Centre for Environment Fisheries & Aquaculture Science

Cefas

*World Class Science for the Marine and Freshwater Environment*

# European Union Reference Laboratory (EURL) Proficiency Testing Scheme

## Enumeration of *Escherichia coli* and the detection of *Salmonella* spp. in bivalve molluscan shellfish (PT 76)

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# Cefas Document Control

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This scheme is intended to provide proficiency testing (PT) samples for laboratories undertaking examination of live bivalve molluscs from production areas in accordance with Regulation (EC) No. 854/2004 and from throughout the production chain in accordance with Regulation (EC) No. 2073/2005.

The scheme is organised by the European Union Reference laboratory (EURL) for monitoring bacteriological and viral contamination of bivalve molluscs. The EURL is designated by the European Union in accordance with Regulation (EU) 2017/625. The scheme is intended to compliment the EURL/PHE Shellfish Scheme through examination of aspects of the methods not covered under the Shellfish Scheme (http://www.hpa.org.uk/ProductsServices/MicrobiologyPathology/ExternalQualityAssessmentProficiencyTesting/EQAPTForFoodWaterAndEnvironmentalMicrobiology/ShellfishScheme/ (initial sample preparation and preparation of initial dilutions) and to provide additional data for laboratories for ISO 17025 accreditation purposes.

The EU stipulated reference method for enumeration of *E. coli* in live bivalve molluscs in 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 (Anon 2015). The EU approved alternative methods for the enumeration of *E. coli* are 'Enumeration of *Escherichia coli* in live bivalve molluscan shellfish by the direct impedance technique using the BacTrac 4300 series analyser' and 'Enumeration of *E. coli* in bivalve molluscan shellfish by the colony-count technique'. Protocols for the application of these methods are available at www.eurlcefas.org

The EU reference method for detection of *Salmonella* spp. in live bivalve molluscs is ISO 6579, Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1 Detection of *Salmonella* spp. (Anon 2017).

A scoring system is used to help assess participants' performance. Details of this system are included as Appendix II of this report. The purpose of scoring is to help the EURL, NRLs and other participating laboratories identify incorrect or outlying results. Further information on the use of scoring in PT and on recommended procedures for following up poor performance can be accessed via the EURL website (www.eurlcefas.org) or obtained by contacting the EURL. The EURL has produced a protocol for management of underperformance in comparative testing and/or lack of collaboration of NRLs with EURLs activities.

If you are experiencing problems with any aspects of these distributions please contact the EURL (contact details below), or alternately refer to the troubleshooting guide included as Appendix III of this report.

Further advice on microbiological testing of bivalve molluscan shellfish can be obtained via the EURL website (www.eurlcefas.org)

Due to the nature of this scheme repeat samples are not available.

**Sample preparation**

**Samples 1**

A single batch of 2000 Pacific Oysters (*Crassostrea gigas*) were collected from a UK commercial harvesting area on the 26th November 2018. Prior to packing the shellfish were placed in a large disinfected container and thoroughly mixed. Sample 1 comprised of approximately 24 randomly selected oysters from this bulk material.

**Sample 2**

A single batch of approximately 700 Pacific Oysters (*Crassostrea gigas*) were collected from a UK commercial harvesting area on the 21st November 2018. On arrival, the oysters were shucked and homogenised before being pooled together to form one homogenate. Sample 2 was aliquoted in 100 ml volumes on the 22nd November and stored at 3±2 °C. Prior to distribution Sample 2 was spiked with *E. coli* ($2.2 \times 10^4$ cfu/sample) and *Salmonella* spp. (*S.* Nottingham at $1.5 \times 10^2$ cfu/sample).

**Sample 3**

A single batch of approximately 700 Pacific Oysters (*Crassostrea gigas*) were collected from a UK commercial harvesting area on the 21st November 2018. On arrival, the oysters were shucked and homogenised before being pooled together to form one homogenate. Sample 3 was aliquoted in 100 ml volumes on the 24th November and stored at 3±2 °C. Prior to distribution Sample 3 was spiked with *E. coli* ($9.6 \times 10^3$ cfu/sample) and *Salmonella* spp. (*S.* Nottingham at $2.9 \times 10^2$ cfu/sample).

**Sample distribution and examination**

Each individual sample was packed in accordance with the Cefas protocol for packaging shellfish for transportation. Samples were despatched at 10:00am on the 26th November 2018 to 41 participating laboratories. Participants were requested to analyse the samples immediately on receipt using their routine methods.

**Sample temperature**

Participants were requested to record the internal sample temperature on arrival. Temperatures recorded by participants are shown in Appendix I.

**Results**

**Reference results - *E. coli***

Ten randomly selected samples were analysed in duplicate on 2 consecutive days (28th and 29th November 2018) under repeatability conditions for *E. coli* using EURL SOP No. 1175 (Table 1). Sample homogeneity was assessed following the procedure described in ISO 22117. The sample material distributed was considered sufficiently homogenous.

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

| Sample No. and type | Analysis date | Range | Median | GM | Median ±3*$SD_T$ |
|---|---|---|---|---|---|
| Sample 1 - Oysters | 27.11.18 | $7.8 \times 10^1 – 4.9 \times 10^2$ | $2.3 \times 10^2$ | $2.4 \times 10^2$ | $4.4 \times 10^1 – 1.2 \times 10^3$ |
| | 28.11.18 | $1.1 \times 10^2 – 7.8 \times 10^2$ | $3.3 \times 10^2$ | $3.2 \times 10^2$ | $6.3 \times 10^1 – 1.7 \times 10^3$ |
| Sample 2 - Homogenate | 27.11.18 | $4.9 \times 10^3 – 5.4 \times 10^4$ | $1.4 \times 10^4$ | $1.3 \times 10^4$ | $2.6 \times 10^3 – 7.1 \times 10^4$ |
| | 28.11.18 | $3.3 \times 10^3 – 3.5 \times 10^4$ | $7.9 \times 10^3$ | $9.4 \times 10^3$ | $1.5 \times 10^3 – 4.1 \times 10^4$ |
| Sample 3 - Homogenate | 27.11.18 | $4.9 \times 10^2 – 1.3 \times 10^4$ | $4.8 \times 10^3$ | $4.4 \times 10^3$ | $9.1 \times 10^2 – 2.5 \times 10^4$ |
| | 28.11.18 | $1.3 \times 10^3 – 1.7 \times 10^4$ | $4.1 \times 10^3$ | $4.7 \times 10^3$ | $7.8 \times 10^2 – 2.2 \times 10^4$ |

GM - geometric mean, $SD_T$ - theoretical standard deviation (0.24 $\log_{10}$)

**Reference results – *Salmonella* spp.**

Ten randomly selected samples were analysed on 2 consecutive days (27th and 28th November 2018) for *Salmonella spp.* using EURL SOP No. 1176 (Table 2).

**Table 2: Reference results**

| Sample No. and type | Date of Analysis | *Salmonella spp.* | No. of replicates giving the expected result |
|---|---|---|---|
| **Sample 1 – Oysters** | 27.11.18 | Absent in 25g | 10 |
| | 28.11.18 | Absent in 25g | 10 |
| **Sample 2 - Homogenate** | 27.11.18 | Present in 25g | 10 |
| | 28.11.18 | Present in 25g | 10 |
| **Sample 3 - Homogenate** | 27.11.18 | Present in 25g | 10 |
| | 28.11.18 | Present in 25g | 10 |

**Note:** Regulation (EC) No. 2073/2005 requires presence/absence testing for *Salmonella* spp. in live bivalve molluscs.

**Participants' results**

Performance assessment was carried out according to the procedures described in the EURL/PHE EQA shellfish scheme for a single distribution, with minor modifications (Appendix II). Participants' results and scores allocated for PT 76 are shown in Tables 3, 4, 5, 6 and Figures 1, 2 and 3.

**Note:** The median and upper and lower limits (±3 SD and ±5 SD) were calculated from participants' results. $SD_T$ 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 3: Participants' results**

GM - geometric mean, $SD_T$ – theoretical standard deviation (0.24)

| Sample and type | *E. coli* MPN/100g | | | |
|---|---|---|---|---|
| | Range | Median | GM | Median±3*$SD_T$ |
| **Sample 1 - Oysters** | $7.8 \times 10^{-1} – 4.9 \times 10^{3}$ | $2.3 \times 10^{2}$ | $2.0 \times 10^{2}$ | $4.4 \times 10^{1} – 1.2 \times 10^{3}$ |
| **Sample 2 - Homogenate** | $7.9 \times 10^{1} – 1.6 \times 10^{5}$ | $1.7 \times 10^{4}$ | $1.8 \times 10^{4}$ | $3.2 \times 10^{3} – 9.0 \times 10^{4}$ |
| **Sample 3 - Homogenate** | $1.1 \times 10^{2} – 3.5 \times 10^{4}$ | $4.9 \times 10^{3}$ | $4.9 \times 10^{3}$ | $9.3 \times 10^{2} – 2.6 \times 10^{4}$ |

**Table 4: Summary statistics of participants' results**

| *E. coli* | Sample 1 - Oysters | Sample 2 - Homogenate | Sample 3 - Homogenate |
|---|---|---|---|
| Participants reporting duplicate results for *E. coli* MPN | 38 | 37 | 37 |
| Participants reporting a single MPN result | 1 | 2 | 2 |
| Participants reporting both replicate MPN results within expected range[1] | 31 | 31 | 31 |
| Participants reporting a single MPN result within expected range[1] | 1 | 2 | 2 |
| Participants reporting one replicate MPN result outside expected range | 5 | 4 | 3 |
| Participants reporting both replicate MPN results outside expected range | 2 | 2 | 3 |
| Participants reporting one replicate MPN results as censored results | 0 | 1 | 0 |
| Participants reporting both replicate MPN results as censored results | 0 | 0 | 0 |
| Participants reporting tube combination and / or MPN results inconsistent with ISO 7218[2] | 6 | 5 | 5 |

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

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

**Table 5: Participants' results and allocated scores for Sample 1, 2 and 3 *E. coli***

| Lab ID | Sample 1 | | | Sample 2 | | | Sample 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* MPN/100g | | | *E. coli* MPN/100g | | | *E. coli* MPN/100g | | |
| | Rep 1 | Rep 2 | Score | Rep 1 | Rep 2 | Score | Rep 1 | Rep 2 | Score |
| 3 * | 1300 | 490 | 9 | 7000 | 35000 | 12 | 7900 | 11000 | 12 |
| 7 * | 130 | 490 | 12 | 24000 | 13000 | 12 | 4900 | 4900 | 12 |
| 9 * | 490 | 330 | 12 | 24000 | 11000 | 12 | 4600 | 11000 | 12 |
| 10 * | 450 | 230 | 12 | 24000 | 7900 | 12 | 3300 | 7900 | 12 |
| 13 * | 170 | 130 | 12 | 17000 | 17000 | 12 | 13000 | 7900 | 12 |
| 17 * | 330 | 330 | 12 | 24000 | 35000 | 12 | 4900 | 7900 | 12 |
| 19 * | 490 | 230 | 12 | 13000 | 22000 | 12 | 3300 | 4900 | 12 |
| 20 [a] | 50 | 50 | 8 | 13000 | 17000 | 8 | 7900 | 13000 | 8 |
| 22 * | 130 | 110 | 12 | 28000 | 35000 | 12 | 2300 | 3300 | 12 |
| 23 * | 690 | 490 | 12 | 35000 | 35000 | 12 | 11000 | 13000 | 12 |
| 27 * | 230 | 130 | 12 | 24000 | 13000 | 12 | 3300 | 7000 | 12 |
| 32 * | 130 | 130 | 12 | 92000 | 54000 | 9 | 22000 | 24000 | 12 |
| 33 * | 93 | 780 | 12 | 92000 | 17000 | 9 | 2700 | 13000 | 12 |
| 35 * | 170 | 330 | 12 | 35000 | 54000 | 12 | 3300 | 13000 | 12 |
| 39 * | 330 | 330 | 12 | 24000 | 24000 | 12 | 4900 | 13000 | 12 |
| 41 * [a] | 230 | 780 | 8 | 24000 | 13000 | 8 | 11000 | 3300 | 8 |
| 42 * | 450 | 3300 | 9 | 11000 | 7900 | 12 | 2300 | 3300 | 12 |
| 43 * | 170 | 210 | 12 | 7000 | 3300 | 12 | 690 | 490 | 6 |
| 44 * [a] | 230 | 230 | 8 | 24000 | 24000 | 8 | 330 | 330 | 2 |
| 47 * | 130 | 170 | 12 | 160000 | 35000 | 9 | 35000 | 17000 | 9 |
| 68 * | 110 | 93 | 12 | 17000 | 24000 | 12 | 13000 | 11000 | 12 |
| 69 * [c] | NE | NE | - | NE | NE | - | NE | NE | - |
| 83 * | 490 | 690 | 12 | 92000 | 92000 | 9 | 35000 | 17000 | 9 |
| 90 * | 78 | 270 | 12 | 17000 | 35000 | 12 | 4900 | 3300 | 12 |
| 92 | 330 | 170 | 12 | 35000 | 35000 | 12 | 2300 | 2300 | 12 |
| 98 | NR | NR | 0 | NR | NR | 0 | NR | NR | 0 |
| 100 | 490 | 330 | 12 | 14000 | 4900 | 12 | 3300 | 7000 | 12 |
| 102 * | 690 | 4900 | 7 | 24000 | NE | 12 | 4900 | NE | 12 |
| 126 | 140 | 20 | 9 | 4900 | 13000 | 12 | 3300 | 4900 | 12 |
| 138 [a] | 170 | 210 | 8 | 54000 | 9400 | 12 | 11000 | 7900 | 12 |
| 144 | 110 | NE | 12 | 9200 | NE | 12 | 9200 | NE | 12 |
| 147 * | 20 | 170 | 9 | 13000 | 17000 | 12 | 2100 | 4900 | 12 |
| 149 | 2300 | 2300 | 6 | 11000 | 7900 | 12 | 3100 | 3300 | 12 |
| 168 | 110 | 78 | 12 | 17000 | 22000 | 12 | 7900 | 7900 | 12 |
| 170 * [b] | 480 | 400 | 8 | 13400 | 12340 | 8 | 4140 | 3860 | 8 |
| 187 | 330 | 330 | 12 | 35000 | 17000 | 12 | 4900 | 4900 | 12 |
| 189 | 110 | 78 | 12 | 17999 | 16000 | 12 | 9200 | 16000 | 12 |
| 203 | 130 | 78 | 12 | 13000 | 92000 | 9 | 7900 | 4900 | 12 |
| 212 * [b] | <67 | <67 | 8 | 11000 | 15000 | 8 | 580 | 2300 | 6 |
| 235 [a] | 0.78 | 0.78 | 0 | 79 | 130 | 0 | 170 | 110 | 0 |
| 245 * [a] | 78 | 230 | 8 | 24000 | 13000 | 8 | 7900 | 4900 | 8 |

* Designated NRL's,

NE – Not examined.

NR – Not returned.

[a] Scores deducted as tube combination inconsistent with rules specified in ISO 7218.

[b] MPN tube combination is not required for this method, the maximum overall score is reduced to reflect this (8).

[C] Samples were not examined as the material was not delivered within the specified time frame.

**Table 6: Participants' results and allocated scores for Sample 1, 2 and 3 *Salmonella spp.***

| Lab ID | Sample 1 | | Sample 2 | | Sample 3 | |
|---|---|---|---|---|---|---|
| | *Sal.* spp. in 25g | | *Sal.* spp. in 25g | | *Sal.* spp. in 25g | |
| | Rep 1 | Score | Rep 1 | Score | Rep 1 | Score |
| **3 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **7 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **9 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **10 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **13 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **17 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **19 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **20** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **22 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **23 *** | Detected | 0 | Detected | 2 | Detected | 2 |
| **27 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **32 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **33 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **35 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **39 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **41 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **42 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **43 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **44 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **47 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **68 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **69 * [a]** | NE | - | NE | - | NE | - |
| **83 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **90 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **92** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **98** | NR | 0 | NR | 0 | NR | 0 |
| **100** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **102 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **126** | NE | - | NE | - | NE | - |
| **138** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **144** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **147 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **149** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **168** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **170 *** | NE | - | NE | - | NE | - |
| **187** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **189** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **203** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **212 *** | NE | - | NE | - | NE | - |
| **235** | Not Detected | 2 | Detected | 2 | Detected | 2 |
| **245 *** | Not Detected | 2 | Detected | 2 | Detected | 2 |

* Designated NRL's,

NE – Not examined.

NR – Not returned.

[a] Samples were not examined as the material was not delivered within the specified time frame.

**General comments**

Forty-one laboratories (26 NRLs and 13 other laboratories) were sent material with 39 laboratories returning results. Information provided by laboratories on the arrival time of the material showed that 1 laboratory received material the day of dispatch, 16 (41%) laboratories received the material the day after dispatch (27th November 2018), with 4 (25%) of these laboratories analysing the material on arrival. Twenty-one laboratories received material on the 28th (16 labs) and 29th (5 Labs). In total 16 (41%) of laboratories analysed the material on the same day as receiving the material. Laboratory 69 did not analyse the material due to delays during distribution causing the sample to arrive on the 6th December (ten days after the dispatch date).

Arrival temperatures were recorded in the range of -0.7 – 10.0°C. All temperature data, arrival and analysis dates and times recorded by participants are shown in Appendix I.

**Sample analyses**

Thirty-nine laboratories returned the report form for this PT distribution. Laboratory 98 did not return the report form with an explanation for why the samples were not examined and were given a score of 0 for all samples. Laboratories 126, 170 and 212 did not analyse the samples for *Salmonella* spp.. Laboratory 102 and 144 did not analyse for *E. coli* in duplicate for one or more of the samples. Laboratory 144 and 189 did not analyse the samples for *E. coli* using the recommended dilutions series. The EURL recommends that all laboratories read the accompanying paperwork before performing any analysis to ensure the correct examinations are performed on the material.

**Sample 1 – Oysters**

***E. coli* –** Thirty-two laboratories returned (single or duplicate) *E. coli* MPN/100g results falling between ±3 SD of the participants' median with 27 laboratories (85%) obtaining full marks. Laboratories 3, 42, 126, 147 and 149 reported 1 replicate result between ±3 and ±5 SD of the participants' median. Laboratory 102 reported one replicate results and Laboratory 235 reported both replicates outside ±5 SD of the participants' median.

Six laboratories (laboratories 20, 41, 44, 138, 235 and 245) had points deducted for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables or for the reporting of an incorrect MPN value for the reported tube combination. Participants are reminded that for official control testing of live bivalve molluscs in the EU, the 5 x 3 MPN tables or MPN calculator in ISO7218:2007/Amd1:2013 and the EURL generic protocol for enumeration of *E. coli* in bivalve molluscs (Issue 14) should be used.

***Salmonella* spp. –** Thirty-six laboratories returned results for *Salmonella* spp. with 35 correctly reporting the absence of *Salmonella* spp. in Sample 1 and received a score of 2. Laboratory 23 detected the presence of *Salmonella* spp. in Sample 1 and received a score of 0. Laboratory 23 carried out serotyping on the isolated colonies and detected *Salmonella* Anatum (10:e,h:1,6).

**Sample 2 – Homogenate**

***E. coli* –** Thirty-three laboratories returned (single or duplicate) *E. coli* MPN/100g results falling between ±3 SD of the participants' median with 29 laboratories (88%) obtaining full marks. Laboratories 32, 33, 47, 83 and 203 reported 1 replicate result between ±3 and ±5 SD of the participants' median. Laboratory 235 reported both replicates outside ±5 SD of the participants' median.

Five laboratories (laboratories 20, 41, 44, 235 and 245) had points deducted for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables or the reporting of incorrect MPN value for the reported tube combination.

***Salmonella* spp. –** Thirty-six laboratories returned results for *Salmonella* spp. with all correctly reporting the

presence of *Salmonella* spp. in Sample 2 and received a score of 2.

**Sample 3 – Homogenate**

***E. coli* –** Thirty-three laboratories returned (single or duplicate) *E. coli* MPN/100g results falling between ±3 SD of the participants' median with 30 laboratories (91%) obtaining full marks. Laboratories 44, 47, 83 and 212 reported 1 replicate result and Laboratory 42 reported both replicates between ±3 and ±5 SD of the participants' median. Laboratory 235 reported both replicates outside ±5 SD of the participants' median.

Five laboratories (laboratories 20, 41, 44, 235 and 245) had points deducted for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables or the reporting of incorrect MPN value for the reported tube combination.

***Salmonella* spp. –** Thirty-six laboratories returned results for *Salmonella* spp. with all correctly reporting the presence of *Salmonella* spp. in Sample 2 and received a score of 2.

**Summary**

Twenty laboratories (52%) achieved full marks for all 3 samples tested for the enumeration of *E. coli*. For this distribution the EURL recommended participants to analyse the sample with 4 dilutions. Laboratories who did not follow the advice given in ISO7218:2007/Amd1:2013 and/or the EURL generic protocol for calculating the MPN value for *E. coli* incurred a deduction (6 laboratories). Laboratories are requested to note from ISO ISO7218:2007/Amd1:2013 that '*In any circumstance when more than three dilutions are made, it is essential that all measured data values be used. It is not scientifically correct to "select" any combination of values on the premise that these values are more "correct" than other combinations. The results from all possible combinations of positive tubes should be recorded and the MPN calculator (http://standards.iso.org/iso/7218/) used to derive MPN values*'.

Those laboratories who achieved <40% of the maximum possible score in this distribution for *E. coli* enumeration (<5 out of the maximum 12 score) and / or *Salmonella* spp. detection (score of 0) should review their laboratory procedures. In the first instance refer to the troubleshooting guide included as Appendix III. However, further guidance is available from the EURL.

**References**

Anon 2001. ISO 16649-2. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44˚C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.

Anon 2007. ISO 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiology examinations.

Anon 2013. ISO 7218:2007/FDAM 1:2013. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations - Amendment 1.

Anon 2010. ISO TS 22117:2010. Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison.

Anon 2015. 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.

Anon 2017. ISO 6579-1. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp..

**Figure 1: Results chart Sample 1 – Pacific oysters**

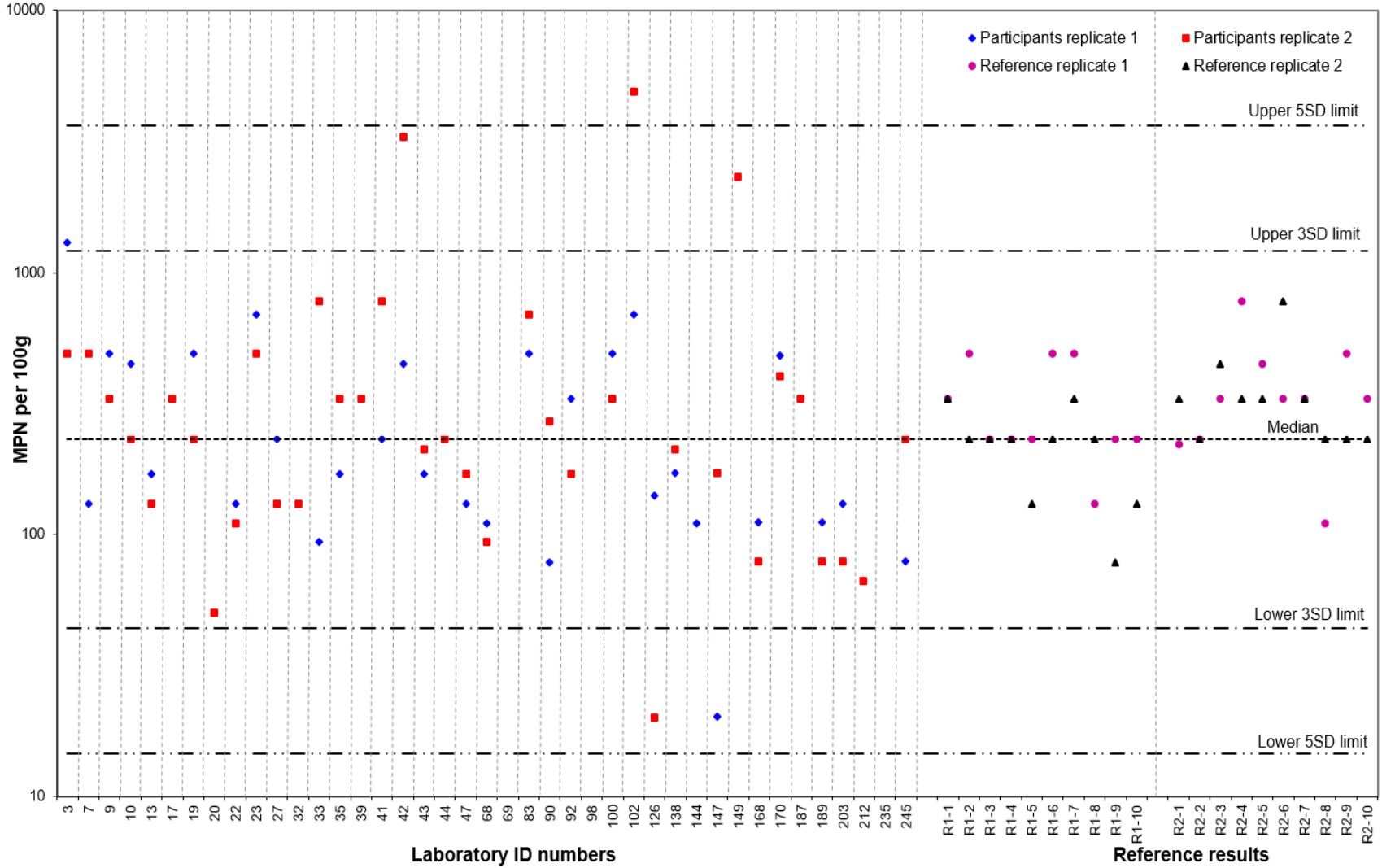


**Figure 2: Results chart Sample 2 – Shellfish homogenate**

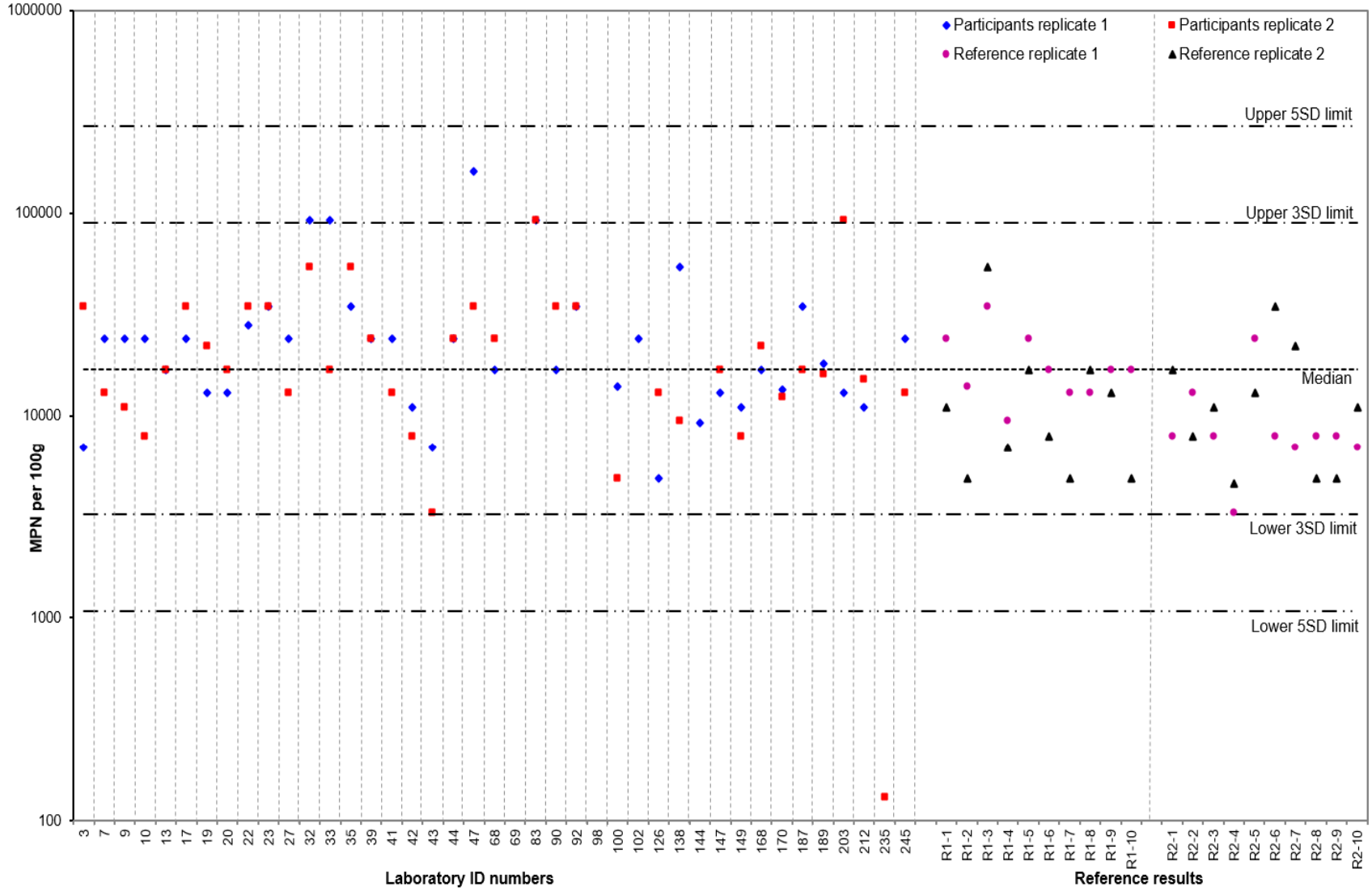
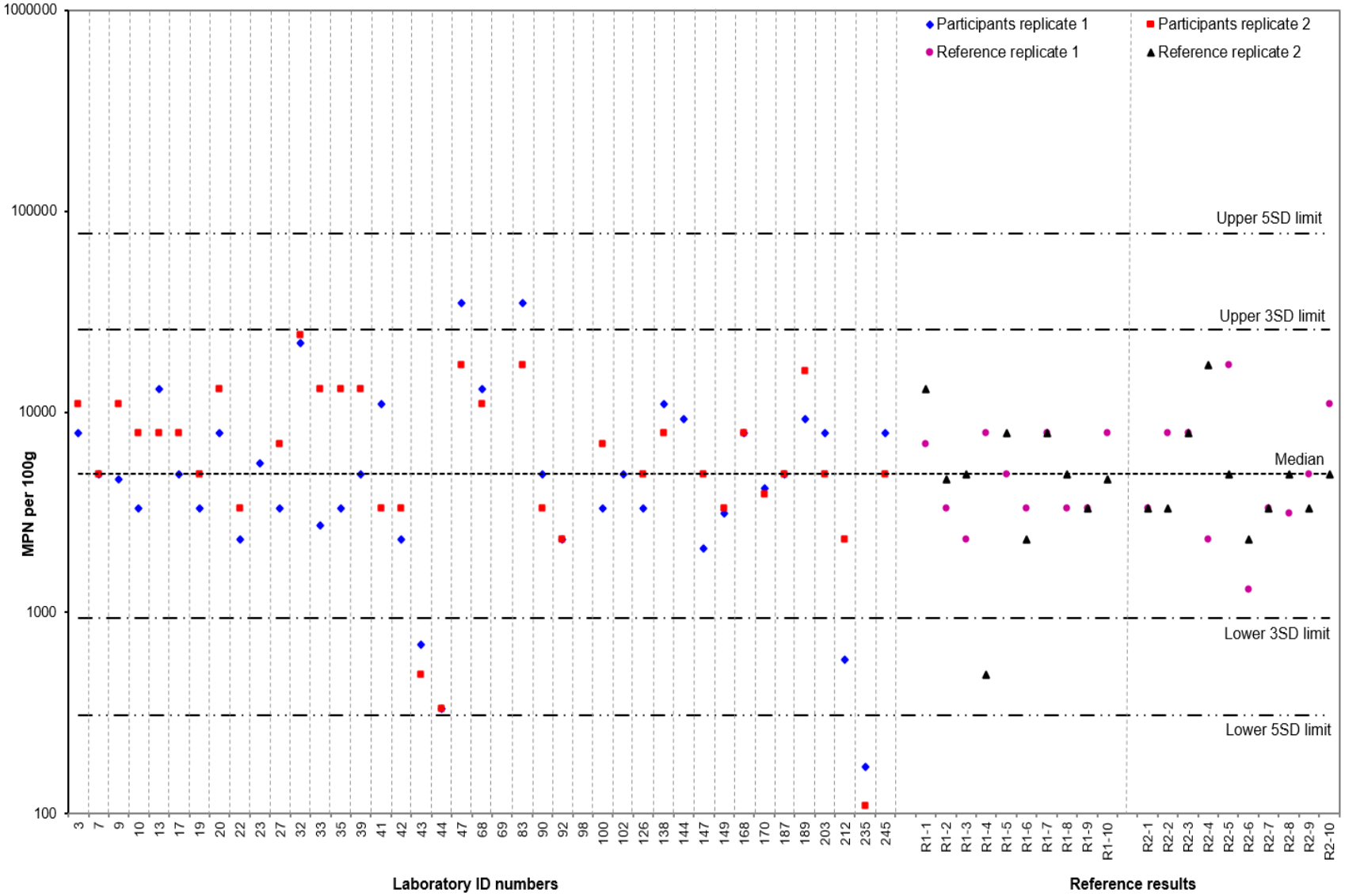


**Figure 3: Results chart Sample 3 – Shellfish homogenate**



**Appendix I**

**Sample arrival and temperature**

| Lab ID | Date arrived | Time of arrival | Sample (°C) | Storage (°C) | Date analysed |
|---|---|---|---|---|---|
| 3 * | 28.11.18 | 18:00 | | | 29.11.18 |
| 7 * | 28.11.18 | 14:30 | 6.4 | 3±2 | 29.11.18 |
| 9 * | 28.11.18 | 13:30 | 2 | 3.0 | 28.11.18 |
| 10 * | 27.11.18 | 11:00 | 1 | N/A | 27.11.18 |
| 13 * | 27.11.18 | 13:05 | 0.5 | 3.0 | 28.11.18 |
| 17 * | 27.11.18 | 16:30 | 4.3 | 3±2 | 28.11.18 |
| 19 * | 27.11.18 | 13:30 | 3.5 | 5.0 | 28.11.18 |
| 20 | 27.11.18 | 09:15 | 5 | 4.0 | 27.11.18 |
| 22 * | 28.11.18 | 13:25 | 4.5 | N/A | 28.11.18 |
| 23 * | 27.11.18 | 11:10 | 2.9 | 4.0 | 28.11.18 |
| 27 * | 28.11.18 | 12:55 | 0.8 | N/A | 28.11.18 |
| 32 * | 28.11.18 | 13:00 | 4 | N/A | 28.11.18 |
| 33 * | 28.11.18 | 14:30 | -0.7 | 3±2 | 29.11.18 |
| 35 * | 27.11.18 | 13:00 | 3.8 | 2.5 | 28.11.18 |
| 39 * | 27.11.18 | 11:30 | 3.9 | 3±2 | 28.11.18 |
| 41 * | 28.11.18 | 11:00 | 2.8 | 3±2 | 28.11.18 |
| 42 * | 28.11.18 | 11:20 | 10 | 5.0 | 03.12.18 |
| 43 * | 29.11.18 | 15:55 | 2 | 4.0 | 03.12.18 |
| 44 * | 28.11.18 | 12:45 | 3.5 | 3±2 | |
| 47 * | 28.11.18 | 11:30 | 1 | N/A | 28.11.18 |
| 68 * | 29.11.18 | 11:05 | 4 | 4.0 | 29.11.18 |
| 69 * | Delays in dispatch. Samples not examined | | | | |
| 83 * | 30.11.18 | 13:00 | 2 | 4.0 | 30.11.18 |
| 90 * | 29.11.18 | 08:45 | 2.9 | 3.9 | 30.11.18 |
| 92 | 29.11.18 | 11:30 | 4.6 | N/A | 29.11.18 |
| 98 | NR | NR | NR | NR | NR |
| 100 | 28.11.18 | 14:30 | 6.8 | 2.0 | 29.11.18 |
| 102 * | 27.11.18 | 11:06 | 1 | 3.0 | 27.11.18 |
| 126 | 27.11.18 | 17:10 | 2.6 | 3.0 | 28.11.18 |
| 138 | 27.11.18 | 16:30 | 1.9 | 6 - 10C | 28.11.18 |
| 144 | 27.11.18 | 13:45 | 2 | 3.0 | 27.11.18 |
| 147 * | 26.11.18 | 13:00 | 4 | 4.0 | 30.11.18 |
| 149 | 28.11.18 | 15:25 | 2.61 | 2.3 | 29.11.18 |
| 168 | 27.11.18 | 12:00 | 10 | 4.0 | 28.11.18 |
| 170 * | 27.11.18 | 13:30 | 3.5 | 5.0 | 28.11.18 |
| 187 | 27.11.18 | 14:45 | 5 | 3.0 | 28.11.18 |
| 189 | 27.11.18 | 11:50 | 2 | 5.0 | 28.11.18 |
| 203 | 28.11.18 | 13:00 | 7.4 | N/A | 28.11.18 |
| 212 * | | | | | 28.11.18 |
| 235 | 28.11.18 | 12:00 | 2.8 | N/A | 29.11.18 |
| 245 * | 29.11.18 | 12:05 | 0.5 | N/A | 29.11.18 |

* Designated NRLs
NR – Not returned

**Appendix II:**

***E. coli* MPN scores allocated to participants returning 2 replicate results**

| Result | Returning of results | Score allocated | | Total score |
|---|---|---|---|---|
| | | Replicate 1 | Replicate 2 | |
| Both replicate MPN results are within the expected range. | 2 | 5 | 5 | 12 |
| One replicate MPN result is outside the expected range and falls between the median ±3SD and median ±5SD values. | 2 | 5 | 2 | 9 |
| Both replicate MPN results are outside the expected range and fall between the median ±3SD and median ±5SD values. | 2 | 2 | 2 | 6 |
| One replicate MPN result is outside the median ±5SD value. | 2 | 5 | 0 | 7 |
| Both replicate MPN results are outside the expected range. The first falls between the median ±3SD and median ±5SD value and the second falls outside the median ±5SD values. | 2 | 2 | 0 | 4 |
| Both replicate MPN results reported are outside the median ±5SD value. | 2 | 0 | 0 | 2 |

***E. coli* MPN scores allocated to participants returning 1 single replicate result**

| Result | Returning of results | Score allocated | Total score |
|---|---|---|---|
| Single replicate MPN result is within the expected range. | 2 | 5 | 7 |
| Single replicate MPN result is outside the expected range and falls between the median ±3SD and median ±5SD values. | 2 | 2 | 4 |
| Single replicate MPN result reported is outside the median ±5SD value. | 2 | 0 | 2 |

***E. coli* score deductions**

| Result | Score deducted | |
|---|---|---|
| | Replicate 1 | Replicate 2 |
| Tube combination inconsistent with MPN reported and / or tube combination selected not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL. | 2 | 2 |
| High censored result (e.g. MPN = >18000 per 100g). | 2 | 2 |
| Sample not examined or results returned late - no explanation received. | 12 | |

***Salmonella* spp. scoring**

| Result | Score allocated |
|---|---|
| Fully correct results | 2 |
| Misleading result, e.g. failure to isolate *Salmonella* | 0 |

**Appendix III:**

**Troubleshooting advice**

1. **Methods –** Ensure that the method used is appropriate for the examination of the sample.

    a. Ensure that any dilutions have been calculated correctly.
    b. Ensure that the dilutions analysed are as specified on the report form.
    c. Ensure that MPN tables (if used) are interpreted correctly.

    **Interpretation of MPN tables**

    Where three dilutions have been tested for a sample, record the number of TBGA/TBX positives for each dilution to give a three figure tube combination number. Use the MPN tables included in ISO 7218 and the EURL generic *E. coli* protocol. Only category 1 or 2 tube combinations are included in the tables and should be reported.

    Where more than three dilutions have been tested for a sample, use the Excel spreadsheet MPN calculator (http://standards.iso.org/iso/7218/) to determine the MPN from all the dilutions tested. Combinations that do not appear in the tables or obtained from the Excel calculator as category 3 are not acceptable and should not be used.

    If the tube combination result is an unacceptable combination, the result is reported as 'void'.

2. **Culture media** - Check the quality control data for media to ensure that they are within specifications and performing adequately.

3. **Equipment** - Check that the equipment used for the procedures (incubators, refrigerators, measuring instruments) are calibrated and performing adequately.

4. **Staff training** - Check that the staff performing the tests are fully trained and familiar with all the procedural steps.

5. **Clerical procedures** - Check that the sample labeling, laboratory numbering and clerical procedures are adequate and that you have procedures for ensuring that test results are reported accurately and on time.

6. **Accreditation**- Check that quality procedures are documented and adhered to at all times.

7. **Internal quality controls (IQC)** – Ensure adequate controls are in place and follow-up procedures are in place to deal with IQC failures.

Further advice can be obtained from the EURL on request.

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