, agg5a, aggr, espi, gad, iha, iucc, iuta, kpse, kpsmii, orf3, orf4, pic, terc* |
| **Strain 6** | *capu, fyua, gad, iha, ipad, ipah9, iucc, iuta, pic, senb, siga, sita, terc, trat, virf* |

## Table 2a: Characteristics of the *E. coli* provided genomes (voluntary exercise)

| ID PT41 | Expected acceptability for sequence quality | Serotype | MLST | Virulence genes profile | *stx1* subtype | *stx2* subtype | Cluster |
|---|---|---|---|---|---|---|---|
| **SEQ_A** | Yes | O80:H2 | ST301 | *eae stx2* | - | *stx2d* | No |
| **SEQ_B** | Yes | O171:H2 | ST332 | *stx2* | - | *stx2b stx2c* | No |
| **SEQ_C** | Yes | O26:H11 | ST29 | *eae stx2* | - | *stx2a* | Yes |
| **SEQ_D** | Yes | O26:H11 | ST29 | *eae stx2* | - | *stx2a* | Yes |
| **SEQ_E** | Yes | O26:H11 | ST29 | *eae stx2* | - | *stx2a* | Yes |
| **SEQ_F** | Yes | O26:H11 | ST29 | *eae stx2* | - | *stx2a* | No |
| **SEQ_G**[1] | No | O26:H11 | ST21 | *eae stx2* | - | *stx2a* | No |
| **SEQ_H**[2] | No | O98:H21 | ST306 | *eae stx1* | *stx1a* | - | No |

[1] The sequence was not acceptable for analysis because the depth of coverage was 14x. For this reason, the characterisation and the cluster analysis for this strain were not requested
[2] The sequence was not acceptable for analysis because the genomic sequence of the *E. coli* strain was contaminated with sequences of *Enterobacter asburiae*. For this reason, the characterisation and the cluster analysis for this strain were not requested

## Table 2b: Virulence genes detected in the *E. coli* provided genomes (voluntary exercise)

| ID PT41 | Additional virulence genes |
|---|---|
| **SEQ_A** | *afaa, afab, afac, afad, afae8, cea, cia, cvac, eae, efa1, ehxa, espa, espb, espf, espp, etsc, gad, hlyf, hra, iron, iss, iucc, iuta, mchb, mchc, mchf, nlea, nleb, nlec, ompt, sita, terc, tir, trat* |
| **SEQ_B** | *asta, cia, espi, espp, gad, hra, iha, iss, lpfa, neuc, ompt, terc, trat* |
| **SEQ_C** | *asta, cba, cif, cma, eae, efa1, ehxa, espa, espb, espf, espj, etpd, fyua, gad, iha, iss, iucc, iuta, lpfa, nlea, nleb, ompt, tccp, terc, tir, trat* |
| **SEQ_D** | *asta, cba, cif, cma, eae, efa1, ehxa, espa, espb, espf, espj, etpd, fyua, gad, iha, iss, iucc, iuta, lpfa, nlea, nleb, ompt, tccp, terc, tir, trat* |
| **SEQ_E** | *asta, cba, cif, cma, eae, efa1, ehxa, espa, espb, espf, espj, etpd, fyua, gad, iha, iss, iucc, iuta, lpfa, nlea, nleb, ompt, tccp, terc, tir, trat* |
| **SEQ_F** | *cba, cif, cma, eae, efa1, ehxa, espa, espb, espf, espj, etpd, fyua, gad, iha, iss, iucc, iuta, lpfa, nlea, nleb, ompT, tccp, terc, tir, trat* |
| **SEQ_G** | *asta, cif, efa1, ehxa, espa, espb, espf, espj, espp, fyua, gad, iha, iss, iucc, katp, lpfa, nlea, nleb, nlec, terc, tir, toxb, trat* |
| **SEQ_H** | *asta, ehxa, espa, espf, espi, espp, fyua, gad, iha, iss, iucc, iuta, lpfa, nlea, nleb, nlec, ompt, tccp, terc, tir, trat* |

### 4.2. Laboratory methods

The laboratories were requested to identify the main STEC and other DEC virulence genes by PCR (conventional or Real Time PCR) using any method applied in the routine testing. Methods for all the assays were also available in the EURL-VTEC website.

The participants were also allowed to submit results obtained with WGS.

As far as the determination of the serogroups is concerned, participants were requested to identify the O-group of the test strains by testing at least for the following 14 serogroups: O26, O45, O55, O80, O91, O103, O104, O111, O113, O121, O128, O145, O146, and O157. Participating laboratories could choose to apply any serological or molecular method in use in their laboratories, including WGS. Procedures based on conventional or Real Time PCR for detecting the genes associated with the serogroups that were in the scope of the PT were made anyway available in the EURL website.

As for the *stx* genes subtyping, an end point PCR method for the identification of the *stx* gene subtypes of the STEC strains, based on the method described by Scheutz *et al.* (*J. Clin. Microbiol. 2012; 50: 2951-63*), is available in the EURL-VTEC website. The participating laboratories could choose to characterise the strains through WGS.

As part of the voluntary exercise on WGS data analysis, participants were asked to report on the quality of the eight sequences provided, on their characterisation in terms of serotype and virulence genes, as well as on their correlation based on analysis of the genomic sequences provided in addition to those of the six PT41 test strains, if produced by the participants. The methods used for these analyses were not indicated and each laboratory could use software and/or webservices of their choice. Cluster analysis could be carried out by SNPs or cgMLST analysis. The Laboratories taking part in this exercise were requested to provide the range of number of SNPs or allelic differences between the genomes forming a cluster. The laboratories also had the possibility to additionally provide a phylogenetic tree.

### 4.3. Collection and elaboration of the results

The results were submitted through a dedicated website developed by the EURL for *E. coli*. The deadline was set at January the 7th, 2025.

### 4.4. Evaluation of the NRLs performance in the identification of the *E. coli* virulence genes and the serogroups

The performance of each NRL in the identification of the characteristics of the *E. coli* test strains was evaluated by assigning penalty points for each incorrect result according to the following scheme:

- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx* genes.
- **2 penalty points** to each incorrect or missing result concerning the identification of the *eae* and the additional DEC virulence genes.
- **2 penalty points** to each incorrect result concerning the identification of the top-14 serogroups.
- **1 penalty point** when the results of the serogroup identification were not uploaded ("null" field) or reported as "Not Done". No penalty points were assigned to the missing identification of O86 serogroup for strain 5 and O124 for strain 6, as they were not included in the 14 serogroups indicated in the EURL-VTEC_Method_003_rev2.
- **1 penalty point** to each missing result or incorrect result concerning the identification of the *stx* genes subtypes.

No penalty points were assigned to incorrect results for the samples provided as genomic sequences (voluntary exercise).

The sum of the penalty points was used to assess the proficiency of the NRLs. A threshold of eight points was set and the laboratories presenting a higher score were considered as under-performant.

## 5. RESULTS

Results were submitted by 29 Laboratories. **Figure 1** shows the number of participating laboratories aggregated according to the methods used to characterise the isolates.

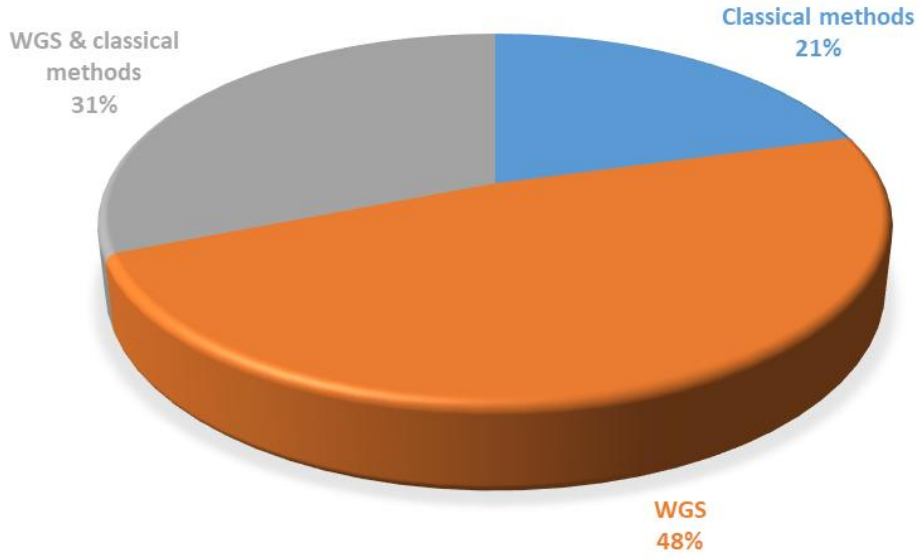


**Figure 1.** Methods applied by the Laboratories to type *E. coli* test strains

## 5.1. Characterisation of the test strains

The individual results reported by the participating laboratories on the characterisation of the test strains are reported in the tables below. The incorrect results are highlighted in red.

**Table 3a. Characterisation of test strain 1**

| Strain 1 | Virulence genes | Serogroup/serotype | *stx* genes subtyping | Penalties | WGS |
|---|---|---|---|---|---|
| Expected result | *stx1* | O146:H21 | *stx1c* | | |
| Labcode | Reported result | Reported result | Reported result | | |
| L002 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L004 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L006 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L007 | *stx1* | O146 | *stx1c* | 0 | * |
| L014 | *stx1* | O146 | *stx1c* | 0 | * |
| L016 | *stx1* | O146 | *stx1c* | 0 | |
| L017 | *stx1* | O146 | *stx1c* | 0 | |
| L018 | *stx1* | O146 | *stx1c* | 0 | |
| L035 | *stx1* | O146 | *stx1c* | 0 | * |
| L144 | *stx1* | O146 | *stx1c* | 0 | * |
| L222 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L256 | *stx1* | O146 | *stx1c* | 0 | * |
| L258 | *stx1* | O146 | *stx1c* | 0 | * |
| L327 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L337 | *stx1* | O146 | *stx1c* | 0 | |
| L370 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L403 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L462 | *stx1* | O146 | *stx1c* | 0 | |
| L615 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L674 | *stx1* | O146 | *stx1c* | 0 | * |
| L697 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L705 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L708 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L758 | *stx1* | O146:H21 | *stx1c* | 0 | * |
| L846 | *stx1* | - | - | 2 | |
| L972 | *stx1* | O146:HNT | *stx1c* | 0 | * |
| L976 | *stx1* | O146 | *stx1c* | 0 | |
| L986 | *stx1* | O146 | *stx1c* | 0 | * |
| L993 | *stx1* | O146 | *stx1c* | 0 | * |

## Table 3b. Characterisation of test strain 2

| Strain 2 | Virulence genes | Serogroup/serotype | *stx* genes subtyping | Penalties | WGS |
|---|---|---|---|---|---|
| **Expected result** | ***eae; stx2*** | **O55:H9** | ***stx2f*** | | |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | | |
| **L002** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L004** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L006** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L007** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L014** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L016** | *eae; stx2* | O55 | *stx2f* | 0 | |
| **L017** | *eae; stx2* | O55 | *stx2f* | 0 | |
| **L018** | *eae* | O55 | *stx2f* | 4 | |
| **L035** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L144** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L222** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L256** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L258** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L327** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L337** | *eae* | O55 | - | 5 | |
| **L370** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L403** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L462** | *eae; stx2* | O55 | *stx2f* | 0 | |
| **L615** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L674** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L697** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L705** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L708** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L758** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L846** | *eae* | - | - | 6 | |
| **L972** | *eae; stx2* | O55:H9 | *stx2f* | 0 | * |
| **L976** | *eae* | O55 | - | 5 | |
| **L986** | *eae; stx2* | O55 | *stx2f* | 0 | * |
| **L993** | *eae; stx2* | O55 | *stx2f* | 0 | * |

## Table 3c. Characterisation of test strain 3

| Strain 3 | Virulence genes | Serogroup/serotype | *stx* genes subtyping | Penalties | WGS |
|---|---|---|---|---|---|
| **Expected result** | ***eae; stx1*** | **O145:H28** | ***stx1a*** | | |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | | |
| **L002** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L004** | *eae; stx1* | O145:H | *stx1a* | 0 | * |
| **L006** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L007** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L014** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L016** | *eae; stx1* | O145 | *stx1a* | 0 | |
| **L017** | *eae; stx1* | O145 | *stx1a* | 0 | |
| **L018** | *eae; stx1* | O145 | *stx1a* | 0 | |
| **L035** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L144** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L222** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L256** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L258** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L327** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L337** | *eae; stx1* | OND | *stx1a* | 1 | |
| **L370** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L403** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L462** | *eae; stx1* | O145 | *stx1a* | 0 | |
| **L615** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L674** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L697** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L705** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L708** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L758** | *eae; stx1* | O145:H28 | *stx1a* | 0 | * |
| **L846** | *eae; stx1* | O145 | - | 1 | |
| **L972** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L976** | *eae; stx1* | O145 | *stx1a* | 0 | |
| **L986** | *eae; stx1* | O145 | *stx1a* | 0 | * |
| **L993** | *eae; stx1* | O145 | *stx1a* | 0 | * |

## Table 3d. Characterisation of test strain 4

| Strain 4 | Virulence genes | Serogroup/serotype | *stx* genes subtyping | Penalties | WGS |
|---|---|---|---|---|---|
| **Expected result** | ***eae; stx2*** | **O26:H11** | ***stx2a*** | | |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | | |
| **L002** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L004** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L006** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L007** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L014** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L016** | *eae; stx2* | O26 | *stx2a* | 0 | |
| **L017** | *eae; stx2* | O26 | *stx2a* | 0 | |
| **L018** | *eae; stx2* | O26 | *stx2a* | 0 | |
| **L035** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L144** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L222** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L256** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L258** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L327** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L337** | *eae; stx2* | O26 | *stx2a* | 0 | |
| **L370** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L403** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L462** | *eae; stx2* | O26 | *stx2a* | 0 | |
| **L615** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L674** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L697** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L705** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L708** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L758** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L846** | *eae; stx2* | O26 | - | 1 | |
| **L972** | *eae; stx2* | O26:H11 | *stx2a* | 0 | * |
| **L976** | *eae; stx2* | O26 | *stx2a* | 0 | |
| **L986** | *eae; stx2* | O26 | *stx2a* | 0 | * |
| **L993** | *eae; stx2* | O26 | *stx2a* | 0 | * |

## Table 3e. Characterisation of test strain 5

| Strain 5 | Virulence genes | Serogroup/serotype | *stx* genes subtyping | Penalties | WGS |
|---|---|---|---|---|---|
| **Expected result** | ***aggR; aaiC*** | **O86:H2[1]** | **-** | | |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | | |
| **L002** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L004** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L006** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L007** | *aggR; aaiC* | O86 | - | 0 | * |
| **L014** | *aggR; aaiC* | O86 | - | 0 | * |
| **L016** | *aggR; aaiC* | - | - | 0 | |
| **L017** | *aggR; aaiC* | Not found | - | 0 | |
| **L018** | *aggR; aaiC* | - | - | 0 | |
| **L035** | *aggR; aaiC* | O86 | - | 0 | * |
| **L144** | *aggR; aaiC* | O86 | - | 0 | * |
| **L222** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L256** | *aggR; aaiC* | O86 | - | 0 | * |
| **L258** | *aggR; aaiC* | O86 | - | 0 | * |
| **L327** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L337** | *aggR; aaiC* | OND | - | 0 | |
| **L370** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L403** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L462** | *aggR; aaiC* | - | - | 0 | |
| **L615** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L674** | *aggR; aaiC* | O86 | - | 0 | * |
| **L697** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L705** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L708** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L758** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L846** | *aggR; aaiC* | - | - | 0 | |
| **L972** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L976** | *aggR; aaiC* | - | - | 0 | |
| **L986** | *aggR; aaiC* | O86:H2 | - | 0 | * |
| **L993** | *aggR; aaiC* | O86 | - | 0 | * |

[1] No penalty points were assigned to the missing identification of O86 serogroup, as it was not included in the 14 indicated in the EURL-VTEC_Method_003_rev2.

## Table 3f. Characterisation of test strain 6

| Strain 6 | Virulence genes | Serogroup/serotype | *stx* genes subtyping | Penalties | WGS |
|---|---|---|---|---|---|
| **Expected result** | ***ipaH*** | **O124:H30[1]** | **-** | | |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | | |
| **L002** | *ipaH* | O124:H30 | - | 0 | * |
| **L004** | *ipaH* | O124:H30 | - | 0 | * |
| **L006** | *ipaH* | O124:H30 | - | 0 | * |
| **L007** | *ipaH* | O124 | - | 0 | * |
| **L014** | *ipaH* | O124 | - | 0 | * |
| **L016** | *ipaH* | - | - | 0 | |
| **L017** | *ipaH* | Not found | - | 0 | |
| **L018** | *ipaH* | - | - | 0 | |
| **L035** | *ipaH* | O124 | - | 0 | * |
| **L144** | *ipaH* | O124 | - | 0 | * |
| **L222** | *ipaH* | O124:H30 | - | 0 | * |
| **L256** | *ipaH* | O124 | - | 0 | * |
| **L258** | *ipaH* | O124 | - | 0 | * |
| **L327** | *ipaH* | O124:H30 | - | 0 | * |
| **L337** | *ipaH* | O 124 | - | 0 | |
| **L370** | *ipaH* | O124:H30 | - | 0 | * |
| **L403** | *ipaH* | O124:H30 | - | 0 | * |
| **L462** | *ipaH* | - | - | 0 | |
| **L615** | *ipaH* | O124:H30 | - | 0 | * |
| **L674** | *ipaH* | O124 | - | 0 | * |
| **L697** | *ipaH* | O124:H30 | - | 0 | * |
| **L705** | *ipaH* | O124:H30 | - | 0 | * |
| **L708** | *ipaH* | O124:H30 | - | 0 | * |
| **L758** | *ipaH* | O124:H30 | - | 0 | * |
| **L846** | *ipaH* | - | - | 0 | |
| **L972** | *ipaH* | O124:H30 | - | 0 | * |
| **L976** | *ipaH* | - | - | 0 | |
| **L986** | *ipaH* | O124:H30 | - | 0 | * |
| **L993** | *ipaH* | O124 | - | 0 | * |

[1] No penalty points were assigned to the missing identification of O124 serogroup, as it was not included in the 14 indicated in the EURL-VTEC_Method_003_rev2.

## 5.2 Characterisation of the test genomes provided as fastq files

Twenty-three laboratories participated in the voluntary exercise on the characterisation of eight additional samples provided as genomic sequences and the individual results provided are reported in the tables below. The incorrect results are highlighted in red.

**Table 4a. Characterisation of SEQ_A**

| SEQ_A | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **Yes** | ***eae; stx2*** | **O80:H2** | ***stx2d*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L004** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L006** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L014** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L017** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L018** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L035** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L144** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L222** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L258** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L327** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L370** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L403** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L462** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L615** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L674** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L697** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L705** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L708** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L758** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L972** | Yes | *eae; stx2* | O80:H2 | *stx2d* |
| **L986** | Yes | *eae; stx2* | O80 | *stx2d* |
| **L993** | Yes | *eae; stx2* | O80 | *stx2d* |

## Table 4b. Characterisation of test SEQ_B

| SEQ_B | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **Yes** | ***stx2*** | **O171:H2** | ***stx2b; stx2c*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L004** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L006** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L014** | Yes | *stx2* | O171 | *stx2b; stx2c* |
| **L017** | Yes | *stx2* | O171 | *stx2b; stx2c* |
| **L018** | Yes | *stx2* | O171 | *stx2b; stx2c* |
| **L035** | Yes | *stx2* | O171 | *stx2b; stx2c* |
| **L144** | Yes | *stx2* | O171 | *stx2d* |
| **L222** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L258** | Yes | *stx2* | O171 | *stx2b; stx2c* |
| **L327** | Yes | *stx2* | O171:H2 | *stx2b* |
| **L370** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L403** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L462** | Yes | *stx2* | O171 | *stx2b; stx2c* |
| **L615** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L674** | Yes | *stx2* | O171 | *stx2c* |
| **L697** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L705** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L708** | Yes | *stx2* | O171:H2 | *stx2b; stx2c* |
| **L758** | Yes | *stx2* | O171:H2 | *stx2c* |
| **L972** | Yes | *stx2* | O171:H2 | *stx2c* |
| **L986** | Yes | *stx2* | O171:H2 | *stx2c* |
| **L993** | Yes | *stx2* | O171 | *stx2b; stx2c* |

## Table 4c. Characterisation of test SEQ_C

| SEQ_C | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **Yes** | ***eae; stx2*** | **O26:H11** | ***stx2a*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L004** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L006** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L014** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L017** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L018** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L035** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L144** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L222** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L258** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L327** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L370** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L403** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L462** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L615** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L674** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L697** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L705** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L708** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L758** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L972** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L986** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L993** | Yes | *eae; stx2* | O26 | *stx2a* |

## Table 4d. Characterisation of test SEQ_D

| SEQ_D | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **Yes** | ***eae; stx2*** | **O26:H11** | ***stx2a*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L004** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L006** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L014** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L017** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L018** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L035** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L144** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L222** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L258** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L327** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L370** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L403** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L462** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L615** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L674** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L697** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L705** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L708** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L758** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L972** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L986** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L993** | Yes | *eae; stx2* | O26 | *stx2a* |

## Table 4e. Characterisation of test SEQ_E

| SEQ_E[1] | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **Yes** | ***eae; stx2*** | **O26:H11** | ***stx2a*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L004** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L006** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L014** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L017** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L018** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L035** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L144** | No | - | - | - |
| **L222** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L258** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L327** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L370** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L403** | Not done | - | - | - |
| **L462** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L615** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L674** | No | - | - | - |
| **L697** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L705** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L708** | No | - | - | - |
| **L758** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L972** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L986** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L993** | No | - | - | - |

[1] This genome was the only one provided as single-end sequence

## Table 4f. Characterisation of test SEQ_F

| SEQ_F | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **Yes** | ***eae; stx2*** | **O26:H11** | ***stx2a*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L004** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L006** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L014** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L017** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L018** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L035** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L144** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L222** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L258** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L327** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L370** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L403** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L462** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L615** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L674** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L697** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L705** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L708** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L758** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L972** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L986** | Yes | *eae; stx2* | O26 | *stx2a* |
| **L993** | Yes | *eae; stx2* | O26 | *stx2a* |

## Table 4g. Characterisation of test SEQ_G

| SEQ_G | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **No[1]** | ***eae; stx2*** | **O26:H11** | ***stx2a*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | No | - | - | - |
| **L004** | No | - | - | - |
| **L006** | No | - | - | - |
| **L014** | No | - | - | - |
| **L017** | No | - | - | - |
| **L018** | No | - | - | - |
| **L035** | No | - | - | - |
| **L144** | No | - | - | - |
| **L222** | No | - | - | - |
| **L258** | No | - | - | - |
| **L327** | Yes | *eae; stx2* | O26:H11 | *stx2a* |
| **L370** | No | - | - | - |
| **L403** | No | *eae; stx2* | O26:H11 | *stx2a* |
| **L462** | No | - | - | - |
| **L615** | No | - | - | - |
| **L674** | No | - | - | - |
| **L697** | No | - | - | - |
| **L705** | No | - | - | - |
| **L708** | No | - | - | - |
| **L758** | No | - | - | - |
| **L972** | No | - | - | - |
| **L986** | No | - | - | - |
| **L993** | No | - | - | - |

[1] The sequence was not acceptable for analysis because the depth of coverage was 14x. For this reason, the characterisation of this strain as well as its inclusion in the cluster analysis were not requested.

## Table 4h. Characterisation of test SEQ_H

| SEQ_H | Expected acceptability for sequence quality | Virulence genes | Serogroup/serotype | *stx* genes subtyping |
|---|---|---|---|---|
| **Expected result** | **No[1]** | ***eae; stx1*** | **O98:H21** | ***stx1a*** |
| **Labcode** | **Reported result** | **Reported result** | **Reported result** | **Reported result** |
| **L002** | No | - | - | - |
| **L004** | No | - | - | - |
| **L006** | No | - | - | - |
| **L014** | No | - | - | - |
| **L017** | Yes | *eae; stx1* | O98:H21 | *stx1a* |
| **L018** | No | - | - | - |
| **L035** | No | - | - | - |
| **L144** | No | - | - | - |
| **L222** | No | - | - | - |
| **L258** | No | - | - | - |
| **L327** | Yes | *eae; stx1* | O98:H21 | *stx1a* |
| **L370** | No | - | - | - |
| **L403** | No | *eae; stx1* | O88/O98:H21 | *stx1a* |
| **L462** | No | - | - | - |
| **L615** | No | - | - | - |
| **L674** | No | - | - | - |
| **L697** | No | - | - | - |
| **L705** | No | - | - | - |
| **L708** | No | - | - | - |
| **L758** | No | - | - | - |
| **L972** | No | - | - | - |
| **L986** | No | - | - | - |
| **L993** | No | - | - | - |

[1] The sequence was not acceptable for analysis because the genomic sequence of the *E. coli* strain was contaminated with sequences of *Enterobacter asburiae*. For this reason, the characterisation of this strain as well as its inclusion in the cluster analysis were not requested.

### 5.3 Cluster analysis

Twenty-two NRLs taking part in the voluntary WGS exercise participated also in the cluster analysis. Twenty NRLs performed the phylogenetic analysis on the test strains and the additional genomic sequences received for PT41, whereas two laboratories, which didn't carry out WGS on the test strains, performed cluster analysis on the sequences provided only. Figure 2 shows the methods used in the cluster analysis exercise, with the proportion of laboratories applying each method.

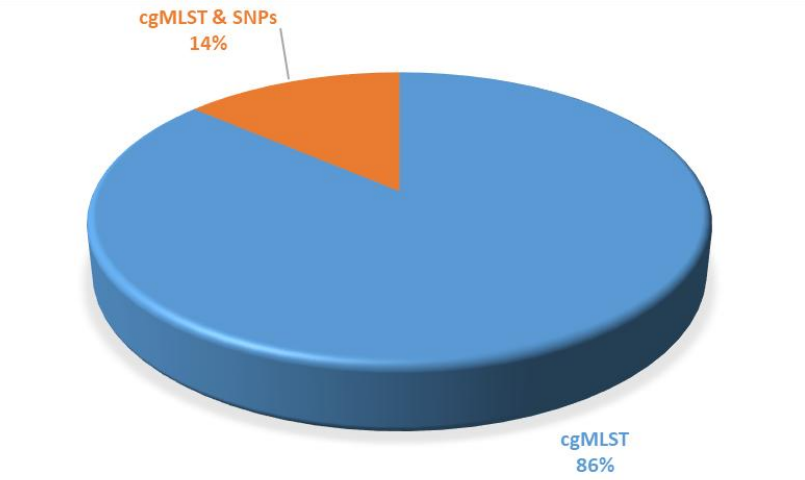


**Figure 2.** Methods applied by the 21 NRLs performing cluster analysis

The results of the cluster analysis exercise are reported in Table 5. The samples SEQ_G and SEQ_H had low quality and should not have been included in the cluster analysis exercise. For this reason, they are not included in Table 5. All the laboratories that compared the whole set of samples could correctly identify that strain 4 and SEQ_C were part of the same cluster. Nevertheless, only six laboratories correctly identified the complete cluster composed by test strain 4, SEQ_C, SEQ_D and SEQ_E.

## Table 5. Cluster analysis results

| | Strain 1 | Strain 2 | Strain 3 | Strain 4 | Strain 5 | Strain 6 | SEQ_A | SEQ_B | SEQ_C | SEQ_D | SEQ_E[1] | SEQ_F | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Expected result** | **No** | **No** | **No** | **Yes** | **No** | **No** | **No** | **No** | **Yes** | **Yes** | **Yes** | **No** | | |
| **Labcode** | **Reported results** | | | | | | | | | | | | **Distance** | **Method** |
| **L002** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | Yes | 1-9 AD | cgMLST |
| **L004** | No | No | No | Yes | No | No | No | No | Yes | Yes | ND[2] | No | 0-10 AD; 0-10 SNPs | cgMLST & SNPs |
| **L006** | No | No | No | Yes | No | No | No | No | Yes | Yes | No | No | 0-15 | cgMLST |
| **L014** | No | No | No | Yes | No | No | No | No | Yes | No | No | No | 0-5 AD | cgMLST |
| **L017[3]** | ND | ND | ND | ND | ND | ND | No | No | Yes | Yes | Yes | Yes | C-D-F (1-10 AD); C-D-E (1-9 AD) | cgMLST |
| **L018[3]** | ND | ND | ND | ND | ND | ND | No | No | Yes | Yes | Yes | No | 0-9 AD | cgMLST |
| **L035** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | No | 0-3 AD | cgMLST |
| **L144** | No | No | No | Yes | No | No | No | No | Yes | Yes | ND[2] | Yes | 0-11 | cgMLST |
| **L222** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | No | 3-17 | cgMLST |
| **L258** | No | No | No | Yes | No | No | No | No | Yes | No | No | No | 0-5 alleles | cgMLST |
| **L327** | No | No | No | Yes | No | No | No | No | Yes | Yes | No | Yes | 0-15 AD | cgMLST |
| **L370** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | No | 0-7 AD | cgMLST |
| **L403** | No | No | No | Yes | No | No | No | No | Yes | No | ND | No | 0-5 AD | cgMLST |
| **L615** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | Yes | 1-12 AD | cgMLST |
| **L674** | No | No | No | Yes | No | No | No | No | Yes | Yes | ND | Yes | 0-29 AD; 0-46 SNP | cgMLST & SNPs |
| **L697[4]** | No | No | No | No | No | No | No | No | Yes | Yes | Yes | No | 1-9 AD | cgMLST |
| **L705** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | No | 0-1 | cgMLST |
| **L708** | No | No | No | Yes | No | No | No | No | Yes | Yes | ND[2] | No | 0-10 AD | cgMLST |
| **L758** | No | No | No | Yes | No | No | No | No | Yes | Yes | No | No | 0-5 AD | cgMLST |
| **L972** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | No | 0-10 AD | cgMLST |
| **L986** | No | No | No | Yes | No | No | No | No | Yes | Yes | Yes | No | 0-10 | cgMLST |
| **L993** | No | No | No | Yes | No | No | No | No | Yes | Yes | ND[2] | No | 0-11 AD; 0-12 SNPs | cgMLST & SNPs |

Red boxes: incorrect results; Orange boxes: cluster was not detected because the strain or the sequence were not analysed; ND: Not Done; AD: Allelic differences; [1]SEQ_E was the only single-end sequence; [2] L004, L144, L708 and L993 commented that sequence quality was not good enough for cluster analysis; [3] L017 and L018 performed cluster analysis only among the sequences received from the EURL; [4] L697 performed cluster analysis separately among the two sets of samples (received as strains and received as genomic sequences)

## 6. Evaluation of the proficiency of the participating Laboratories

The proficiency of the Laboratories has been assessed as described in 4.4. The participating laboratories presenting a score higher than eight were considered as under-performant (red bars in **Figure 3**).

**Figure 3.** Number of penalty points per NRL

## 7. CONCLUDING REMARKS

1. A high level of participation was recorded, proving the willingness of NRLs in participating in the EURL for *E. coli* PT scheme.
2. WGS was carried out by 79% of the laboratories participating in the study, most of which exhibited an excellent performance, confirming the high level of adoption of NGS in the NRLs for *E. coli* network and the efficacy of this approach.
3. The proficiency of all the participating laboratories was satisfactory, with the only exception of L846, that accumulated most of the penalty points due to lack of reporting of *stx* subtyping results. Such laboratory explained in the notes they had a problem in performing WGS on time and for this reason didn't have results on *stx* subtyping.
4. Three laboratories, using classical methods, missed the identification of *stx2f* subtype in strain 2 and one additional laboratory correctly identified *stx2f* subtype, but failed to report positivity for *stx2*. Such subtype is not detected by the Real Time PCR described in ISO TS 13136, but it is in the scope of a dedicated method developed by EURL for *E. coli* (EURL-VTEC_Method_10) and its identification is relevant as outbreaks and sporadic cases of human disease associated with such strains, including Hemolytic Uremic Syndrome cases, have been described.
5. All laboratories could correctly identify the virulence genes characteristic of Enteroaggregative and Enteroinvasive *E. coli* present in strains 5 and 6, respectively, regardless the method used.
6. A total of 22 NRLs participated in the cluster analysis exercise, including two laboratories (L017 and L018) that did not performed WGS on the set of samples delivered as bacterial strains live cultures, demonstrating a high level of interest of the network for this exercise.
7. The two participants, L017 and L018, that didn't perform WGS on the test strains and couldn't include them in the cluster analysis, obviously missed the identification of strain 4 as belonging to the cluster.
8. All the participating NRLs correctly identified the unacceptable quality of sequences SEQ_G and SEQ_H, with the only exception of two laboratories. On the other hand, four laboratories reported unacceptable quality for SEQ_E, which was instead a good quality sequence produced with a single end sequencing protocol.

9. All the 23 participating NRLs correctly performed the characterisation of all the provided sequences, with only three exceptions in the identification of two *stx* subtypes in the same sample (SEQ_B), once more highlighting a very good performance of the labs in genomic analysis of *E. coli* strains.
10. Four NRLs (L004, L144, L708 and L993) commented that sequence quality of sample SEQ_E was not good enough for cluster analysis. Such sample was the only single end sequence provided and when analysed at EURL *E. coli* it resulted not contaminated, had 163x coverage, and showed very good statistics with chewBBACA tool for cgMLST analysis (2345 exact matches). Optimization of pipelines for the analysis of single end sequences may be needed by these laboratories.
11. Several NRLs had problems in identifying the complete cluster, mainly incorrectly including in the cluster SEQ_F which belonged to the same serogroup (O26) or because of lack of analysis of SEQ_E, which was the only single-end sequence provided. Attention will be posed to these topics in training courses dedicated to WGS analysis.