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Report of the sixth inter-laboratory study on the enumeration of *E. coli* (PT44) – 2025

Edited by:

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1. INTRODUCTION AND OBJECTIVES OF THE STUDY

This exercise was intended to provide proficiency testing (PT) samples to the laboratories performing the analysis of live bivalve molluscs from production areas in accordance with Regulation (EC) N° 854/2004 and from throughout the production chain in accordance with Regulation (EC) N° 2073/2005.

PT44 employed a freeze-dried mixed culture composed by the *beta*-glucoronidase positive ATCC strain 25922 and a field isolate of a *beta*-glucoronidase negative *E. coli* strain, to be assayed with the ISO 16649-3 ***"Microbiology of the food chain - Horizontal method for the enumeration of β-glucuronidase-positive* Escherichia coli *Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide"***.

This document represents the evaluation report of the PT44 study. The study was conducted according to the International Standard ISO/IEC 17043:2010 "Conformity assessment – General requirements for proficiency testing".

2. PARTICIPANTS

Twenty-five NRLs, representing 16 EU Member States plus two EFTA countries and one third country, accepted the invitation to participate. The participation of the Third Country laboratory was self-supported.

Each NRL was assigned to an individual laboratory numerical code, which was used to label the laboratories in the results tables.

The Laboratories participating in the study were:

Belgium	Laboratory of Foodborne Pathogens (SCIENSANO)
Bulgaria	National Diagnostic and Research Veterinary Institute (NDRVMI)
Croatia	Croatian Veterinary Institute HVI - VETERINARSKI ZAVOD SPLIT
Croatia	Laboratory for Food Microbiology, Croatian Veterinary Institute
Cyprus	Laboratory for the Control of Food of Animal Origin (LCFAO)
Denmark	Microbiological laboratory Ringsted (FVST)
Germany	NRL *E. coli*, Bundesinstitut für Risikobewertung (BfR)
Greece	Department of Food Hygiene of Athens (NRL Greece for *E.coli* in LBM)
Greece	Veterinary Laboratory of Kavala
Greece	NRL-*Salmonella*, The Veterinary Laboratory of Chalkida, Hellenic Ministry of Rural Development and Food
Iceland	Matís ohf /Icelandic Food and Biotech R&D
Ireland	Shellfish Microbiology Unit, Marine Institute (MARINE)
Italy	Istituto Superiore di Sanità - ISS

Italy	IZS Umbria e Marche, Sezione di Ancona
Netherlands	RIVM
Norway	Institute of Marine Research - HMR
Poland	National Veterinary Research Institute (NVRI)
Romania	Institute for Diagnoses and Animal Health (IDAH)
Slovakia	State veterinary and food institute - SVPU
Slovenia	University of Ljubljana, Veterinary Faculty (Unit for food safety)
Spain	Centro Nacional de Alimentación - AESAN
Spain	National Plant Health Laboratory
Sweden	Swedish Food Agency, The Biology department
UK	CEFAS

We report on the analysis of twenty-four laboratories that submitted results. One didn’t return the results (L674).

3 MATERIALS AND METHODS

3.1. Sample preparation

The freeze-dried cultures were prepared as follows: a mix of two live cultures of *E. coli* strains composed by the ATCC-25922 and a *beta*-glucoronidase negative strain (ECOR6) was prepared by refreshing each culture separately and let grow until reaching the concentration of 10^8 cells/ml. The cultures were centrifuged and the pellets re-suspended in 5% sucrose to the final concentration of 10^9/ml, each. 800 µl of a mixture of the sucrose re-suspended cultures in the proportions of 99,5% of strain ECOR6 and 0,5% of ATCC 25922 strain was added to a lyophilization vial and frozen at -20 °C for two days. The vials were then freeze-dried into an "Alpha 1-2 LSC Basic" lyophilizer apparatus for three days under the following conditions: (i) -60°C; (ii) 0.01 mbar. The vials were eventually sealed. A random selection of the samples was assayed using the Part 3 of the ISO 16649 method to determine the MPN of the ATCC 25922 *beta*-glucoronidase positive strain.

On 25th of November 2025, the samples were shipped to the participating laboratories by courier. The participants were requested to reconstitute the lyophilized culture with 1 ml of TSB (Tryptone Soya broth) and proceed according to ISO 16649 part 3.

3.2. Collection and Elaboration of the NRLs Results

The results were submitted using the on-line service of the EURL for *E. coli*. The participants were requested to fill in both (i) the Evaluation form (notes field to report any problem with the samples delivery/packaging) and (ii) the Sample Results section.

3.3. Analysis of the NRLs' results

3.3.1 Parameters used for the assignment of the scores

A scoring system is used to assess the participant's performance. *E. coli* MPN scores allocated to participants are detailed in the Table 1.

Table 1: *E. coli* MPN scores - *the expected range is represented by participants' Median ±3SD (SD stands for Theoretical Standard Deviation = 0,24). The expected range values are reported in detail in Table 3 (Results Section).

Results	Returning of results	Replicate 1	Replicate 2	Score
Both replicates MPN results are within the expected range*	2	5	5	12
One replicate MPN result reported is outside the expected range and falls between the median ± 3 SD and the median ± 5 SD value	2	5	2	9
One replicate MPN result reported is outside the median ± 5 SD value	2	5	0	7
Both replicates MPN results reported are outside the expected range and fall between the median ±3SD and the median ± 5 SD value	2	2	2	6
Both replicate MPN results reported are outside the expected range: one replicate MPN results fall between the median ±3SD and the median ± 5 SD value and one replicate MPN result reported is outside the median ± 5 SD value	2	2	0	4
Both replicates MPN results reported are outside the median ± 5 SD value	2	0	0	2
Sample not examined or results returned late, or no explanation received	0	0	0	0
High censored result (i.e. MPN => 18000)	-	-	-	**Samples excluded from the analysis**

4. RESULTS

4.1. Reference results

Freeze-dried mixed cultures are characterized by an intrinsic high level of variability (large confidence intervals). In the assessment of the proficiency of the laboratories, we have thus used the median and standard deviation computed with the results provided by the participating laboratories to set the threshold limits for the laboratories' proficiency assessment. To contain the amplitude of the acceptance intervals, we have excluded the values reported by laboratories that were outliers.

4.1. Participants' results

Performance assessment was carried out according to the scoring parameters reported in Table 1 – Section Materials and Methods. Participants' results and scores are shown in Tables 2, 3, 4 and Figure 1.

Table 2: Summary statistics of participants' results (total results received 24 laboratories).

***E. coli* MPN – summary statistics'**	**Sample**
Participants reporting duplicate results for *E. coli* MPN	24
Participants reporting a single MPN result	0
Participants reporting both replicate MPN results within expected range*	18/24
Participants reporting both replicate MPN results outside expected range	0/24
Participants reporting one replicate MPN result outside expected range	3/24
Participants reporting "high censored result" (i.e. MPN => 18000 per g)	3/24

****expected range**: Participants' Median ±3SD – SD stands for Theoretical Standard Deviation = 0,24
******points deducted from participants returning results with incorrect tube combinations and/or inconsistent with ISO 7218.

Table 3: *E. coli* MPN/g participants' results.

Sample Number	Range (*E.coli* MPN/g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample	110	1,4E+04	1700	8,92E+03	324	2,7E+04	1,1E+02

Note: The median and upper and lower limits (± 3 SD and ± 5 SD) were calculated from participants' results. SDT calculations were based on the inherent variability of the 5 x 3 MPN method (0.24 $\log_{10}$). **Reference values were excluded from the calculation of the participants' median.**

Table 4. Details of the analysis performed by the Laboratories and scores obtained.

Lcode	*E.coli* MPN/g				Score
	Replicate 1	Rarity Category	Replicate 2	Rarity Category	
L004	3300	1	2300	1	12
L014	2300	1	2300	1	12
L017	3500	1	9200	1	12
L025	7000	1	7000	1	12
L037	490	1	790	1	12
L038	1600	1	1600	1	12
L126	1600	1	1600	1	12
L131	7900	1	7900	1	12
L140	1800	1	920	1	12
L142	350	1	170	1	9
L148	7000	1	7900	1	12
L155	7900	1	4900	1	12
L222	1600	1	540	1	12
L256	1600	1	540	1	12
L337	14000	1	1500	1	9
L615	2400	1	1300	1	12
L697	1600	1	1600	1	12
L705	4900	1	1700	1	12
L708	350	1	110	1	9
L758	4900	1	1700	1	12
L986	2400	1	2400	1	12

The three Laboratories that reported both replicates with MPN => 18000; were excluded from the analysis and are not shown.

Figure 1: results dot graph – lyophilized culture - lenticule