PT36

Inter-laboratory study on the enumeration of *Escherichia coli*

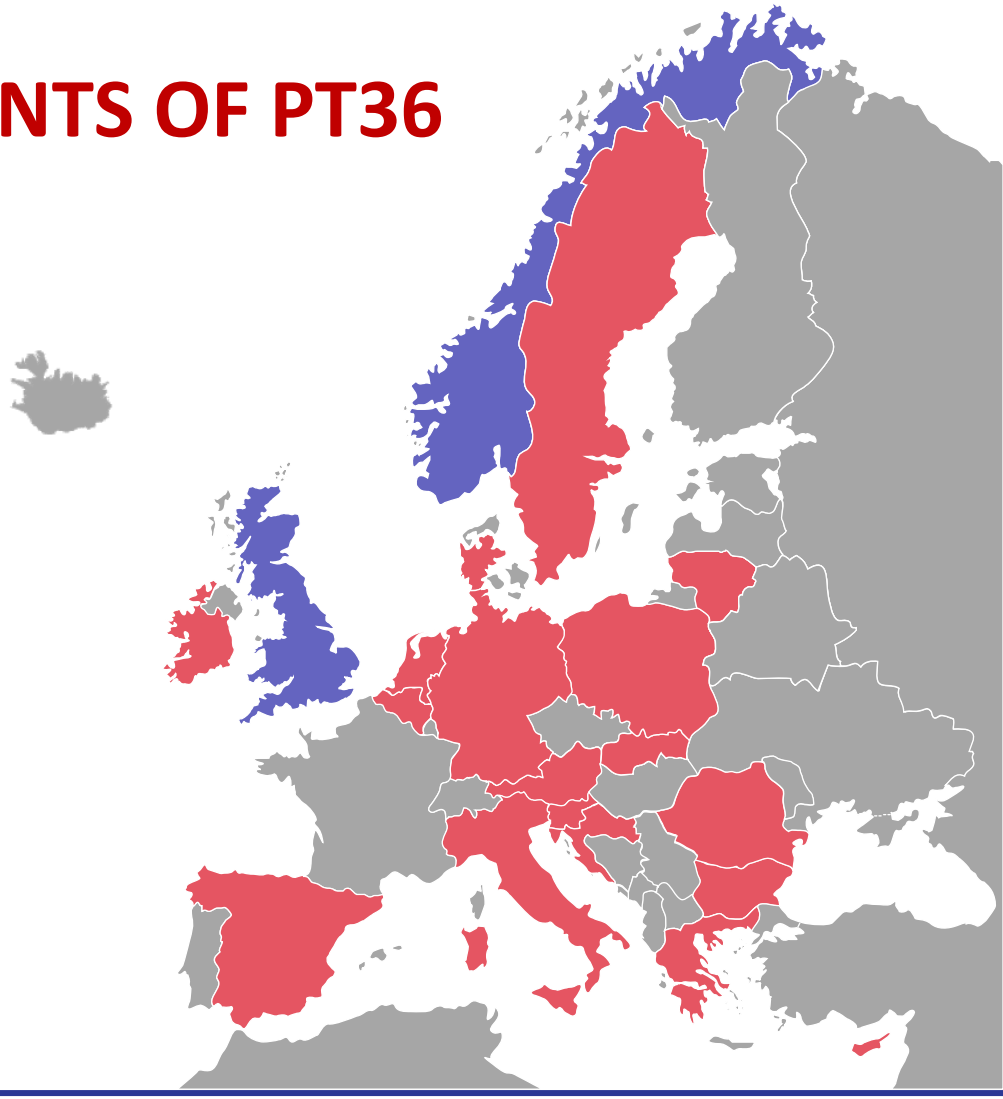
Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Dep.
European Reference Laboratory for *Escherichia coli*

The main objective of the study was:

performing the analysis of live bivalve molluscs from establishment labeled as Area C, with no further treatments for depuration applied, in accordance with **Regulation (EC) N°854/2004** and from throughout the production chain in accordance with **Regulation (EC) N°2073/2005**

PT36 employed a freeze-dried culture of the ATCC strain (25922)

PARTICIPANTS OF PT36



30 NRLs representing

EU MS (19) NON EU MS (2)

PT36: Design of the study (Part I)

A live culture of the ATCC strain refreshed at 10^8 cells/ml, was pelleted and re-suspended in 5% sucrose with a bacterial load of 10^{10}/ml. A volume of 800 µl of the re-suspended culture was distributed into each vial and frozen at -20 °C for a minimum of two days. The vials were then placed into the "Alpha 1-2 LSC Basic" lyophilizer apparatus and freeze-dried for a minimum of three days under the following conditions: (i) -60°C; (ii) 0.01 mbar

The resulting samples were evaluated using the Part 3 of the ISO 16649 method

An acceptable MPN value (5.6 x 10^5) was achieved by diluting the freeze-dried samples 1:2000 before starting the ISO 16649-3 analytical procedure

PT36: Design of the study (Part II)

On 20th of November 2023 samples were shipped to the participating laboratories

Reconstitute the lyophilized culture with 1 ml of TSB

Equilibrate the culture for 5 min then prepare a 1:20 dilution putting the 1 ml of the reconstituted culture in a falcon tube containing 19 ml of TSB, from this dilution perform other two serial dilutions 1:10 to the final dilution rate 1:2000

Put 10 ml of the 1:2000 diluted culture in 200 ml of MRD medium and proceed according to the Part 3 of the ISO 16649 method.

Parameters used for the assignment of the scores PT36

Results	Returning of results	Score allocated		Score
		Replicate 1	Replicate 2	
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
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
One replicate MPN result reported is outside the median ± 5 SD value	2	5	0	7
Both replicates MPN results reported are outside the median ± 5 SD value	2	0	0	2
Single MPN result reported only	2	5	0	7
Tube combination incosistent with MPN reported (only one replicate)	2	7		9
Tube combination incosistent with MPN reported (both replicates)	2	5		7
Sample not examined or results returned late, or no explanation received	0	0	0	0
High censored result (*i.e.* MPN => 18000 per 100g)	Score not assigned			

Max value

Min value

***expected range**: 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 OF PT36: *E. coli* MPN

References' results

Sample Number - Type	Range (*E.coli* MPN/100g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample 1	24000	160000	92000	4,83E+05	17530,24	1,46E+06	5804,81

SDT stands for Theoretical Standard Deviation = 0,24

Note: 4,28 E+03 stands for 4,28 x 10^3 which is 4,28 times 10 (E) to the 3rd power (+03)

Participants' results

Sample Number	Range (*E.coli* MPN/100g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample 1	20	3,5E+06	135950	7,13E+05	25905	2,2E+06	8,6E+03

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.

RESULTS OF PT36: Summary statistics

Table 3: Summary statistics of participants' results (total results received 25 laboratories).

E. coli MPN – summary statistics'	Sample 1
Participants reporting duplicate results for *E. coli* MPN	25
Participants reporting a single MPN result	0
Participants reporting both replicate MPN results within expected range*	22/25
Participants reporting both replicate MPN results outside expected range	1/25
Participants reporting one replicate MPN result outside expected range	2/25
Participants reporting one replicate MPN results as censored results	0
Participants reporting both replicate MPN results as censored results	0
Participants reporting tube combination and/or MPN results inconsistent with ISO 7218*	0

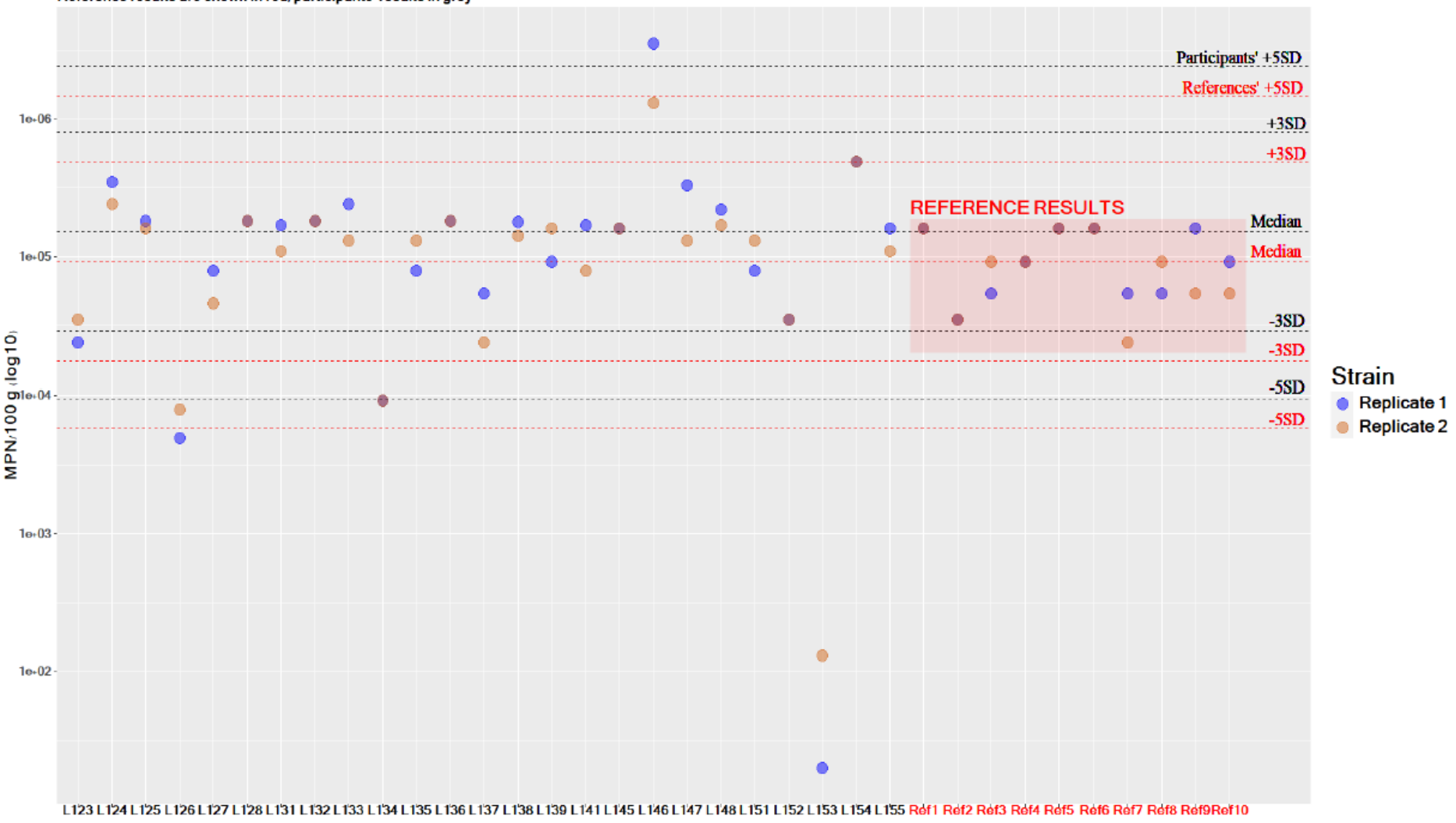
****expected range**: Participants' Median ±3SD – SD stands for Theoretical Standard Deviation = 0,24
****points deducted from participants returning results with incorrect tube combinations and/or inconsistent with ISO 7218.

RESULTS OF PT36: replicate' values

Lcode	*E.coli* MPN/100g				Score
	Replicate 1	Rarity Category	Replicate 2	Rarity Category	
L123	24000	1	35000	1	9
L124	350000	1	240000	1	12
L125	180000	1	160000	1	12
L126	4900	1	7900	1	2
L127	79000	1	46000	1	12
L128	180000	1	180000	1	12
L131	170000	1	110000	1	12
L132	180000	1	180000	1	12
L133	240000	1	130000	1	12
L134	9200	1	9200	1	6
L135	79000	1	130000	1	12
L136	180000	1	180000	1	12
L137	54000	1	24000	1	9
L138	178200	1	141900	1	12
L139	92000	1	160000	1	12
L141	170000	1	79000	1	12
L145	160000	1	160000	1	12
L146	3500000	1	1300000	1	4
L147	330000	1	130000	1	12
L148	220000	1	170000	1	12
L151	79000	1	130000	1	12
L152	35000	1	35000	1	12
L153	20	1	130	1	2
L154	490000	1	490000	1	12

Figure 1: Results' Dot Graph - lyophilized culture-lenticule

Reference results are shown in red, participants' results in grey



CONCLUDING REMARKS – PT36

The procedure for analyzing the freeze-dried ATCC strain (25922) cultures provided by the EURL was not immediately clear to the participants that requested support

Some laboratories had issues with the samples' treatment prior to the application of the method: problem of communication of the procedure

The scoring scheme is proposed for self-evaluation not be used to identify the underperformance

Use of a mixed preparation including both beta - glucuronidase positive and negative strains will be considered to facilitate the application of the ISO 16649-3 method

THANKS FOR THE ATTENTION!