

## PT29

# 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 production areas 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  $\beta$ -glucuronidase-positive *Escherichia coli* Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide”



## PARTICIPANTS OF PT29

30 NRLs representing

● EU MS (28) ● NON EU MS (2)



## PT29: Design of the study

### Sample Preparation

A single batch of **23.5 kg common mussels** (*M. gallusprovincialis*) were collected from a harvesting area class C in Italy, on **28<sup>th</sup> September 2021**; 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 **30 boxes** were shipped and dispatched on **28<sup>th</sup> September 2021 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 29<sup>th</sup> September 2021 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 PT29

Results	Returning of results	Score allocated		Score	
		Replicate 1	Replicate 2		
Both replicates MPN results are within the expected range*	2	5	5	12	<u>Max value</u>
One replicate MPN result reported is outside the expected range and falls between the median $\pm 3$ SD and the median $\pm 5$ SD value	2	5	2	9	
Both replicates MPN results reported are outside the expected range and fall between the median $\pm 3$ SD and the median $\pm 5$ SD value	2	2	2	6	
One replicate MPN result reported is outside the median $\pm 5$ SD value	2	5	0	7	
Both replicates MPN results reported are outside the median $\pm 5$ SD value	2	0	0	2	
Single MPN result reported only	2	5	0	7	
Tube combination inconsistent with MPN reported (only one replicate)	2	7		9	
Tube combination inconsistent with MPN reported (both replicates)	2	5		7	
Sample not examined or results returned late, or no explanation received	0	0	0	0	<u>Min value</u>
High censored result ( <i>i.e.</i> MPN $\Rightarrow$ 18000 per 100g)	<u>Score not assigned</u>				

\***expected range**: Participants' Median  $\pm 3$ SD – SD stands for Theoretical Standard Deviation = 0,24  
 The expected range values are reported in detail in Table 3 (Results Section).



# RESULTS OF PT29: *E. coli* MPN/100g



- 29 out of 30 laboratories reporting results
- samples were received within 24 hours

## References' results

Sample Number - Type	Range ( <i>E.coli</i> MPN/100g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample 1 - Mussels	0	330	85,5	4,49E+02	16,29	1,36E+03	5,39

## Participants' results

Sample Number - Type	Range ( <i>E.coli</i> MPN/100g)		Median	Median±3SDT*		Median±5SDT*	
	Minimum Value	Maximum Value					
Sample 1 - Mussels	0	3,3E+02	78	4,09E+02	15	1,2E+03	4,9E+00

# RESULTS OF PT29: Summary statistics



**Table 3: Summary statistics of participants' results (total results received: 29 laboratories).**

<i>E. coli</i> MPN – summary statistics'	Sample 1
Participants reporting duplicate results for <i>E. coli</i> MPN	29
Participants reporting a single MPN result	0
Participants reporting both replicate MPN results within expected range*	25/29
Participants reporting both replicate MPN results outside expected range	3/29
Participants reporting one replicate MPN result outside expected range	1/29
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  $\pm 3SD$  – SD stands for Theoretical Standard Deviation = 0.24



# RESULTS OF PT29



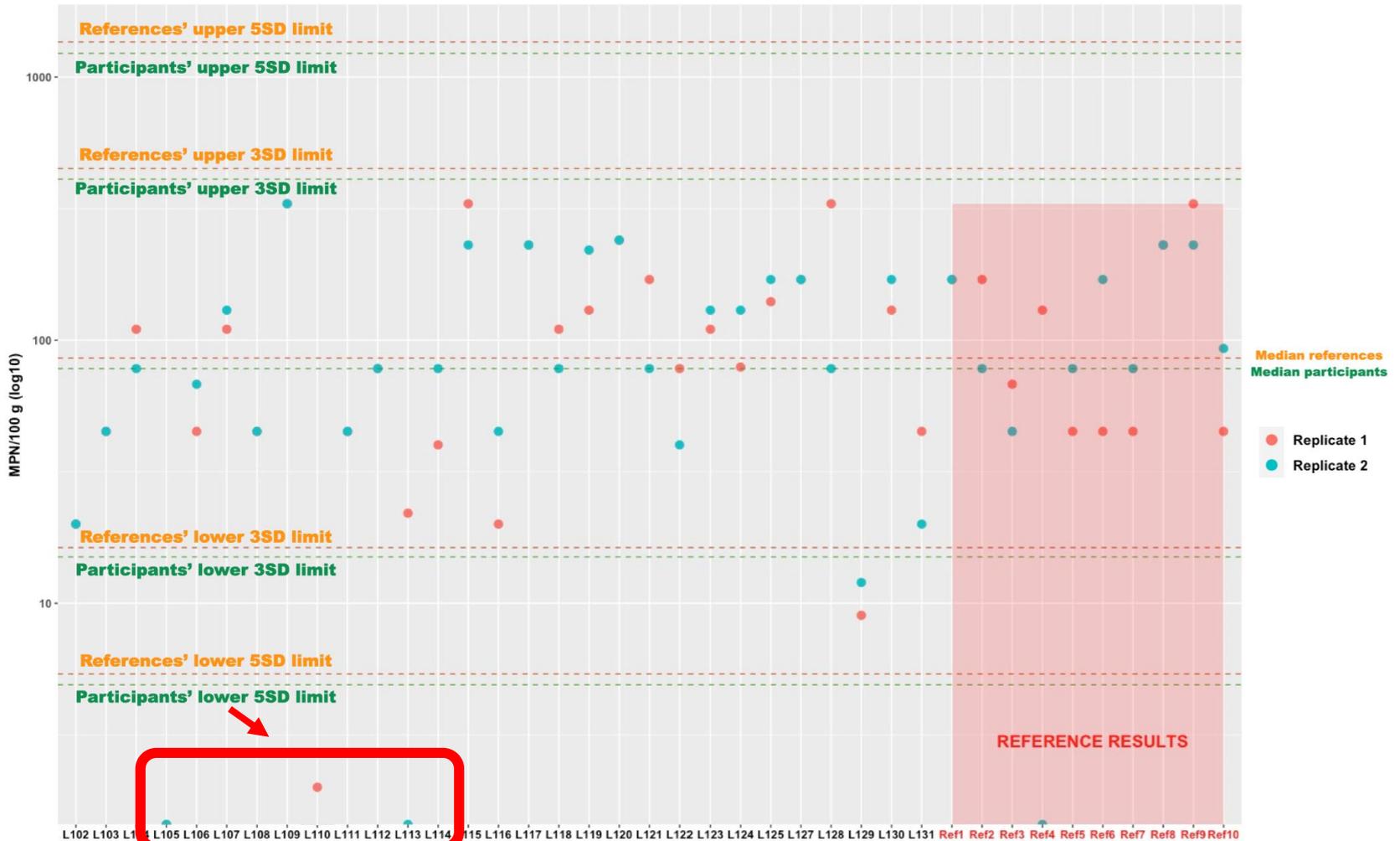
Lcode	Shipment Date	Start Date	E.coli MPN/100g						Score
			Replicate 1	Rarity Category	Rarity Index	Replicate 2	Rarity Category	Rarity Index	
L102	28/09/2021	29/09/2021	20	1	1	20	1	1	12
L103	28/09/2021	30/09/2021	45	1	1	45	1	1	12
L104	28/09/2021	30/09/2021	110	1	0,565	78	1	1	12
L105	28/09/2021	29/09/2021	0	1	1	0	1	1	2
L106	28/09/2021	29/09/2021	45	1	1	68	1	0,354	12
L107	28/09/2021	29/09/2021	110	1	0,564	130	1	1	12
L108	28/09/2021	29/09/2021	45	1	1	45	1	1	12
L109	28/09/2021	29/09/2021	330	1	1	330	1	1	12
L110	28/09/2021	29/09/2021	2	1	1	2,2	3	0,009	2
L111	28/09/2021	29/09/2021	45	1	1	45	1	1	12
L112	28/09/2021	29/09/2021	78	1	1	78	1	1	12
L113	28/09/2021	29/09/2021	22	1	1	0	1	1	7
L114	28/09/2021	29/09/2021	40	1	0,205	78	1	1	12
L115	28/09/2021	29/09/2021	330	1	1	230	1	0,772	12
L116	28/09/2021	29/09/2021	20	1	1	45	1	1	12
L117	28/09/2021	29/09/2021	230	1	0,772	230	1	0,772	12
L118	28/09/2021	30/09/2021	110	1	0,564	78	1	1	12
L119	28/09/2021	29/09/2021	130	1	1	220	1	0,315	12
L120	28/09/2021	29/09/2021	240	1	0,744	240	1	0,744	12
L121	28/09/2021	29/09/2021	170	1	0,981	78	1	1	12
L122	28/09/2021	30/09/2021	78	1	1	40	1	0,205	12
L123	28/09/2021	29/09/2021	110	1	0,564	130	1	1	12
L124	28/09/2021	30/09/2021	79	1	1	130	1	1	12
L125	28/09/2021	29/09/2021	140	1	0,147	170	1	0,083	12
L127	28/09/2021	30/09/2021	170	2	0,013	170	2	0,013	12
L128	28/09/2021	29/09/2021	330	1	1	78	1	1	12
L129	28/09/2021	30/09/2021	9	1	1	12	1	0,051	6
L130	28/09/2021	30/09/2021	130	1	1	170	1	0,918	12
L131	28/09/2021	29/09/2021	45	1	1	20	1	1	12

25 out of the 29 participants successfully took part to this PT obtaining the best score (max value = 12)



# RESULTS OF PT29: reference and participants results

Results' Dot Graph - Sample 1 Mussels



## CONCLUDING REMARKS - PT29

As a whole 25 out of the 29 laboratories that contributed the results for PT29 reported MPN results for both replicates falling within the expected range of median  $\pm$  the theoretical 3 SD, configuring a **good overall performance of the network in this second round!**



**Only two labs = final score 2 - because both replicate MPN result were outside the median  $\pm$  5 SD value (< 4,9).**