



European Union Reference Laboratory (EURL) and Public Health England (PHE) EQA Shellfish Scheme

Escherichia coli and *Salmonella* spp. EQA

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Article 32 of Regulation (EC) 882/2004 sets out the organisational responsibilities for EU Reference Laboratories (EURL) with respect to comparative proficiency testing (PT).

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

The scheme is organised in collaboration with the Public Health England (PHE) (<http://www.hpa.org.uk/ProductsServices/InfectiousDiseases/ExternalQualityAssessmentProficiencyTesting/EQAPTForFoodWaterAndEnvironmentalMicrobiology/ShellfishScheme/>).

The EU reference method for enumeration of *E. coli* in raw bivalve molluscs is 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. 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 *Escherichia coli* in bivalve molluscan shellfish by the colony-count technique'. Protocols for the application of these methods are available at www.eurlcefafas.org

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).

These methods must be used for official control testing of live bivalve molluscs for compliance with EU Regulations.

Performance assessments are valuable tools to help laboratories identify any ongoing problems with their procedures or analyses. Scores are given for each distribution to assess participants' performance and to highlight any incorrect or outlying results.

If you are experiencing problems please contact the EURL, or alternately refer to the troubleshooting guide included as Appendix VI of this report. Further advice on microbiological testing of bivalve molluscan shellfish can be obtained via the EURL website www.eurlcefafas.org

Methodology

Sample preparation - PHE EQA

Samples distributed consisted of LENTICULE™ discs containing fully characterised bacterial isolates. The proportions and types of organisms were designed to mirror those found in freshly harvested bivalve molluscs. Samples were packaged according to IATA regulations and distributed with report forms.

Sample preparation – EURL PT

Two shellfish samples comprising of common mussels (*M. edulis*) and Pacific oysters (*C. gigas*) both originating from a UK commercial harvesting area were distributed in November 2015. Samples were packaged according to IATA regulations and distributed with instructions and report forms.

Reference results

For each distribution 10 reference samples were examined by the organising laboratory. Reference analyses were performed using ISO TS 16649-3 for the enumeration of *E. coli* and ISO 6579 for the detection of *Salmonella* spp..

Participants' analysis and scoring system

Reported *E. coli* MPN values were compared to the median MPN from all participants' results, reference results were omitted from the calculation. The acceptable limits were calculated as the participants' median ± 2.68 standard deviation (SD) and ± 4 SD above and below the participants' medium. Reported MPN values were \log_{10} transformed before being compiled into charts are shown in Appendix 1 to 3. Performance assessment was according to the algorithm in Appendix 5.

Participation in statutory determinands

All samples were analysed using participants official control methods i.e. those methods routinely used for official control analysis of live bivalve molluscs. Table 1 shows the participation of NRLs for 2015 with 84% participating in the EURL matrix distribution and 1 or more EQA distributions. Currently there is no active participation from a designated NRL in Malta, Cyprus, The Czech Republic or Estonia. NRLs in Bulgaria, Denmark, Finland, Greece and Latvia did not participate in the mandatory number of EQA distributions and the EURL matrix distribution per year agreed in Resolution 8 of the NRLs annual workshop 2012. The EURL recommends that NRLs not registered to the EQA scheme should join this scheme.

Table 1: Participation by NRLs in 2015 for *E. coli* and *Salmonella* spp. determinands.

Country	Austria	Belgium and Luxembourg	Bulgaria	Croatia	Denmark	Finland	France	Germany	Greece	Hungary	Iceland	Ireland	Italy	Latvia	Lithuania	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	United Kingdom
EURL PT 60	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PHE EQA distributions	0		✓			✓			✓					✓											
	1									✓	✓				✓						✓				
	2		✓		✓	✓	✓	✓					✓				✓		✓				✓	✓	
	3	✓										✓				✓		✓		✓		✓			✓

Performance Assessment

A cumulative performance assessment was undertaken on participant's results for both *E. coli* and *Salmonella* spp. from the EURL matrix distribution (PT 60) (Appendix 4) and 3 EQA distributions (March to November 2015). The allocated scores are summarised in Tables 2 and 3 respectively. Good performance is identified where a cumulative score of >70% is achieved. Participants who achieved <70% for *E. coli* enumeration and/or *Salmonella* spp. detection should in the first instance refer to the troubleshooting guide included as Appendix 6.

E. coli MPN assessment

Twenty-one laboratories participated in the EURL matrix scheme (Appendix 4) and 1 or more EQA distributions in 2015 and were therefore subject to a full performance assessment. All 21 laboratories that were subjected to the full assessment achieved a cumulative total of >70% for the two or more distributions analysed. NRLs Bulgaria, Denmark, Finland, Greece and Latvia did not participate in the mandatory number of EQA distributions (1) and the EURL matrix distribution per year agreed in Resolution 8 of the NRLs annual workshop 2012.

Table 2: Summary of participants' performance in the EURL matrix scheme and the EQA scheme - *E. coli*

Lab no. ^a	PT 60		Distribution SF050		Distribution SF051		Distribution SF052		All distributions		
	Sample 1	Sample 2	SF0108	SF0109	SF0110	SF0111	SF0112	SF0113	Cumulative score	Max score	%
121 [19]	12	12	12	12	12	8	12	5	85	96	89
391 [9]	-	12	12	12	-	-	12	12	60	60	100
403 [86] ^b	-	-	-	-	12	12	7	2	-	-	0
413 [35]	12	12	12	12	12	12	12	12	96	96	100
493 [22] ^b	12	12	-	-	-	-	-	-	-	-	0
583 [32]	12	12	9	12	-	-	12	12	69	72	96
597 [41]	12	9	12	12	12	12	-	-	69	72	96
649 [68]	8	-	12	10	-	-	12	12	54	60	90
651 [43]	12	12	10	12	12	12	12	12	94	96	98
653 [39]	12	12	8	8	9	12	12	12	85	96	89
658 [23]	8	12	-	-	-	-	12	12	44	48	92
701 [10]	12	12	12	12	12	12	12	12	96	96	100
703 [3]	12	9	12	10	12	12	12	12	91	96	95
715 [21]	12	12	-	-	5	12	12	12	65	72	90
718 [7]	6	12	12	5	12	12	12	8	79	96	82
720 [90]	12	12	12	12	12	12	-	-	72	72	100
744 [47]	12	12	12	12	-	-	12	12	72	72	100
983 [44] ^b	8	8	-	-	-	-	-	-	-	-	0
1498 [27]	12	12	-	-	-	-	12	12	48	48	100
1527[170]	12	12	8 ^c	8 ^c	8 ^c	8 ^c	8 ^c	8 ^c	72	72	100
1578 [13]	12	12	12	12	-	-	-	-	48	48	100
1798 [147]	4	12	12	12	-	-	12	12	64	72	89
1859 [83]	7	12	-	-	-	-	12	12	43	48	90
2118 [33]	8	8	12	12	-	-	-	-	40	48	83
[42] ^b	9	12	-	-	-	-	-	-	-	-	0
[102] ^b	-	-	-	-	-	-	-	-	-	-	0
[212] ^d	9	12	-	-	-	-	-	-	-	-	-

^a NRL Lab number from PHE EQA scheme. ID number in [x] taken from EURL PT scheme.

^b Full performance assessment was not carried out as NRL did not participate in PT 60 and 1 EQA during 2015.

^c The reporting of MPN tube combinations is not required for this method, the overall score is reduced to reflect this (8).

^d EQA material cannot be analysed using this method, therefore a full assessment is not completed.

Salmonella spp. assessment

Twenty-one laboratories participated in 1 or more EQA distributions in 2015 and were therefore subject to a full performance assessment. All laboratories that were subjected to the full assessment achieved a cumulative total of >70% for the one or more distributions analysed. NRLs Bulgaria, Finland, Greece and Latvia did not participate in the mandatory number of EQA distributions (1) per year agreed in Resolution 8 of the NRLs annual workshop 2012.

Table 3: Summary of participants' performance in the EQA scheme – *Salmonella* spp.

Lab no. ^a	Distribution SF050		Distribution SF051		Distribution SF052		All distributions		
	SF0108	SF0109	SF0110	SF0111	SF0112	SF0113	Cumulative score	Max score	%
121 [19]	2	2	2	2	2	2	12	12	100
391 [9]	2	2	-	-	2	2	8	8	100
403 [86]	-	-	2	2	2	2	8	8	100
413 [35]	2	2	2	2	2	2	12	12	100
493 [22] ^b	-	-	-	-	-	-	-	-	0
583 [32]	2	2	-	-	2	2	8	8	100
597 [41]	2	2	2	2	-	-	8	8	100
649 [68]	2	2	-	-	2	2	8	8	100
651 [43]	2	2	2	2	2	2	12	12	100
653 [39]	2	2	2	2	2	2	12	12	100
658 [23]	-	-	-	-	2	2	4	4	100
701 [10]	2	2	2	2	2	2	12	12	100
703 [3]	-	-	2	2	-	-	4	4	100
715 [21]	-	-	2	2	2	2	8	8	100
718 [7]	2	2	2	2	2	2	12	12	100
720 [90]	2	2	2	2	-	-	8	8	100
744 [47]	2	2	-	-	2	2	8	8	100
983 [44] ^b	-	-	-	-	-	-	-	-	0
1498 [27]	-	-	-	-	2	2	4	4	100
1527[170] ^c	-	-	-	-	-	-	-	-	-
1578 [13]	2	2	-	-	-	-	4	4	100
1798 [147]	2	2	-	-	2	2	8	8	100
1859 [83]	-	-	-	-	2	2	4	4	100
2118 [33]	2	2	-	-	-	-	4	4	100
[42] ^b	-	-	-	-	-	-	-	-	0
[102] ^b	-	-	-	-	-	-	-	-	0
[212] ^c	-	-	-	-	-	-	-	-	-

^a NRL Lab number from PHE EQA scheme. ID number in [x] taken from EURL PT scheme.

^b Full performance assessment was not carried out as NRL did not participate in 1 EQA during 2015.

^c *Salmonella* spp. analyses is not performed by these NRLs.

References

Anon 2005. ISO TS 16649-3:2005. 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 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. Geneva, Switzerland.

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

European Communities 2004. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. Eur. Communities* L 165, 30.4.04 : 1-141.

European Communities 2004. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. *Off. J. Eur. Communities* L 226, 25.6.04 : 83-127.

European Communities 2005. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Communities* L338, 22.12.05 : 1-26.

Appendix 1

Distribution SF050

Sample SF0108 contents - *E. coli* (10^3 - 10^4) (wild strain), *S. Indiana* 1,4,12:z:1,7 (10^2) (wild strain), *B. pumilus* (10^2) (wild strain), *E. cloacae* (10^5) (wild strain).

Reference results - *E. coli* MPN – 3.47×10^3 - 8.33×10^4 per 100g. *Salmonella* spp. – Detected in 25g.

Analysed February / March 2015 – Seventeen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 403, 658, 715, 1498 and 1859 did not participate in this distribution.

Table 4: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0108

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	1.3×10^4	2.6×10^3	1.2×10^3	6.5×10^4	1.4×10^5
Participants results	1.7×10^4	3.4×10^3	1.6×10^3	8.5×10^4	1.9×10^5

Participants' results - SF0108

Table 5: Results reported by participants and scores allocated - SF0108 (Figure 1)

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	17000	13000	12	Detected	2
391	22000	17000	12	Detected	2
403	DNP	DNP	-	DNP	-
413	22000	22000	12	Detected	2
493	DNR	DNR	-	DNR	-
583	92000	28000	9	Detected	2
597	17000	33000	12	Detected	2
649	17000	33000	12	Detected	2
651	13000	7900	10	Detected	2
653	6900	11000	8	Detected	2
658	DNP	DNP	-	DNP	-
701	7800	11000	12	Detected	2
703	24000	13000	12	NE	-
715	DNP	DNP	-	DNP	-
718	13000	35000	12	Detected	2
720	17000	11000	12	Detected	2
744	49000	33000	12	Detected	2
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	10420	9600	8 *	NE	-
1578	33000	33000	12	Detected	2
1798	24000	35000	12	Detected	2
1859	DNP	DNP	-	DNP	-
2118	35000	7900	12	Detected	2

DNR – NRL did not register for the scheme

DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

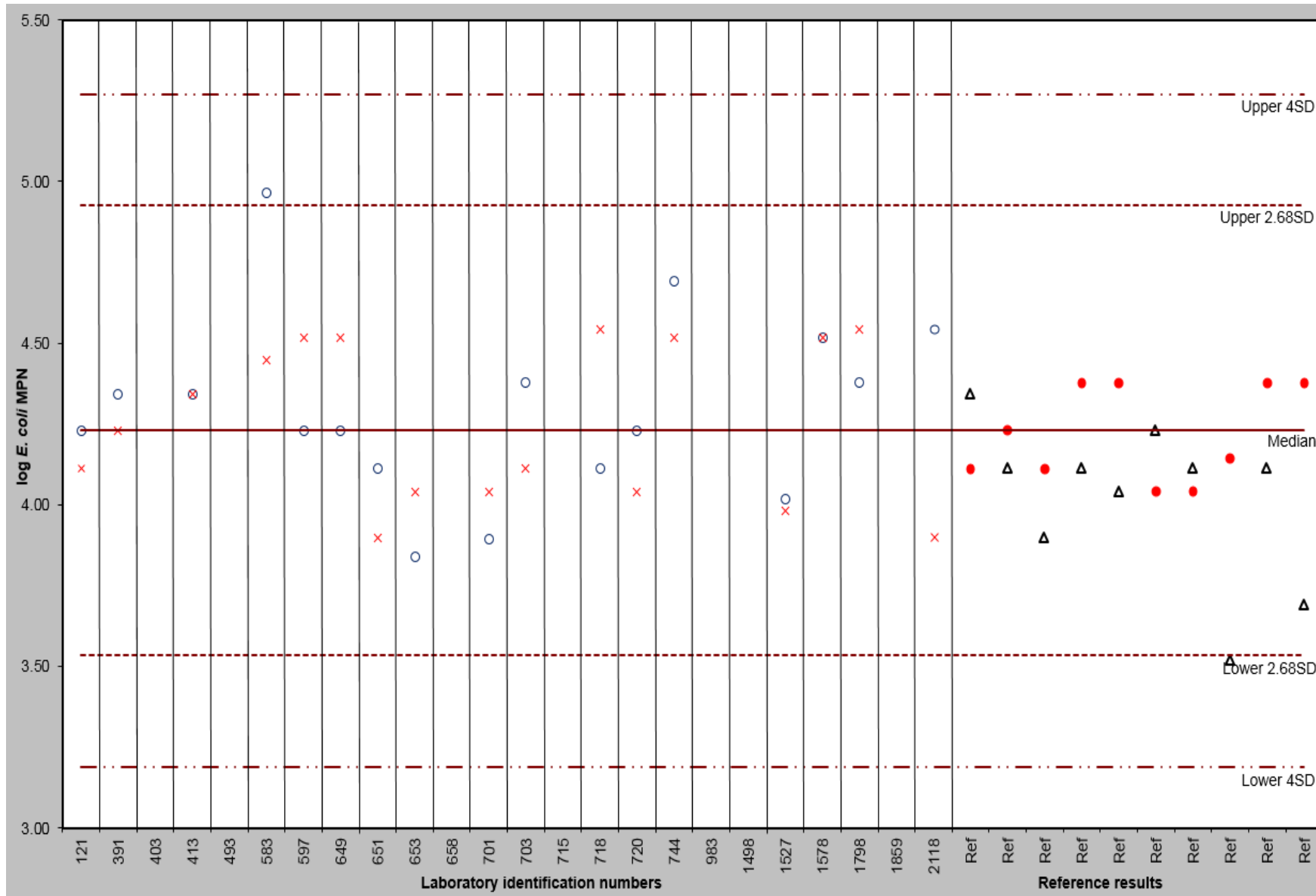
* Score calculated out of 8 rather than 12 as tube combination not reported using this method.



***E. coli* MPN** – Sixteen laboratories reported replicate results within ± 2.68 SD of the participants' medium with 14 receiving a maximum score. Laboratory 583 reported one replicate result within ± 2.68 SD of the participants' medium and received a maximum score of 9. Laboratories 651 and 653 were deducted points for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables.

***Salmonella* spp.** – All 15 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Figure 1. Distribution SF050: Sample SF0108



Distribution SF050

Sample SF0109 contents – *E. coli* ($10 - 10^2$), *S. Typhimurium* 1,4,[5],12:i:1,2 (10^2) (wild strain), *K. oxytoca* (10^4) (wild strain), *E. faecalis* (10) (wild strain).

Analysed February / March 2015 – Seventeen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 403, 658, 715, 1498 and 1859 did not participate in this distribution.

Reference results - *E. coli* MPN – $<18 - 3.82 \times 10^2$ per 100g. *Salmonella* spp. – Detected in 25g.

Table 6: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0109

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	7.8×10^1	1.6×10^1	7.1×10^0	3.9×10^2	8.6×10^2
Participants results	7.8×10^1	1.6×10^1	7.1×10^0	3.9×10^2	8.6×10^2

Participants results - SF0109 (Figure 2)

Table 7: Results reported by participants and scores allocated - SF0109

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	45	130	12	Detected	2
391	110	78	12	Detected	2
403	DNP	DNP	-	DNP	-
413	170	170	12	Detected	2
493	DNR	DNR	-	DNR	-
583	40	68	12	Detected	2
597	110	140	12	Detected	2
649	20	93	10	Detected	2
651	78	220	12	Detected	2
653	68	20	8	Detected	2
658	DNP	DNP	-	DNP	-
701	78	140	12	Detected	2
703	68	18	10	NE	-
715	DNP	DNP	-	DNP	-
718	16	9	5	Detected	2
720	130	45	12	Detected	2
744	78	170	12	Detected	2
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	<200	<200	8 *	NE	-
1578	170	78	12	Detected	2
1798	78	110	12	Detected	2
1859	DNP	DNP	-	DNP	-
2118	170	78	12	Detected	2

DNR – NRL did not register for the scheme

DNP – NRL registered for EQA scheme but did not participate in this distribution

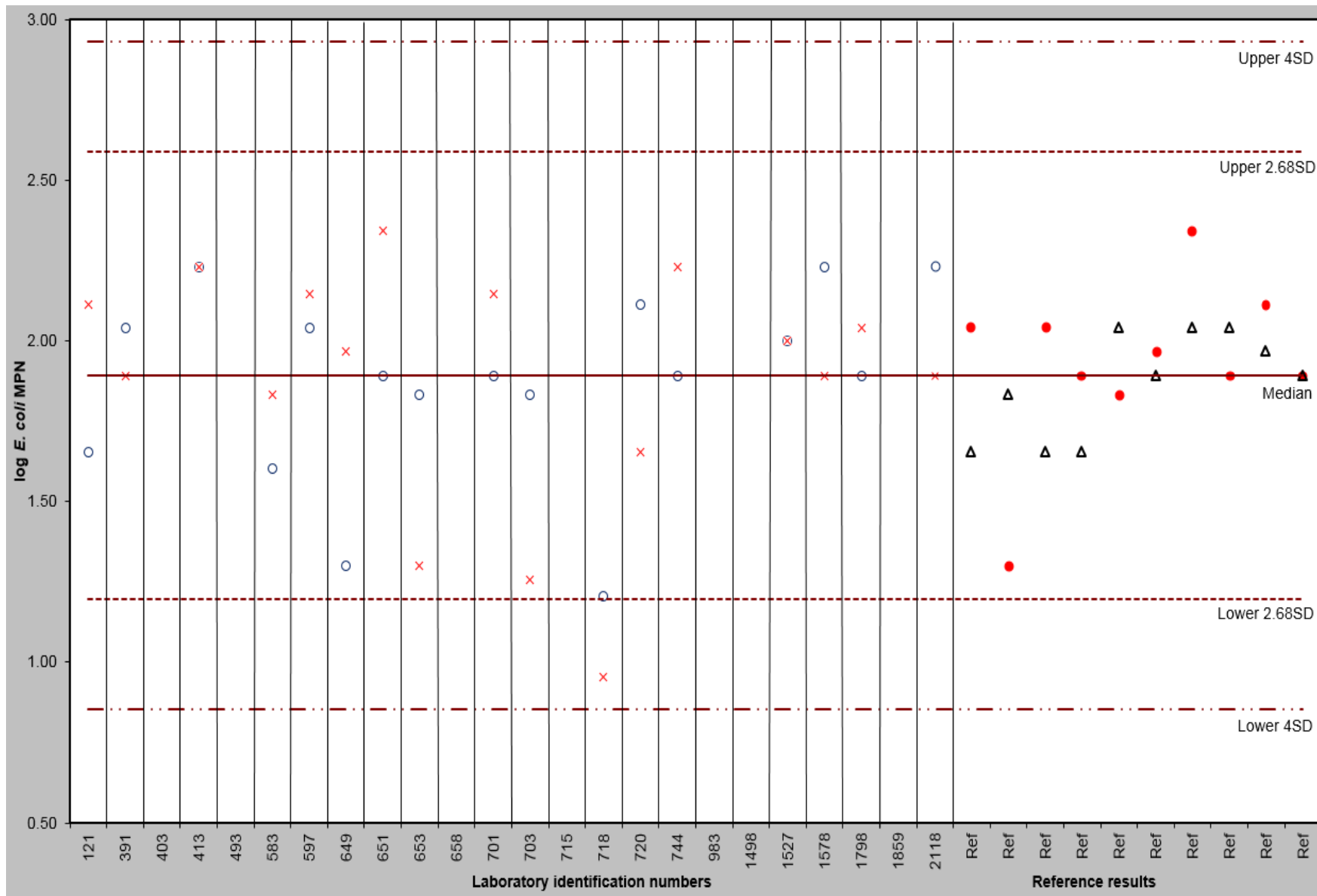
NE – Not examined

* Score calculated out of 8 rather than 12 as tube combination not reported using this method.

***E. coli* MPN** – Sixteen laboratories reported replicate results within ± 2.68 SD of the participants' median with 13 received a maximum score. Laboratory 718 reported one replicate result between ± 2.68 and $\pm 4SD$ of the participants' median and reported tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables and received an overall score of 5. Laboratories 649, 653 and 703 were deducted points for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables.

***Salmonella* spp.** – All 15 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Figure 2. Distribution SF050: Sample SF0109



Appendix 2

Distribution SF051

Sample SF0110 contents – *E. coli* (10^3 - 10^4) (wild strain), *S. London 3*, {10}{15}:l,v:1,6 (30) (wild strain), *K. oxytoca* (10^5) (wild strain), *P. agglomerans* (10^5) (wild strain).

Analysed June / July 2015 – Twelve laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 583, 649, 658, 744, 1498, 1578, 1798, 1859 and 2118 did not participate in this distribution.

Reference results - *E. coli* MPN – 4.49×10^3 - 1.08×10^5 per 100g. ***Salmonella* spp.** - Detected in 25g.

Table 8: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0110

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	3.5×10^4	7.0×10^3	3.2×10^3	1.7×10^5	3.8×10^5
Participants results	1.9×10^4	3.9×10^3	1.8×10^3	9.6×10^4	2.1×10^5

Participants results - SF0110 (Figure 3)

Table 9: Results reported by participants and scores allocated - SF0110

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	17000	33000	12	Detected	2
391	DNP	DNP	-	DNP	-
403	17000	24000	12	Detected	2
413	23000	23000	12	Detected	2
493	DNR	DNR	-	DNR	-
583	DNP	DNP	-	DNP	-
597	49000	33000	12	Detected	2
649	DNP	DNP	-	DNP	-
651	7800	6900	12	Detected	2
653	3300	7800	9	Detected	2
658	DNP	DNP	-	DNP	-
701	23000	23000	12	Detected	2
703	27000	7800	12	Detected	2
715	3500	9200	5	Detected	2
718	33000	14000	12	Detected	2
720	33000	22000	12	Detected	2
744	DNP	DNP	-	DNP	-
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	6500	7500	8 *	NE	-
1578	DNP	DNP	-	DNP	-
1798	DNP	DNP	-	DNP	-
1859	DNP	DNP	-	DNP	-
2118	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

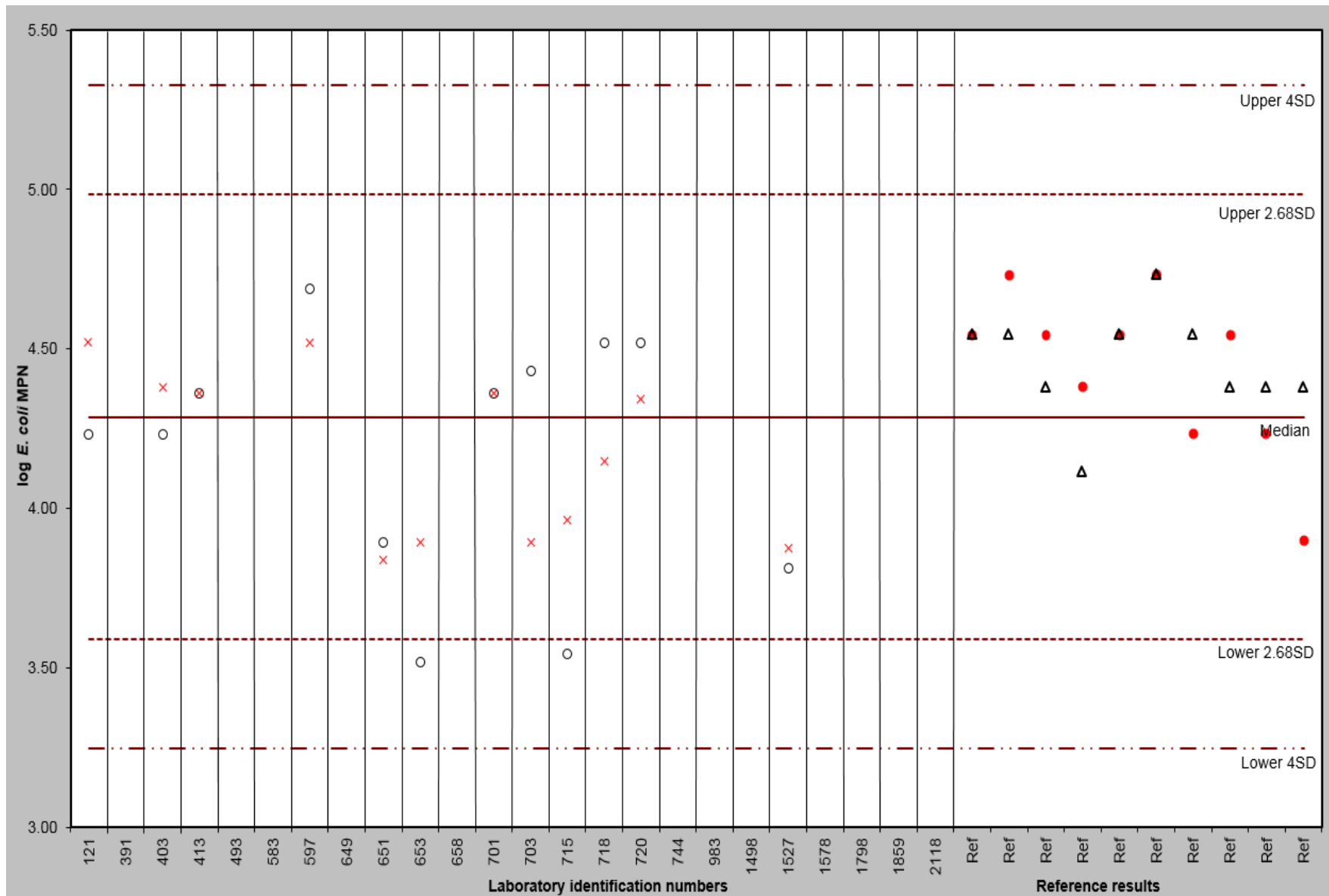
* Score calculated out of 8 rather than 12 as tube combination not reported using this method.



***E. coli* MPN** – Ten laboratories reported replicate results within ± 2.68 SD of the participants' median with 10 receiving a maximum score. Laboratory 653 reported one replicate result between ± 2.68 and $\pm 4SD$ of the participants' median and received a score of 9. Laboratory 715 reported one replicate result between ± 2.68 and $\pm 4SD$ of the participants' median and reported both tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables and received an overall score of 5.

***Salmonella* spp.** – All 11 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Figure 3. Distribution SF051: Sample SF0110



Distribution SF051

Sample SF0111 contents – *E. coli* (10^2 - 10^3) (wild strain), *S. Anatum* 3, {10}{15}{15,34} e,h:1,6 [z₆₄] (25) (wild strain), *A. hydrophila* (<10) (wild strain), *E. faecium* (10^3) (wild strain).

Analysed June / July 2015 – Twelve laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 583, 649, 658, 744, 1498, 1578, 1798, 1859 and 2118 did not participate in this distribution.

Reference results

E. coli MPN – 4.49×10^2 - 1.08×10^4 per 100g. *Salmonella* spp. – Detected in 25g.

Table 10: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0111

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	2.2×10^3	4.4×10^2	2.0×10^2	1.1×10^4	2.4×10^4
Participants results	1.7×10^3	3.4×10^2	1.6×10^2	8.5×10^3	1.9×10^4

Participants results - SF0111 (Figure 4)

Table 11: Results reported by participants and scores allocated - SF0111

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	1400	2200	8	Detected	2
391	DNP	DNP	-	DNP	-
403	1300	1100	12	Detected	2
413	2300	2300	12	Detected	2
493	DNR	DNR	-	DNR	-
583	DNP	DNP	-	DNP	-
597	1700	1700	12	Detected	2
649	DNP	DNP	-	DNP	-
651	1700	780	12	Detected	2
653	780	690	12	Detected	2
658	DNP	DNP	-	DNP	-
701	1300	7900	12	Detected	2
703	2300	1700	12	Detected	2
715	490	790	12	Detected	2
718	3300	3300	12	Detected	2
720	2200	3300	12	Detected	2
744	DNP	DNP	-	DNP	-
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	1300	1600	8 *	NE	-
1578	DNP	DNP	-	DNP	-
1798	DNP	DNP	-	DNP	-
1859	DNP	DNP	-	DNP	-
2118	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

* Score calculated out of 8 rather than 12 as tube combination not reported using this method.



***E. coli* MPN** – Twelve laboratories reported replicate results within ± 2.68 SD of the participants' medium with 11 receiving a maximum score. Laboratory 121 was deducted points for reporting tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables.

***Salmonella* spp.** – All 11 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Appendix 3

Distribution SF052

Sample SF0112 contents – *E. gallinarum* (3.6×10^4) (wild strain), *M. varians* (3.8×10^3) (wild strain).

Analysed November / December 2015 – Eighteen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 403 reported 1 replicate result for *E. coli*. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 597, 720, 1578 and 2118 did not participate in this distribution.

Reference results

***E. coli* MPN** – <18 per 100g. ***Salmonella* spp.** – Not detected in 25g.

Participants results - SF0112

Table 12: Results reported by participants and scores allocated - SF0112

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	0	0	12	Not Detected	2
391	<18	<18	12	Not Detected	2
403	<18	NE	7	Not Detected	2
413	0	0	12	Not Detected	2
493	DNR	DNR	-	DNR	-
583	<18	<18	12	Not Detected	2
597	DNP	DNP	-	DNP	-
649	<18	<18	12	Not Detected	2
651	<18	<18	12	Not Detected	2
653	<18	<18	12	Not Detected	2
658	0	0	12	Not Detected	2
701	<18	<18	12	Not Detected	2
703	<18	<18	12	NE	-
715	<18	<18	12	Not Detected	2
718	<18	<18	12	Not Detected	2
720	DNP	DNP	-	DNP	-
744	<18	<18	12	Not Detected	2
983	DNR	DNR	-	DNR	-
1498	<18	<18	12	Not Detected	2
1527	<200	<200	8 *	NE	-
1578	DNP	DNP	-	DNP	-
1798	<18	<18	12	Not Detected	2
1859	<18	<18	12	Not Detected	2
2118	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

* Score calculated out of 8 rather than 12 as tube combination not reported using this method.

***E. coli* MPN** – Seventeen laboratories reported the absence of *E. coli* in this sample and received a maximum score. Laboratory 403 reported the absence of *E. coli* in one replicate result and received a maximum score of 7.

***Salmonella* spp.** – All 16 laboratories that reported a result for *Salmonella* spp. correctly reported that *Salmonella* spp. was not detected and received a maximum score of 2.

Distribution SF052

Sample SF0113 contents – *E. coli* (1×10^2 – 1×10^3) (wild strain), *S. Pensacola* 1,9,12:m,t:[1,2] (70) (wild strain), *B. pumilus* (1.4×10^4) (wild strain), *P. putida* (3.3×10^3) (wild strain)

Analysed November / December 2015 – Eighteen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 403 reported 1 replicate result for *E. coli*. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 597, 720, 1578 and 1859 did not participate in this distribution.

Reference results

E. coli MPN – 1.59×10^2 - 3.82×10^3 per 100g. *Salmonella* spp. – Detected in 25g.

Table 13: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0113

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	4.9×10^2	9.8×10^1	4.5×10^1	2.4×10^3	5.4×10^3
Participants results	7.8×10^2	1.6×10^2	7.1×10^1	3.9×10^3	8.6×10^3

Participants results - SF0113

Table 14: Results reported by participants and scores allocated - SF0113

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	4900	3300	5	Detected	2
391	1300	780	12	Detected	2
403	13000	NE	2	Detected	2
413	780	780	12	Detected	2
493	DNR	DNR	-	DNR	-
583	780	1700	12	Detected	2
597	DNP	DNP	-	DNP	-
649	460	790	12	Detected	2
651	330	3100	12	Detected	2
653	690	1100	12	Detected	2
658	490	490	12	Detected	2
701	690	330	12	Detected	2
703	780	450	12	NE	-
715	930	490	12	Detected	2
718	450	680	8	Detected	2
720	DNP	DNP	-	DNP	-
744	1700	490	12	Detected	2
983	DNR	DNR	-	DNR	-
1498	230	330	12	Detected	2
1527	180	260	8 *	NE	-
1578	DNP	DNP	-	DNP	-
1798	330	1100	12	Detected	2
1859	1300	780	12	Detected	2
2118	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

DNP – NRL registered for EQA scheme but did not in this distribution

NE – Not examined

* Score calculated out of 8 rather than 12 as tube combination not reported for this method.

***E. coli* MPN** – Sixteen laboratories replicate results within ± 2.68 SD of the participants' medium with 15 receiving a maximum score. Laboratory 403 reported a single replicate result which fall outside ± 4 SD of the participants' median and scored 2. Laboratory 121 reported one replicate result between ± 2.68 and ± 4 SD of the participants' median and reported both tube combinations inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables and received an overall scored 5. Laboratory 718 had scores deducted as the tube combinations reported were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

***Salmonella* spp.** – All 16 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Appendix 4:
EURL PT 60 - NRL results and allocated scores

Note: PHE shellfish EQA scheme laboratory identifications given in square brackets

Lab ID	<i>E. coli</i> MPN/100g					
	Sample 1 – Common mussels			Sample 2 – Pacific oysters		
	Replicate 1	Replicate 2	Score	Replicate 1	Replicate 2	Score
3 [703]	690	330	12	230	330	9
7 [718]	2300	1700	6	110	45	12
9 [391]	NE	NE	-	230	170	12
10 [701]	230	110	12	78	110	12
13 [1578]	330	330	12	78	20	12
19 [121]	780	330	12	0	0	12
21 [715]	230	490	12	20	45	12
22 [493]	780	920	12	40	78	12
23 [658]	45	68	8	0	0	12
27 [1498]	330	130	12	20	20	12
32 [583]	170	330	12	<18	45	12
33 [2118]	490	1100	8	45	78	8
35 [413]	170	170	12	130	78	12
39 [653]	330	450	12	68	40	12
41 [597]	330	220	12	130	330	9
42	130	230	8	20	68	8
43 [651]	490	220	12	40	40	12
44 [983]	45	68	8	20	20	8
47 [744]	330	230	12	78	78	12
68 [649]	230	110	8	NE	NE	-
83 [1859]	330	Void	7	45	130	12
86 [403]	-	-	-	-	-	-
90 [720]	230	220	12	45	20	12
102	-	-	-	-	-	-
147 [1798]	20	<18	4	130	78	12
170 [1527]	<200	<200	12	<200	<200	12
212	760	2500	9	<67	<67	12

Appendix 5:
Scoring for the PHE/EQA and EURL matrix scheme
***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 $\pm 3SD$ and median $\pm 5SD$ values	2	5	2	9
Both replicates MPN results are outside the expected range and fall between the median $\pm 3SD$ and median $\pm 5SD$ values	2	2	2	6
One replicate MPN result is outside the median $\pm 5SD$ value	2	5	0	7
Both replicates MPN results are outside the expected range. The first falls between the median $\pm 3SD$ and median $\pm 5SD$ values and the second falls outside the median $\pm 5SD$ values	2	2	0	4
Both replicates MPN results is outside the median $\pm 5SD$ 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 $\pm 3SD$ and median $\pm 5SD$ values	2	2	4
Single replicate MPN result is outside the median $\pm 5SD$ 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/Amd1:2013 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 <i>Salmonella</i>	0

Appendix 6:**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 Medium - 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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