# European Union Reference Laboratory (EURL)
# Proficiency Testing Scheme

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

### EURL PT reference number: PT 54

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This scheme is intended to provide proficiency testing 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 (EC) No. 882/2004. The scheme is intended to compliment the EURL/PHE Shellfish Scheme (http://www.hpa.org.uk/ProductsServices/MicrobiologyPathology/ExternalQualityAssessmentProficiencyTesting/EQAPTForFoodWaterAndEnvironmentalMicrobiology/ShellfishScheme/ through examination of aspects of the methods not covered under the Shellfish Scheme (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 TS 16649-3, Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (Anon 2005). The EU reference method for detection of *Salmonella* spp. in live bivalve molluscs is ISO 6579, Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Anon 2002).

A scoring system is used to help assess participants' performance. Details of this system are included as Appendix I 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 proficiency testing 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 European Union 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 II of this report.

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

## Sample preparation

### Sample 1

Approximately 4,000 (60kg) Manila clams (*T. phillipinarum*) comprising a single batch were collected from a UK commercial harvesting area. Sample 1 provided to participating laboratories comprised of 50 randomly selected Manila clams from this bulk material.

### Sample 2

Approximately 3,200 (45kg) common mussels (*Mytilus edulis*) comprising a single batch were collected from a UK commercial harvesting area. Sample 2 provided to participating laboratories comprised of 40 randomly selected common mussels from this bulk material.

## Sample distribution and examination

Samples were packed in accordance with Cefas protocol for packaging shellfish for transportation and distributed at 10:30am on the 21st October 2014 to 39 participating laboratories. Participants were requested to analyse the material in duplicate immediately on receipt using their routine laboratory procedures for the enumeration of *E. coli* and the detection of *Salmonella* spp..

## 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 (22.10.14 and 23.10.14) for *E. coli* using EURL SOP No. 1175 (Table 1). Sample homogeneity was assessed following the procedure described in ISO 22117. On analysing the MPN values it was concluded that the common mussel (*M. edulis*) material used for sample 2 was not homogeneous and that further analysis of participants' results data would not be valid. Subsequent investigations showed that mussels which were harvested on two separate days had been combined by the supplier, this is contrary to the identified procedure.

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

| Sample type | Analyses dates | Range | Median | GM | Median ±3*$SD_T$ |
|---|---|---|---|---|---|
| Sample 1 – Manila clams | 22.10.14 | $3.1 \times 10^2$ – $4.9 \times 10^3$ | $1.6 \times 10^3$ | $1.9 \times 10^3$ | $3.0 \times 10^2$ - $8.1 \times 10^3$ |
| | 23.10.14 | $2.3 \times 10^2$ – $7.9 \times 10^3$ | $1.7 \times 10^3$ | $1.2 \times 10^3$ | $3.2 \times 10^2$ - $8.9 \times 10^3$ |

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

### Reference results – *Salmonella* spp.

Ten randomly selected samples were analysed on 2 consecutive days (22.10.14 and 23.10.14) for *Salmonella spp.* using EURL SOP No. 1176 (Table 2). Given that the faecal indicator (*E. coli*) results for sample 2 were not homogenous, it was decided that analysis of participants' data for the detection of *Salmonella* spp. in that sample would not be valid.

**Table 2: *Salmonella* spp. reference results**

| Sample type | Analyses dates | *Salmonella spp.* | No. of replicates giving negative results |
|---|---|---|---|
| Sample 1 – Manila clams | 22.10.14 | *Salmonella* spp. not detected in 25g | 10 |
| | 23.10.14 | *Salmonella* spp. not detected 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 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 54 are shown in Tables 3, 4, 5 and Figure 1.

**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 participants' median.

**Table 3: Participants results**

| Sample type | *E. coli* MPN/100g | | | |
|---|---|---|---|---|
| | Range | Median | GM | Median±3*$SD_T$ |
| Sample 1 – Manila clams | $9.0 \times 10^1$ - $1.7 \times 10^4$ | $1.3 \times 10^3$ | $1.6 \times 10^3$ | $2.5 \times 10^2$ - $6.8 \times 10^3$ |

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

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

| *E. coli* | Sample 1 – Manila clams |
|---|---|
| Participants reporting duplicate results for *E. coli* MPN | 37 |
| Participants reporting a single MPN result | 0 |
| Participants reporting MPN results within the expected range for both replicates[1] | 27 |
| Participants reporting MPN results outside the expected range for one replicate | 7 |
| Participants reporting MPN results outside the expected range for both replicates | 3 |
| Participants reporting MPN results as censored results for one replicate | 0 |
| Participants reporting MPN results as censored results for both replicates | 0 |
| Participants reporting MPN results inconsistent with ISO 7218 (Anon 2007)[2] | 25 |

| *Salmonella* spp. summary statistics | |
|---|---|
| Participants reporting results for *Salmonella* spp. | 35 |
| Participants reporting the presence of *Salmonella* spp. | 3 |
| Participants reporting the absence of *Salmonella* spp. | 32 |

| | |
|---|---|
| Participants not returning results | 2 |
| Participants not receiving material due to problems at customs | 1 |

[1] expected range = participants' median ± theoretical 3SD,
[2] points deducted from participants returning results inconsistent with ISO 7218 who reported using the reference method.

**Table 5: Participants' results and allocated scores**

| Lab ID | Sample 1 – Manila clams | | | | | Lab ID | Sample 1 – Manila clams | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* MPN/100g | | | *Salmonella* spp. in 25g | | | *E. coli* MPN/100g | | | *Salmonella* spp. in 25g | |
| | Replicate 1 | Replicate 2 | Score | Result | Score | | Replicate 1 | Replicate 2 | Score | Result | Score |
| **3 *** | 13000 | 4600 | 9 | NE | - | **48** [a] | 2200 | 2200 | 8 | Not detected | 2 |
| **7 *** [a] | 2800 | 4600 | 8 | Not detected | 2 | **68 *** | 4600 | 1700 | 12 | Not detected | 2 |
| **9 *** [a] | 790 | 1100 | 8 | Not detected | 2 | **69** [a] | 1100 | 330 | 8 | Not detected | 2 |
| **10 *** | 780 | 2200 | 12 | Not detected | 2 | **72** | 490 | 690 | 12 | Not detected | 2 |
| **13 *** [a] | 790 | 1300 | 8 | Not detected | 2 | **83** [a] | 16000 | 9200 | 2 | Not detected | 2 |
| **17 *** [a] | 1300 | 1700 | 8 | Not detected | 2 | **86 *** [a] | 1300 | 2400 | 8 | Not detected | 2 |
| **19 *** [a b] | 2200 | 90 | 3 | Not detected | 2 | **90 *** | 2300 | 4900 | 12 | Not detected | 2 |
| **21 *** [a] | 790 | 1100 | 8 | Not detected | 2 | **92** | 4900 | 4600 | 12 | Not detected | 2 |
| **22 *** [a] | 1300 | 1300 | 8 | Not detected | 2 | **96** | NR | NR | 0 | NR | 0 |
| **23 *** [a] | 330 | 230 | 5 | Not detected | 2 | **98** [a] | 330 | 1100 | 8 | Not detected | 2 |
| **27 *** [a] | 1300 | 2400 | 8 | Not detected | 2 | **100** | 13000 | 17000 | 6 | Not detected | 2 |
| **32 *** | 780 | 780 | 12 | Present | 0 | **131** | 780 | 780 | 12 | Not detected | 2 |
| **33 *** [a] | 490 | 490 | 8 | Not detected | 2 | **147 *** [a] | 230 | 230 | 2 | Not detected | 2 |
| **35 *** [a] | 5400 | 9200 | 5 | Present | 0 | **170 *** | 1300 | 1340 | 12 | Not detected | 2 |
| **39 *** [a] | 3500 | 3500 | 8 | Not detected | 2 | **177** | IP | IP | 0 | IP | 0 |
| **41 *** | 7000 | 1700 | 9 | Not detected | 2 | **203** [a] | 2400 | 3500 | 8 | Not detected | 2 |
| **42 *** [a] | 16000 | 16000 | 2 | Not detected | 2 | **212 *** | 1400 | 2800 | 12 | NE | - |
| **43 *** [a] | 7000 | 5400 | 5 | Not detected | 2 | **236** [a b] | 790 | 330 | 6 | Not detected | 2 |
| **44 *** [a] | 490 | 490 | 8 | Not detected | 2 | **245** [a] | 490 | 490 | 8 | Not detected | 2 |
| **47 *** [a] | 490 | 330 | 8 | Present | 0 | | | | | | |

[a] Scores deducted as tube combination inconsistent with rules specified in ISO 7218.
[b] Score deducted as incorrect MPN value given for recorded tube combination.
* Designated NRL's,
IP – Laboratory experienced import problems and was unable to receive material.
NE – Not examined,
NR – Not returned.

**General comments**
Thirty-nine laboratories (27 NRL and 12 other laboratories) were sent material with only one laboratory not receiving the material due to problems at customs. Laboratory 96 did not return their results. Information provided by laboratories on the temperature and arrival time of the material showed that 82% (32) of laboratories received the material the day after dispatch (22.10.14) with 13% (5) of these laboratories analysing the material on arrival. Temperature data, arrival and analysis dates and times recorded by participants are shown in Appendix I.

**Sample preparation**
The homogenising of shellfish material for both *E coli* and *Salmonella* spp. testing is predominately performed using a stomacher with 62% (23 laboratories) of participants using this procedure.

**Methodology**
Thirty-seven laboratories provided information on the methodology used for *E. coli* and *Salmonella* spp. analyses and are shown in Tables 6 and 7.

***E. coli***
Ninety-two percent (24 NRLs and 10 other laboratories) of laboratories cited ISO TS 16649-3 (Anon 2005) as their laboratory method for the enumeration of *E. coli.* Twenty-seven laboratories stated the MPN tables provided by the EURL were used to calculate the MPN value. Of these only 4 laboratories correctly included all MPN dilutions to obtain the MPN value. Eight laboratories obtained the MPN value using the MPN calculator of for which 2 laboratories did not use the correct tube combination to obtain the MPN value.

**Table 6: *E. coli* methodology**

| ***E. coli* methods** | **Number of laboratories** |
|---|---|
| **ISO TS 16649-3 (Anon 2005)** | 34 |
| **NMKL 96** | 1 |
| **BACTRAC 4300** | 1 |
| **ISO 16649-2 (Anon 2001)** | 1 |

***Salmonella* spp.**
Eighty-three percent (21 NRLs and 8 other laboratories) of laboratories cited ISO 6579 (Anon 2005) as their laboratory method for the detection of *Salmonella* spp. with a further 3 laboratories citing ISO 6579 with supplementary confirmation tests as their laboratory method.

**Table 7: *Salmonella* spp. methodology**

| ***Salmonella spp.* methods** | **Number of laboratories** |
|---|---|
| **ISO 6579 (Anon 2002)** | 32 |
| **NMKL 71** | 2 |
| **VIDAS *Salmonella* (SLM)** | 1 |
| **Rapid sal. AFNOR** | 1 |
| **Rapid sal. BioRad** | 1 |
| **PCR** | 1 |

**Sample analyses**
Thirty-seven laboratories returned the report form for this PT distribution. Laboratory 96 did not return their results and Laboratory 177 experienced problems at customs. Laboratory 3 and 212 did not examine the material for *Salmonella* spp..

**Sample 1 – Manila Clams**
***E. coli***
Twenty-seven laboratories returned duplicate *E. coli* MPN/100g results falling between ±3 SD of the participants' median with 9 laboratories obtaining a score of 12. Laboratories 3, 19, 23, 35, 41 and 43 reported one replicate

result within ±3 SD of the participants' median. Laboratories 42, 83, 100, and 147, reported both replicate results outside ±3 SD of the participants' median.

Twenty-five laboratories (laboratories 7, 9, 13, 17, 19, 21, 22, 23, 27, 33, 35, 39, 42, 43, 44, 47, 48, 69, 83, 86, 98, 147, 203, 236 and 245) were deducted points for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables. Laboratory 19 reported MPN values inconsistent with the tube combination provided. 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/FDAM1:2013 and the EURL generic protocol for enumeration of *E. coli* in bivalve molluscs (Issue 10) should be used.

***Salmonella* spp.**

Thirty-two laboratories returned results for *Salmonella* spp. and reported the absence of *Salmonella* spp. in sample 1 as expected. Laboratories 32, 35 and 47 reported the presence of *Salmonella* spp. Laboratories 3 and 212 did not examine this sample for *Salmonella* spp.. Laboratory 96 did not return any results.

**Summary**

Nine laboratories (24%) achieved full marks for results reported for the enumeration of *E. coli*. For this distribution the EURL recommended participants to analyse the sample with 4 dilutions. Laboratories who incurred a deduction (25 laboratories) in score were due to the advice given in ISO7218:2007/FDAM1:2013 and/or the EURL generic protocol for calculating the MPN value for *E. coli* was not followed. Of these laboratories, 24 specified the methodology used to calculate the MPN value was the reference method (ISO 16649-3) and the MPN tables. Laboratories are requested to note in ISO ISO7218:2007/FDAM1:2013 it states '*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*'.

There was no apparent association between the incorrect reporting of *Salmonella* spp. and the sample analysis and methodology data reported for the detection of *Salmonella* spp..

For 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 (<1 out of the maximum 2 score) should in the first instance refer to the troubleshooting guide included as Appendix III.

Note: The EURL will perform further analyses on the data and methodology reported in this distribution to assess the performance of Stomaching and blending of shellfish material.

**References**

Anon 2005. ISO TS 16649-3. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. Geneva, Switzerland.

Anon 2002. ISO 6579. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Geneva, Switzerland.

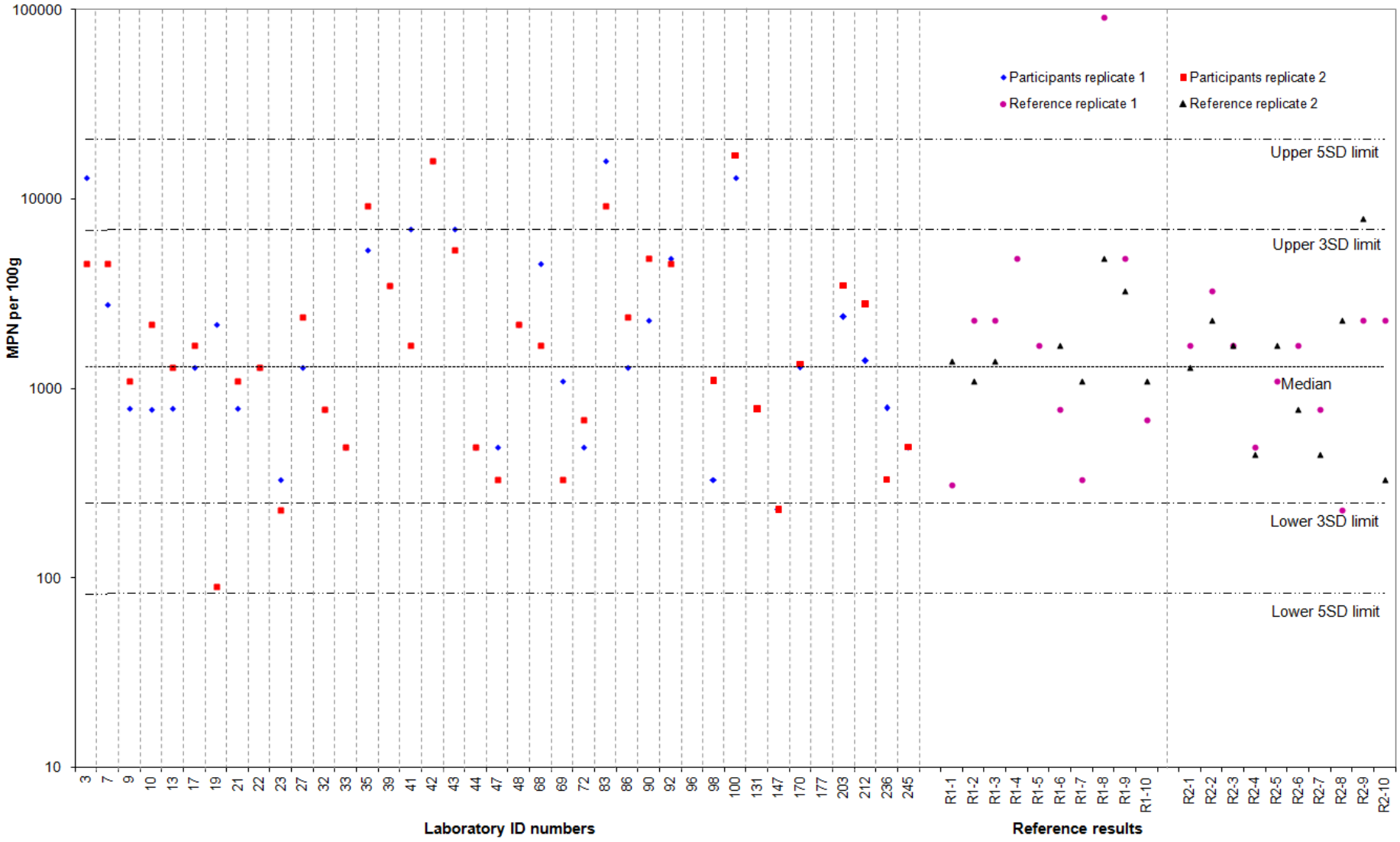
Anon 2013 ISO 7218:2007/FDAM 1:2013, Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations - Amendment 1. International Organization for Standardization, Geneva.

Anon 2001. ISO TS 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. Geneva, Switzerland.

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

## Figure 1: Results chart sample 1 – Manila clams

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



## Appendix I

### Sample arrival and temperature

| Lab ID | Date arrived | Time of arrival | Sample (°C) | Storage (°C) | Date analysed | Time of analysis |
|---|---|---|---|---|---|---|
| **3 *** | 23.10.14 | 09:40 | 3.8 | | 23.10.14 | 11:30 |
| **7 *** | 23.10.14 | 13:20 | 4 | 3 ± 2 | 24.10.14 | 10:30 |
| **9 *** | 22.10.14 | 15:30 | 7 | 4 - 5 | 22.10.14 | 16:30 |
| **10 *** | 22.10.14 | 11:59 | 7.7 | | 22.10.14 | 14:30 |
| **13 *** | 27.10.14 | 12:15 | 5.5 | 5 | 27.10.14 | 13:00 |
| **17 *** | 22.10.14 | 14:00 | 4 | | 22.10.14 | 14:00 |
| **19 *** | 22.10.14 | 13:10 | 3.2 | 4 | 22.10.14 | 15:00 |
| **21 *** | 22.10.14 | 13:15 | 4 | 4 | 22.10.14 | 13:15 |
| **22 *** | 22.10.14 | 15:45 | 4.8 | 6.8 | 23.10.14 | 10:00 |
| **23 *** | 22.10.14 | 12:30 | 1.4 | 5 | 23.10.14 | 13:00 |
| **27 *** | 22.10.14 | 12:45 | 3.8 | 5 ± 3 | 23.10.14 | 10:00 |
| **32 *** | 22.10.14 | 09:00 | 4 | 4 | 22.10.14 | 09:30 |
| **33 *** | 22.10.14 | 15:40 | 1.4 | 2 | 23.10.14 | 08:15 |
| **35 *** | 22.10.14 | 12:30 | 2.8 | 1.8 | 22.10.14 | 14:30 |
| **39 *** | 22.10.14 | 12:30 | 3 | 3 ± 2 | 22.10.14 | 14:00 |
| **41 *** | 22.10.14 | 11:45 | 3.2 | 4 | 22.101 | 13:45 |
| **42 *** | 22.10.14 | 12:15 | 4 | 4 | 22.10.14 | 14:00 |
| **43 *** | 22.10.14 | 15:30 | 4 | 3 - 5 | 28.10.14 | 14:30 |
| **44 *** | 22.10.14 | 16:30 | 2 | 3 ± 2 | 23.10.14 | 10:00 |
| **47 *** | 22.10.14 | 13:40 | 2.2 | 3 | 22.10.14 | 18:10 |
| **48** | 23.10.14 | 09:00 | 1.5 | 4 - 5 | 23.10.14 | 13:00 |
| **68 *** | 22.10.14 | 12:30 | 1.6 | | 22.10.14 | 12:45 |
| **69** | 22.10.14 | 12:03 | 4 | | 22.10.14 | 13:00 |
| **72** | 22.10.14 | 13:30 | 1.6 | | 22.10.14 | 13:30 |
| **83** | 22.10.14 | 13:10 | 4 | 5 | 22.10.14 | 16:00 |
| **86 *** | 22.10.14 | 15:00 | 4.5 | 5 | 23.10.14 | 12:10 |
| **90 *** | 23.10.14 | 09:00 | 6 | 7 | 23.10.14 | 11:30 |
| **92** | 23.10.14 | 14:40 | 3.5 | 3.1 | 24.10.14 | 10:05 |
| **96** | 22.10.14 | 14:55 | | | | |
| **98** | 22.10.14 | 14:00 | 6.7 | 4 | 22.10.14 | 14:30 |
| **100** | 22.10.14 | 14:15 | 4.5 | | 22.10.14 | 14:30 |
| **131** | 22.10.14 | 11:00 | 1.3 | 4.5 | 22.10.14 | 14:30 |
| **147 *** | 22.10.14 | 13:00 | 4 | 4 | 22.10.14 | 16:00 |
| **170 *** | 22.10.14 | 13:10 | 3.2 | 4 | | |
| **177** | | | | | | |
| **203** | 22.10.14 | 13:00 | <5 | | 22.10.14 | 13:25 |
| **212 *** | 22.10.14 | 11:45 | 3.2 | 4 | | |
| **236** | 22.10.14 | 12:00 | | 3 ± 2 | 23.10.14 | |
| **245** | 22.10.14 | 13:30 | 1.6 | 2 - 4 | 22.10.14 | 10:30 |

* Designated NRL's

## Appendix II:

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

| Reported results | Returning of results | Replicate 1 | Replicate 2 | Total score |
|---|---|---|---|---|
| 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 replicates 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 replicates MPN results are outside the expected range. The first falls between the median ±3SD and median ±5SD values and the second falls outside the median ±5SD values | 2 | 2 | 0 | 4 |
| Both replicates MPN results is outside the median ±5SD value | 2 | 0 | 0 | 2 |

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

| 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 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/FDAM1:2013 or MPN tables provided by the EURL. | 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**

    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 have you 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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