

EU Reference Laboratory for *E. coli* Department of Food Safety, Nutrition and Veterinary Public Health Microbiological food safety and food-borne diseases Unit Istituto Superiore di Sanità



# Report of the First inter-laboratory study on the enumeration of *E. coli* in Live Bivalve Molluscs (PT24) –

2019

#### Edited by:

Silvia Arancia, Arianna Boni, Gianfranco Brambilla, Paola Chiani, Clarissa Ferreri, Fabio Galati, Federica Gigliucci, Arnold Knijn, Antonella Maugliani, Valeria Michelacci, Fabio Minelli, Margherita Montalbano Di Filippo, Stefano Morabito, Rosangela Tozzoli

#### 1. INTRODUCTION AND OBJECTIVES OF THE STUDY

As an effect of Regulation (EU) 222/2018, the European Union Reference Laboratory for *E. coli* (EURL-VTEC) has taken over the activities carried out by the former EU reference laboratory for monitoring the bacteriological contamination of bivalve molluscs as regards the analytical tests for *E. coli*. The duties of the EURL include the organization of proficiency tests (PT) to assess the performance of the designated National Reference Laboratories (NRLs) for the microbiological contamination of bivalve molluscs in the EU and to assist the EFTA Member States, EU Candidate Countries and third countries in using the method for the enumeration of *E. coli* in live bivalve molluscan shellfish. The EU reference method for enumeration of *E. coli* in live bivalve molluscs is 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".

This scheme is intended to provide proficiency testing (PT) samples for 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.

The first round organized by the EURL-VTEC, PT24, was carried out on commercial common live mussels, without any additional contamination. The live molluscs were obtained from establishment labeled as Area C, with no further treatments for depuration applied.

The main objective of this study was to provide data on the proficiency of the laboratories for ISO 17025 accreditation purposes.

This document represents the evaluation report of the PT24 study. The study was conducted according to the International Standard ISO/IEC 17043:2010 "Conformity assessment –General requirements for proficiency testing".

## **1. PARTICIPANTS**

Twenty-four NRLs, representing 19 EU Member States plus Norway, accepted the invitation to participate. Each NRL received its own individual laboratory numerical code, which was used to label the laboratories in the result tables.

The Laboratories participating in the study were:

- AGES, Institut für Lebensmittelsicherheit Wien, Gruppe Mikrobiologie, Wien, Austria

- Sciensano, Foodborne Pathogens, Brussels, Belgium
- National Diagnostic and Research Veterinary Institute, National center of food safety (NDRVMI-BFSA), Sofia, Bulgaria
- Croatian Veterinary Institute, Veterinary Institute SPLIT (HVI-VZS), Laboratory for microbiology of food and animal feeding stuffs (S-2), Split, Croatia
- Croatian Veterinary Institute, Department Rijeka, Laboratory for food and feed microbiology, Rijeka, Croatia
- Croatian Veterinary Institute, Laboratory for Food Microbiology, Zagreb, Croatia
- Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services, Nicosia, Cyprus
- Veterinary Research Institute, Brno, Czech Republic
- Microbiological Laboratory Ringsted, Ringsted, Denmark
- German Federal Institute for Risk Assessment, Unit Food Microbiology, Host-Pathogen-Interaction, Department Biological Safety, Berlin, Germany
- Department of Food Hygiene of Athens, Ministry of Rural Development and Food, Athens, Greece
- Veterinary Laboratory of Kavala, Ministry of Rural Development & Food, Kavala, Greece
- Marine Institute, Rinville, Oranmore, Co. Galway, Ireland
- IZS Umbria e Marche "Togo Rosati", Sezione di Ancona, Italy
- BIOR, Riga, Latvia
- Institute of Marine Research, Laboratory for molecular biology, Bergen, Norway
- National Veterinary Research Institute (NVRI), Department of Hygiene of Food of Animal Origin, Pulawy, Poland
- Institute for Diagnoses and Animal Health, Bucharest, Romania
- Department of Food Hygiene, Veterinary and food institute, Dolny Kubin, Slovakia
- UL, Veterinary faculty / National veterinary institute, Institute for food safety, feed and environment – Unit for food safety, Ljubljana, Slovenia
- Microbiology Food Department, Agencia Española de Seguridad Alimentaria y Nutrición, Spanish Food Safety and Nutrition Agency, National Center for Food -Centro Nacional de Alimentación (CNA), Madrid, Spain
- RIVM, Bilthoven, The Netherlands
- Laboratory Food and Feed Safety, Netherlands Food and Consumer Product Safety Authority, Wageningen, The Netherlands

#### - Cefas, Weymouth, UK

We report the analysis of 23 laboratories that submitted results. One lab (L112) didn't take part in the study because the samples were received in non-optimal conditions due to problems occurred during the transport.

## 2. MATERIALS AND METHODS

## 3.1. Sample preparation

A single batch of 20 kg common mussels (*M. gallusprovincialis*) were collected from a commercial harvesting area class C in Italy, on July 10-11<sup>th</sup> 2019. Prior to packing, the shellfish were placed in a large disinfected container and thoroughly mixed. Samples of approximately 500 g of randomly selected mussels were aliquoted and the parcels were shipped to the participating laboratories by courier.

#### 3.2. Sample distribution and examination

Each individual sample was packed in a polystyrene refrigerate box containing one plastic pocket with a stomacher bag containing approximately 500 g of common mussels. A total of 24 boxes were shipped. Nineteen samples were despatched on  $11^{th}$  July 2019 morning to the participants. The remaining five samples were stored at  $3 \pm 2$  °C and shipped on Monday  $15^{th}$  July 2019, to avoid the delivery of the samples to the laboratory during the weekend. Participants were requested to analyse 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.

#### 3.3. Collection and Elaboration of the NRLs Results

An instruction sheet, comprising a step by step procedure about all the details on how to handle and prepare the samples, and how to submit the results through the web platform (<u>https://w3.iss.it/site/PT\_MB/</u>) was sent by email to all the participants on July 10<sup>th</sup> 2019.

All the data collected were submitted by the NRLs using their own User IDs and passwords. The participants were requested to fill in both the Evaluation form (information on: arrival date/time/condition/temperature, Storage temperature, analysis start date/time and notes field in order to specify any problem with the samples delivery/packaging) and the Sample Results section.

## 3. RESULTS

## 4.1. Reference results

Ten randomly selected samples from the shipment lot consisting in approximately 1 kg of mussels, were analysed in duplicate on 11<sup>th</sup> July 2019 using the ISO 16649-3 method. Sample homogeneity was assessed according with the requirements of ISO 17043:2010. The sample material distributed was considered sufficiently homogenous. The reference results are reported in Table 1.

Sample N° and type	Analysis Date	Range ( <i>E. coli</i> MPN/100 g)	Median	Median ± 3*SDT
Sample 1- Mussels	11/07/2019	0 – 4.5 x 10 <sup>1</sup>	2 x 10 <sup>1</sup>	3.8 – 1 x 10²

## Table 1: E. coli MPN/100 g reference results

\*SDT - theoretical standard deviation (0.24 log<sub>10</sub>)

#### 4.2. Participants' results

The median and upper and lower limits ( $\pm$  3 SD and  $\pm$  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<sub>10</sub>). The results are reported in Table 2. Reference values were excluded from the calculation of the participants' median.

Performance assessment was carried out according to the scoring parameters reported in Table 3. Details of the analysis performed, results and scores are reported in Table 4. The results of all participants were compared with the Median  $\pm$  3 SDT and Median  $\pm$  5 SDT and are shown in Figure 1.

Summary statistics of participants' results for PT24 are shown in Table 5.

# Table 2: Participants' results

Sample N° and type	Range ( <i>E. coli</i> MPN/100 g)	Median	Median ± 3*SDT	Median ± 5*SDT
Sample 1- Mussels	0 – 6.9 x 10²	6.8 x 10 <sup>1</sup>	1.3 x 10 <sup>1</sup> - 3.6 x 10 <sup>2</sup>	4.3 – 1.1 x 10 <sup>3</sup>

\*SDT – theoretical standard deviation (0.24 log<sub>10</sub>)

## Table 3. Parameters used for the assignment of the scores adopted in PT24

Results	Points assigned
Return of results	2
All replicate MPN results within the expected range	10
Or	-
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	7
Or	
Both replicate MPN results reported are outside the expected range and fall between the median $\pm 3$ SD and the median $\pm 5$ SD value	4
Or	
One replicate MPN result reported is outside the median $\pm 5$ SD value	5
Or	
Both replicate MPN results reported are outside the median $\pm 5$ SD value	0
Or	
Single MPN result reported only	5
Or	
Tube combination inconsistent with MPN reported (one replicate)	7
Or	
Tube combination inconsistent with MPN reported (both replicates)	5
Or	
Sample not examined or results returned late- no explanation received	0
Or	
High censored result ( <i>e.g.</i> MPN => 18,000 per 100 g)	Score not assigned

	Shipment Date	Start Date	E.coli MPN/ 100g				<b>C c c c c c c c c c c</b>
LCode			Replicate1	Rarity Category	Replicate2	Rarity Category	Score
L101*	11/07/2019	12/07/2019	44	2	90	1	12
L102	15/07/2019	16/07/2019	170	1	140	1	12
L103	11/07/2019	15/07/2019	78	1	20	1	12
L104	11/07/2019	12/07/2019	68	3**	45	1	12
L105	11/07/2019	12/07/2019	20	1	0	1	12
L106	11/07/2019	12/07/2019	45	1	690	2	9
L107	11/07/2019	15/07/2019	45	1	45	1	12
L108	11/07/2019	12/07/2019	130	1	20	1	12
L109	15/07/2019	17/07/2019	130	1	110	1	12
L110	15/07/2019	16/07/2019	130	1	20	1	12
L111	11/07/2019	16/07/2019	68	1	ND	ND	6
L113	11/07/2019	12/07/2019	0	1	0	1	12
L114	11/07/2019	12/07/2019	230	1	230	1	12
L115	11/07/2019	12/07/2019	20	1	0	1	12
L117	15/07/2019	17/07/2019	170	ND	330	ND	12
L118	11/07/2019	12/07/2019	170	1	78	1	12
L119	11/07/2019	12/07/2019	5	1	2	1	7
L120	11/07/2019	12/07/2019	20	1	110	1	12
L121	11/07/2019	12/07/2019	330	1	45	1	12
L122	11/07/2019	13/07/2019	220	1	230	1	12
L123	11/07/2019	12/07/2019	45 <b>A</b>	1	0 <b>A</b>	1	11
L125	11/07/2019	12/07/2019	0	1	0	1	12
L126	11/07/2019	12/07/2019	130	1	93	1	12

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

6 dilutions performed
 Theoretically Not Valid
 A Values deducted from excel file 7218 MPN calculation form

ND Not Determinable because of the absence of the excel file 7218 MPN calculation form

## Table 5. Summary statistics of participants' results

<i>E. coli</i> MPN	Sample 1
Participants reporting duplicate results for <i>E. coli</i> MPN	22
Participants reporting a single MPN result	1
Participants reporting both replicate MPN results within	21
expected range <sup>1</sup>	
Participants reporting both replicate MPN results outside	1
expected range	
Participants reporting one replicate MPN result outside	1*
expected range	
Participants reporting one replicate MPN results as censored	None
results	
Participants reporting both replicate MPN results as censored	None
results	
Participants reporting tube combination and / or MPN results	1
inconsistent with ISO 7218 <sup>2</sup>	
Total results received	23

1 expected range = participants' median ± theoretical 3 SD.

2 points deducted from participants returning results with incorrect tube combinations and/or inconsistent with ISO 7218.

\* This is the same laboratory that submitted MPN results inconsistent with ISO 7218.



Figure 1. Results chart Sample 1 - Mussels

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#### 5. CONCLUDING REMARKS

- Twenty-four laboratories joined the study, 23 of them sent back the results. One Laboratory (L112) did not perform the analysis because of the bad conditions of the received samples (Temperature 25.4 °C).
- 2. The samples were shipped in two different dates as to avoid the delivery to some participating laboratories during the weekend. The samples were received within 24 hours by all the participants, except two, that were conferred 48 hours after the dispatch.
- 3. Twenty-three laboratories returned the analyses results. One laboratory reported the results for only one replicate (L111). For L123, the test results have been deducted from the excel file "7218 MPN calculation form" as this laboratory didn't report them using the online interface; L106 reported just one replicate outside the expected range and falling between the median ± 3 SD and the median ± 5 SD values. Finally, L119 submitted values considered as inconsistent (instead of outlier) because of the wrong dilution factor reported in the "7218 MPN calculation form" and this affected the final results. A correct use of the MPN calculation Form is a requirement of the analytical determination and will be recommended in the next PTs.
- 4. Two laboratories (L101 and L106) reported results falling in the rarity category 2 for one replicate (Table 4). As mentioned in ISO 7218, this results' category can be included in the final evaluation. In this report, we also included both the replicates of L104, even if for one of them the rarity category was 3, because the related values falled between the median ± 3 SD and the median ± 5 SD. Besides, it is interesting to note that one of the laboratories reporting results into the rarity category 2 performed six dilutions instead of the requested 4, and this could have affected the result.
- 5. Nineteen out of the 23 participants successfully took part to this PT obtaining the best score (12). These included L109 e L110, although these laboratories received the mussels dead and open. A reduced score (1 out of 2) was assigned to L111 (final score 6) and L123 (final score 11), as these laboratories submitted twice the result of 1 only replicate or because the results were not reported using the online form but were instead deducted from ISO "7218 MPN calculation form", respectively. Two other participants received a low score. In particular, L106 got a final score of 9, because "one replicate MPN result was outside the expected range and falled between the median ± 3 SD and the median ± 5 SD value"; and L119 received a final score of 7 because of

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"Tube combination inconsistent with MPN reported (both replicates)", as mentioned in point 2.

6. As a whole 21 out of the 23 laboratories that contributed the results for PT24 reported MPN results for both replicates falling within the expected range of median ± the theoretical 3 SD, configuring a good overall performance of the network in this first round of PT on LBM organized by EURL VTEC.