PT32

Inter-laboratory study on the enumeration of *Escherichia coli* in live molluscs

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**
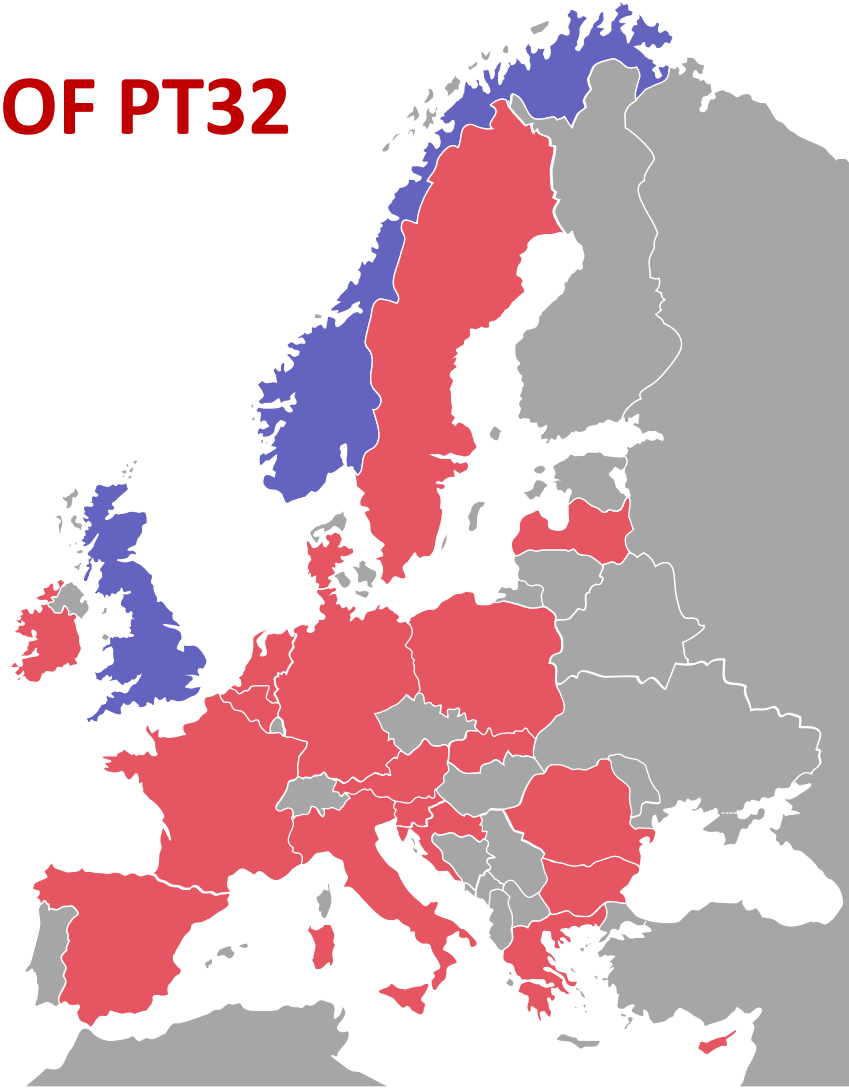
❯ **ISO 16649-3** *"Microbiology of the food chain - Horizontal method for the enumeration of β-glucuronidase-positive* Escherichia coli *Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide"*

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

EU-RL VTEC

PARTICIPANTS OF PT32

25 NRLs representing

● EU MS (23) ● NON EU MS (2)

Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Dep.
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PT32: Design of the study

Sample Preparation

A single **batch of 25 kg common mussels** (*M. gallusprovincialis*) were collected from a harvesting area class C in Italy, **on 17th May 2022**; samples of approximately **500 g of randomly selected mussels were aliquoted and the parcels were shipped to the participating laboratories** by courier

Sample distribution and examination

A total of **25 boxes were shipped and dispatched on 17th May 2022 morning** to the participants. **Participants were requested to analyze the samples as soon as possible after receipt, and in all cases within 24 hours of arrivals, using the Part 3 of the method ISO 16649**

Production of Reference Results

Ten randomly selected samples from the shipment lot consisting in approximately 1 kg of mussels (100g each sample), were **analyzed in duplicate on 18th May 2022 using the ISO 16649-3 method**; **Sample homogeneity was assessed according with the requirements of ISO 17043:2010**

Parameters used for the assignment of the scores PT32

Results	Returning of results	Score allocated		Score
		Replicate 1	Replicate 2	
Both replicates MPN results are within the expected range*	2	5	5	12	**Max value**
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	**Min value**
High censored result (*i.e.* MPN => 18000 per 100g)	Score not assigned			

***expected range**: Participants' Median ±3SD – <u>SD stands for Theoretical Standard Deviation = 0,24</u>
The <u>expected range values are reported in detail in **Table 3** (Results Section)</u>.

RESULTS OF PT32: *E. coli* MPN/100g

References' results

Sample Number - Type	Range (*E.coli* MPN/100g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample 1 - Mussels	0	78	32,5	1,71E+02	6,19	5,15E+02	2,05

SDT stands for Theoretical Standard Deviation = 0,24

Note: 1,71E+02 stands for $1,71 \times 10^2$ which is 1,71 times 10 (E) to the 2nd power (+02)

Participants' results

Sample Number - Type	Range (*E.coli* MPN/100g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample 1 - Mussels	0	6,8E+01	0	-	-	-	-

Note: The median and upper and lower limits (± 3 SD and ± 5 SD) could not be calculated from participants' results.

RESULTS OF PT32: 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*	4/25
Participants reporting both replicate MPN results outside expected range	16/25
Participants reporting one replicate MPN result outside expected range	5/25
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: note without Participants' Median ±3SD, the expected range is Reference Median ±3SD.
**points deducted from participants returning results with incorrect tube combinations and/or inconsistent with ISO 7218.
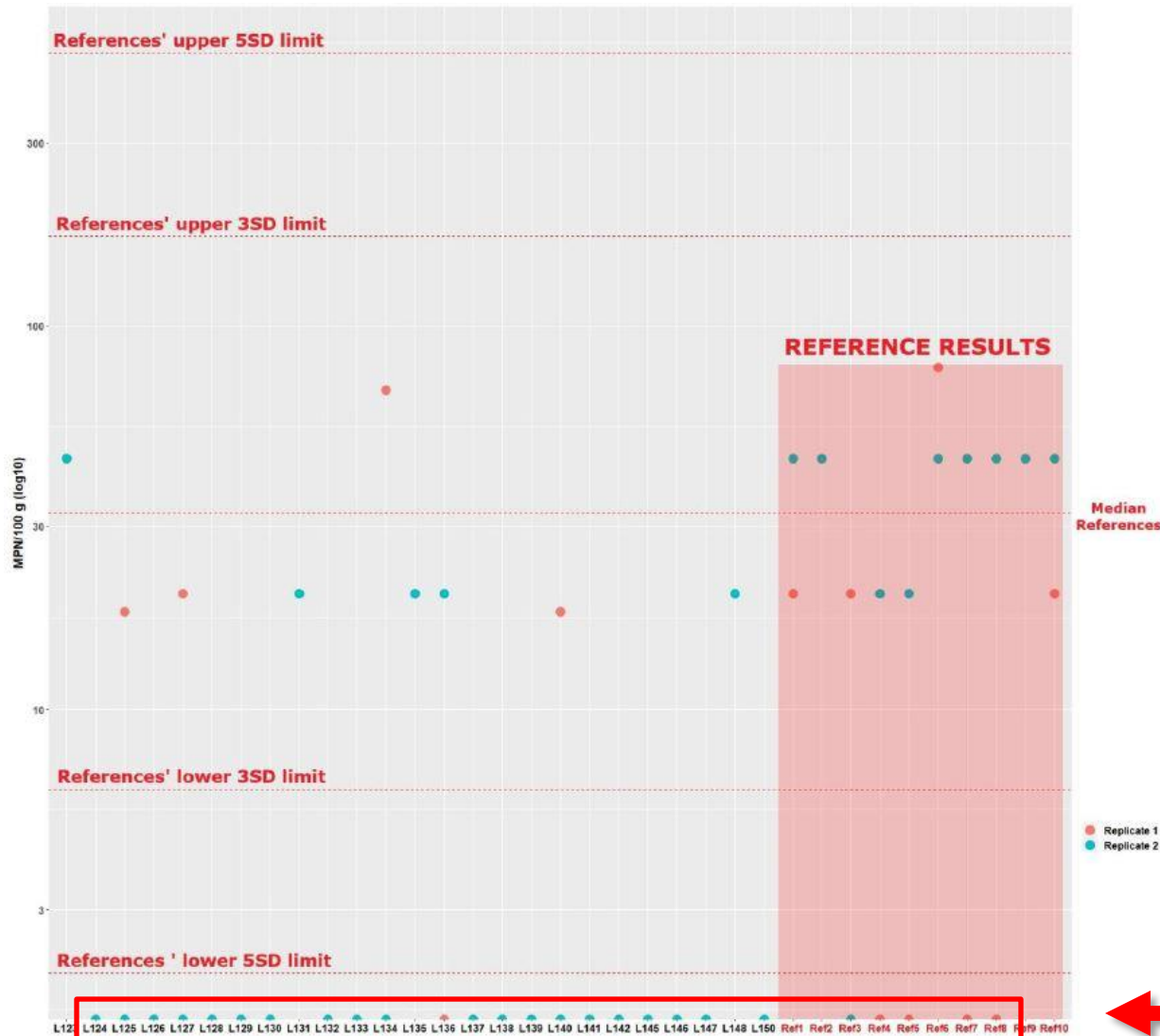
RESULTS OF PT32: replicate' values

Lcode	Shipment Date	Start Date Analysis and Temperature (°C) samples	E.coli MPN/100g			
			Replicate 1	Rarity Category	Replicate 2	Rarity Category
L123	17/05/2022	18/05/2022 - 4°C	45	1	45	1
L124	17/05/2022	19/05/2022 - 13°C	0	1	0	1
L125	17/05/2022	18/05/2022 - 12°C	18	1	0	0,092
L126	17/05/2022	19/05/2022 - 23°C	0	1	0	1
L127	17/05/2022	18/05/2022 - 14°C	20	1	0	1
L128	17/05/2022	18/05/2022 - 13°C	0	1	0	1
L129	17/05/2022	18/05/2022 - 5°C	0	1	0	1
L130	17/05/2022	18/05/2022 - 12°C	0	1	0	1
L131	17/05/2022	18/05/2022 - 16°C	20	1	20	1
L132	17/05/2022	19/05/2022 - 14°C	0	1	0	1
L133	17/05/2022	19/05/2022 - 22°C	0	1	0	1
L134	17/05/2022	18/05/2022 - 13°C	68	0,354	0	1
L135	17/05/2022	18/05/2022 - 13°C	20	1	20	1
L136	17/05/2022	18/05/2022 - 9°C	0	1	20	1
L137	17/05/2022	20/05/2022 - 21°C	0	1	0	1
L138	17/05/2022	19/05/2022 - 16°C	0	1	0	1
L139	17/05/2022	19/05/2022 - 7°C	0	1	0	1
L140	17/05/2022	18/05/2022 - 7°C	18	1	0	1
L141	17/05/2022	18/05/2022 - 6°C	0	1	0	1
L142	17/05/2022	19/05/2022 - 13°C	0	1	0	1
L145	17/05/2022	18/05/2022 - 9°C	0	1	0	1
L146	17/05/2022	18/05/2022 - 7°C	0	1	0	1
L147	17/05/2022	18/05/2022 - 12°C	0	1	0	1
L148	17/05/2022	20/05/2022 - 22°C	20	1	20	1
L150	17/05/2022	18/05/2022 - 14°C	0	1	0	1

REFERENCE RESULTS

Median References

Replicate 1
Replicate 2

Note: The median and upper and lower limits (± 3 SD and ± 5 SD) were calculated only from references' results. Sample replicates (Replicate 1 and Replicate 2) are represented by red and blue dots.

Considering how above, the proficiency of the participant laboratories, particularly of those **reporting <18 MPN, could not be assessed**!

CONCLUDING REMARKS – PT32

- The **contamination of the molluscs with *E. coli* was that naturally present in the animals and was very low**; in addition, a further reduction could be observed probably due to the shipment conditions
- The **low contamination level** was the main feature affecting the **analysis of the participants' proficiency**
- The observed randomness in the contamination levels of the animals collected in the wild and the frailty of the samples made up by live molluscs suggests using frozen or lyophilized materials for the next PTs as test matrix.

THANKS FOR THE ATTENTION!