Report of the 39th inter-laboratory study on the detection of Shiga toxin-producing *E. coli* (STEC) in cheese (PT39) – non-EU NRLs – 2024

Edited by:

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1. INTRODUCTION

PT39 consisted in the detection of STEC in cheese samples, with the aim of consolidating the preparedness of the NRLs in testing such matrix 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”, and this document represents the full evaluation report of PT39 for the NRLs from non-EU Countries.

2. DESIGN AND OBJECTIVES OF THE STUDY

The study consisted in the examination of artificially contaminated cheese samples and the **objectives** were:

- to improve the preparedness of the NRLs towards testing cheese samples for the detection and isolation of STEC according 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 O157 serogroup;
- to give support to the NRLs for the accreditation of the ISO TS 13136:2012.

3. PARTICIPANTS

Three NRLs from non-EU Countries participated in the study. The participating laboratories are listed below:

- ✓ Macedonia, Faculty of Veterinary Medicine Skopje
- ✓ Egypt, The Central Laboratory of residues analysis of pesticides and heavy metals in food
- ✓ UK, United Kingdom Health Security Agency (UKHSA), Porton
- ✓ UK, United Kingdom Health Security Agency (UKHSA), York

After reporting the results, each NRL received its own individual report of participation indicating the expected and the reported results per each sample.

4. MATERIALS AND METHODS

4.1. Sample preparation

The cheese 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 225 ml of 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 by applying the ISO TS 13136:2012 method. Both samples were negative for all the target genes at the screening.

Two test samples (1 and 2), each consisting of 25 g of cheese potentially contaminated with STEC, were sent in the blind to the NRLs. The characteristics of the contaminated samples are reported in Table 1.

The artificial contamination of the samples was carried out on 17 May 2024, using appropriate dilutions of an exponential liquid culture (0.5 OD read at 600 nm) of the STEC strain C1188-02. An uncertainty of measurement of 0.209 log CFU/ml was associated to the standardized inoculum, calculated using the procedure described in the ISO TS 19036:2006. Serial dilutions of the inoculum suspensions of strain C1188-02 used to spike the samples were streaked onto MacConkey agar plates to confirm the titer.

The test samples were labeled with randomly generated numerical codes, different for each laboratory, and stored at 4°C until shipped refrigerated on 20 May 2024 by courier. The NRLs were requested to record date of delivery and sample temperature upon reception and to start the analyses immediately upon receipt.

Table 1: Characteristics of the cheese samples assessed in the study

Contaminant (*Genotype*)	**Contamination level in:**	
	Sample 1	**Sample 2**
Strain C1188-02, STEC O26:H11 *(stx1+, stx2+, eae+*)	-	2 CFU/25 g

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.

Stability was assessed using samples spiked on 13 February 2024 and tested by ISO TS 13136:2012 after 0, 2, 6, and 9 days since the initial contamination. The Real Time PCR screening for the STEC target genes and isolation were both successful for all the samples spiked at all the time points.

Six specimens were randomly selected for homogeneity testing among the spiked samples prepared for shipment. All were enriched at 37°C and analyzed by Real Time PCR for the presence of the contaminating STEC on 20 May 2024. The Real Time PCR screening for

the STEC target genes was positive, and the O26 STEC strain was isolated from all six replicates. Similarly, all the six replicates selected from non-spiked samples were negative for the presence of STEC.

4.3. Laboratory methods

Laboratories were requested to identify the presence of STEC by applying the ISO TS 13136:2012 method.

4.4. Collection and elaboration of the NRL results

The results were submitted through a dedicated Microsoft Form. The participating laboratories had to indicate in the Form their Lab code, provide the information on the arrival date, temperature, and quality of the samples, as well as the results obtained for each test of the blind samples.

4.5. Analysis of the NRL results

4.5.1. Scoring of the Real Time PCR screening step

The results reported were categorized by assigning penalty points. Four penalty points to each incorrect or missing result concerning the identification of *stx1* and *stx2* genes, and two penalty points for the incorrect identification of *eae* gene as well as the top-5 serogroups-associated genes.

4.5.2. Scoring of the isolation and characterization of the STEC strains isolated from the PCR-positive enrichment cultures

The results reported were categorized by assigning two penalty points in case of lack of isolation of STEC from sample 2. As for strain characterization, four penalties have been assigned to incorrect detection of Stx-coding genes, two penalties to lack of identification of *eae* gene, and two for the identification of a serogroup different from that of the STEC strain used to contaminate the sample (O26).

4.5.3. Evaluation of the NRL performance

The sum of the penalty points obtained in the different steps of the procedure originated a total score, used to evaluate the performance of the NRLs in the PT. Laboratories that obtained a score higher than eight were considered underperforming.

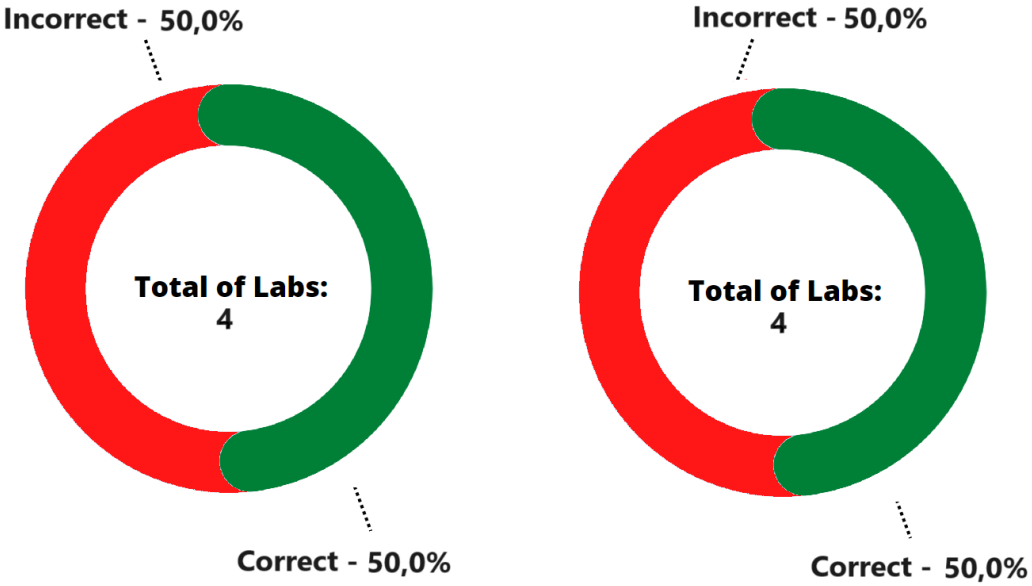
5. RESULTS

All the four Laboratories returned results via the web platform.

The parcels containing the specimens were sent on the 20th of May 2024 and were received by two participants on May the 23rd, while the samples were delivered to the remaining two labs on the 25th and 27th of May 2024 (L563 and L987, respectively).

The reported temperature at delivery was > 15 °C for all the participating NRLs (ranging from 15.1°C to 28°C), likely due to the fact that the parcels were not received promptly for custom clearance process. Three Laboratories reported that the conditions of the test samples were good, and one fair. The results submitted by the participating laboratories are summarized in **Figures 1 – 3**.

Figure 1. Percentage of Laboratories reporting the correct results for the screening of samples (a) and isolation of the STEC strain (b) (green: correct result; red: incorrect result).



Screening Step: % of Laboratories correctly detecting STEC in the spiked sample (green: correct results; red: incorrect results).

Isolation Step: % of Laboratories that successfully isolated the STEC strain detected in the screening step (green: correct results; red: incorrect results).

Figure 2. Real-time PCR detection of virulence and serogroup-associated genes in the enrichment cultures per laboratory (values in the yellow boxes are the gold standards; green boxes: correct results; red boxes: incorrect results).

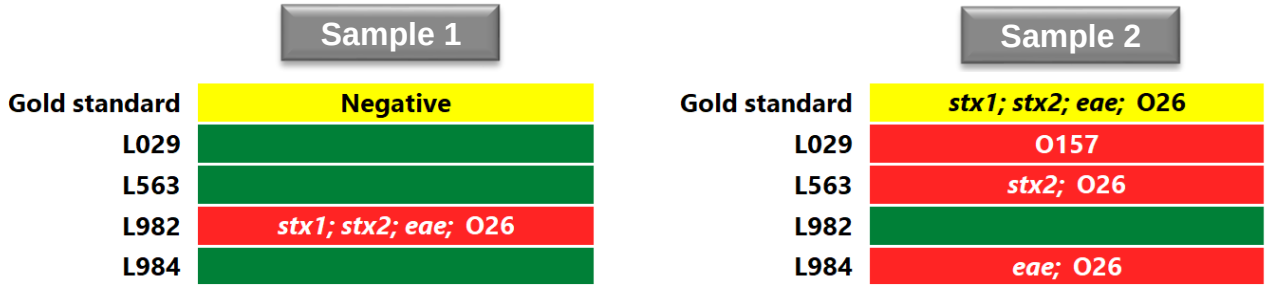
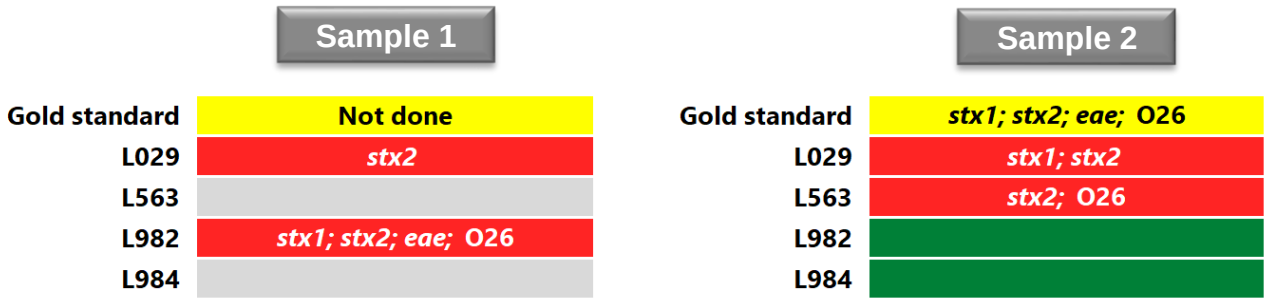
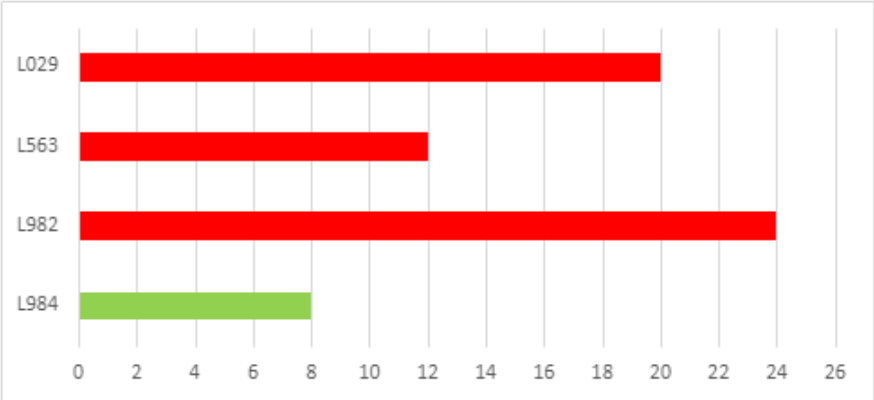


Figure 3. Isolation and genotyping of STEC strains from the cheese samples (values in the yellow boxes are the gold standards; green boxes: correct results; red boxes: incorrect results).



For each NRL, the number of penalty points was determined using the criteria described in section 4.5. **Figure 4** shows the score achieved by each NRL. Only one Laboratory showed a satisfactory performance.

Figure 4. Evaluation of the NRLs performance.



6. CONCLUDING REMARKS

The consolidation of the use of the ISO TS 13136:2012 standard is crucial to ensure a harmonized approach to food testing for STEC throughout the EU.

PT39 concerned the application of the ISO TS 13136:2012 on cheese samples.

The analysis of the results provided by the four NRLs from non-EU Countries participating in PT39 induces the following conclusions:

1. The majority of the participants obtained scores corresponding to unsatisfactory performance.

2. One laboratory (L982) detected the presence of STEC and could isolate it from both test samples. This NRL reported in the notes field that it was evident that the the amount of STEC in one of the two saples was much higher than the amount in the other, suggesting that cross contamination may have occurred.
3. The remaining three laboratories provided incorrect results concerning the detection of the virulence genes either in the screening step, or in the isolated STEC strain. In addition, one laboratory mis-detected the serogroup-associated gene in the scrrening.
4. It has to be considered that the participants received the test samples in a range of 3 to 7 days from the shipment, the latter being at the limit or out of the estimated range of stability (9 days). The samples were received not refrigerated, but all the NRLs reported to have isolated a STEC strain from one of the two samples, suggesting that the samples were still stable for the analysis.
5. The possibility to attend training stages is open to non-EU NRLs too, and the underperforming Laboratories may exploit the participation to these trainings to fix technical problems they may encounter when dealing with STEC analyses.