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World Class Science for the Marine and Freshwater Environment

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

Escherichia coli and Salmonella spp. (PT 78)

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This PT scheme is intended to provide comparative test 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. Article 94 of Regulation (EU) 2017/625 sets out the organisational responsibilities for EU Reference Laboratories (EURL) with respect to comparative proficiency testing (PT).

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

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'.

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).

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.

Methodology

Sample preparation - PHE EQA

Samples comprising of LENTICULE™ discs containing fully characterised bacterial isolates were distributed during February, June and October 2018 (SF059, SF060 and SF061 respectively). 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

Three shellfish samples comprising of whole Pacific oysters (*Crassostrea gigas*) and homogenised Pacific oysters (*C. gigas*) originating from a UK commercial harvesting area were distributed in November 2018 (EURL PT 76). Samples were packaged according to IATA regulations and distributed with instructions and report forms.

Reference results

For each distribution a minimum of 6 reference samples were examined by the organising laboratory. Reference analyses were performed using ISO 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' median for EQA distributions and ± 3 SD and ± 5 SD above and below the participants' median for EURL whole animal distributions. Reported MPN values were \log_{10} transformed before being compiled into charts as shown in Appendix 1 to 3. Performance assessment was carried out according to the algorithm in Appendix 5.

Participation in PT for 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 NRL participation for 2018; 24 NRLs (NRL Denmark has nominated an OCL to take part in bacteriological PT schemes on their behalf and NRL France and NRL Netherlands participate using two *E. coli* reference methods) participated in both the EURL matrix distribution and 1 or more PHE EQA distribution in 2018 as agreed in Resolution 8 of the NRLs annual workshop 2012 (currently there are no NRLs designated in Malta, Cyprus, the Czech Republic or Estonia). NRL Norway nominated an OCL to take part in bacteriological PT schemes on their behalf but was unable to participate in the EURL whole animal distribution due to transportation issues. Participation of NRLs and designated OCLs in EURL PT 76 and PHE EQA distribution SF059 was funded by the EURL; participation in the other PHE EQA distributions was self-funded.

Table 1: Participation by NRLs in 2018 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
EUURL PT 76	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Number of PHE/EQA distributions	1		✓	✓		✓	✓		✓	✓				✓	✓		✓	✓			✓	✓	✓		
	2				✓			✓			✓		✓					✓	✓					✓	
	3	✓	✓									✓				✓									✓

Performance Assessment

A cumulative performance assessment was undertaken on participants' results for both *E. coli* and *Salmonella* spp. from the EUURL matrix distribution (PT 76) (Appendix 4) and 1 EQA distribution (SF059). The allocated scores are summarised in Tables 2 and 3 respectively. Scores for SF060 and SF061 are also included with cumulative scores assigned for information only. 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-four laboratories, except laboratory 1891, participated in the EUURL matrix scheme (Appendix 4) and 1 or more PHE/EQA distributions in 2018 and were therefore subject to a full performance assessment. Of these, one NRL participated using 2 methods (the reference method and an approved alternative method) for both EUURL and PHE/EQA distributions. One NRL participated using 2 methods (the reference method and an approved alternative) in the EUURL distribution only (LENTICULES used in the PHE/EQA scheme are not suitable for analyses using this alternative method). In both cases different lab ID numbers are provided for each individual method used. From the 25 sets of reported results for PT 76 and SF059, 24 laboratories (96%) achieved a cumulative total of >70% for the 2 or more distributions analysed (the lab using both the reference and an alternative method scored >70% using both methods). Laboratory 983 achieved a cumulative total of <70%.

***Salmonella* spp. assessment**

Twenty-three laboratories participated in the EUURL matrix scheme and 1 or more EQA distributions in 2018 and were therefore subject to a full performance assessment with all laboratories achieving a cumulative total of >70% for the distributions analysed. Laboratory 1891 did not examine PT 76 for *Salmonella* spp. and laboratory 703 did not examine SF059 for *Salmonella* spp..

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

Lab no. ^a	Performance assessment								Provided for information only ^f						
	PT 76			Distribution SF059		Cumulative score ^e	Max score	%	Distribution SF060		Distribution SF061		Cumulative score	Max score	%
	S - 1	S - 2	S - 3	SF0126	SF0127				SF0128	SF0129	SF0130	SF0131			
121 [19]	12	12	12	12	12	60	60	100	NE	NE	NE	NE	60	60	100
391 [9]	12	12	12	12	12	60	60	100	-	-	12	12	84	84	100
413 [35]	12	12	12	12	12	60	60	100	12	12	12	12	108	108	100
493 [22]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
583 [32]	12	9	12	12	12	57	60	95	-	-	12	12	81	84	96
597 [41]	8	8	8	12	12	48	60	80	-	-	-	-	48	60	80
649 [68]	12	12	12	12	12	60	60	100	-	-	12	12	84	84	100
651 [43]	12	12	6	12	12	54	60	90	-	-	12	12	78	84	93
653 [39]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
658 [23]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
701 [10]	12	12	12	12	12	60	60	100	12	12	12	12	108	108	100
703 [3]	9	12	12	9	12	54	60	90	12	12	12	12	102	108	94
718 [7]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
720 [90]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
744 [47]	12	9	9	12	12	54	60	90	-	-	12	9	75	84	89
983 [44]	8	8	2	8	8	34	60	57	-	-	-	-	34	60	57
1498 [27]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
1527[170] ^b	8	8	8	8	8	40	40	100	8	8	8	8	72	72	100
1578 [13]	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
1798 [147]	9	12	12	12	12	57	60	95	12	12	8	8	97	108	90
1859 [83]	12	9	9	12	12	54	60	90	-	-	12	12	78	84	93
1891 [69] ^c	-	-	-	12	12	-	-	-	-	-	12	12	48	48	100
1892 [245]	8	8	8	12	12	48	60	80	12	12	-	-	72	84	86
2118 [33]	12	9	12	12	12	57	60	95	-	-	-	-	57	60	95
2340 [42]	9	12	12	12	12	57	60	95	-	-	-	-	57	60	95
2341 [102]	7	12	12	NE	12	43	48	90	-	-	-	-	43	48	90
[212] ^{b,d}	8	8	6	-	-	-	-	-	-	-	-	-	-	-	-

^a NRL ID number from PHE EQA scheme [ID number from EURL PT scheme in brackets].

^b The reporting of MPN tube combinations is not required for the alternative method used by this laboratory, the maximum overall score is reduced to reflect this (8).

^c PT 76 samples were not examined as the material was not delivered within the specified time frame.

^d EQA material cannot be analysed using the alternative method used by this laboratory, therefore a full assessment cannot be completed.

^e Cumulative score is based on scores from PT 76 and distribution SF059 only.

^f Additional EQA results provided for information only. Cumulative score based on all distributions examined.

NE = Not Examined

'-' = NRL did not receive material for this distribution

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

Lab no. ^a	Performance assessment									Provided for information only ^e					
	PT 76			Distribution SF059		Cumulative score ^d	Max score	%	Distribution SF060		Distribution SF061		Cumulative score	Max score	%
	S - 1	S - 2	S - 3	SF0126	SF0127				SF0128	SF0129	SF0130	SF0131			
121 [19]	2	2	2	2	2	10	10	100	NE	NE	NE	NE	10	10	100
391 [9]	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
413 [35]	2	2	2	2	2	10	10	100	2	2	2	2	18	18	100
493 [22]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
583 [32]	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
597 [41]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
649 [68]	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
651 [43]	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
653 [39]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
658 [23]	0	2	2	2	2	8	10	80	-	-	-	-	8	10	80
701 [10]	2	2	2	2	2	10	10	100	2	2	2	2	18	18	100
703 [3]	2	2	2	-	-	-	-	-	2	0	2	2	12	14	86
718 [7]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
720 [90]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
744 [47]	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
983 [44]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
1498 [27]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
1527[170] ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1578 [13]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
1798 [147]	2	2	2	2	2	10	10	100	2	0	2	2	16	18	89
1859 [83]	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
1891 [69] ^c	-	-	-	2	0	-	-	-	-	-	2	2	6	8	75
1892 [245]	2	2	2	2	2	10	10	100	2	2	-	-	14	14	100
2118 [33]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
2340 [42]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
2341 [102]	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
[212] ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a NRL ID number from PHE EQA scheme [ID number from EURL PT scheme in brackets].

^b Lab ID number is used only for *E. coli* analysis using an alternative method.

^c PT 76 samples were not examined as the material was not delivered within the specified time frame.

^d Cumulative score is based on scores from PT 76 and distribution SF059 only.

^e Additional EQA results provided for information only. Cumulative score based on all distributions examined.

NE = Not Examined

'-' = NRL did not receive material for this distribution

References

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 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..

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.

European Union 2017. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. *Off. J. Eur. Union* L95, 7.4.2017, 1–142.

ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing

Appendix 1

Distribution SF059

Sample SF0126 contents – *Escherichia coli* 1.3×10^3 - 3.3×10^3 (wild strain), *Enterococcus faecalis* 4.9×10^4 (wild strain), *Staphylococcus hominis* 2.4×10^4 (wild strain)

Reference results - *E. coli* MPN – 290 - 6900 per 100g. *Salmonella* spp. – Not detected in 25g.

Analysed February / March 2018 – Twenty-six sets of results were returned for this distribution and were included in the assessment. Laboratory 2341 did not examine the sample for *E. coli* and laboratories 703 and 1527 did not examine the sample for *Salmonella* spp..

Table 4: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0126

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	2300	462	210	11443	25219
Participants' results	1700	342	155	8458	18640

Participants' results - SF0126 (Figure 1)

Table 5: Results reported by participants and scores allocated - SF0126

Lab No.	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	780	1100	12	Not Detected	2
391	2200	1700	12	Not Detected	2
413	2300	2300	12	Not Detected	2
493	1700	1700	12	Not Detected	2
583	1700	1300	12	Not Detected	2
597	490	3300	12	Not Detected	2
649	1700	2300	12	Not Detected	2
651	3100	1100	12	Not Detected	2
653	2300	1300	12	Not Detected	2
658	1300	1700	12	Not Detected	2
701	2300	1700	12	Not Detected	2
703	490	330	9	NE	-
718	3300	2300	12	Not Detected	2
720	1300	4900	12	Not Detected	2
744	1300	1400	12	Not Detected	2
983	3300	4900	8	Not Detected	2
1498	1300	3300	12	Not Detected	2
1527 *	1000	1180	8	NE	-
1578	1300	690	12	Not Detected	2
1798	1700	1300	12	Not Detected	2
1859	1300	2200	12	Not Detected	2
1891	2300	1300	12	Not Detected	2
1892	2300	3300	12	Not Detected	2
2118	780	780	12	Not Detected	2
2340	1100	4600	12	Not Detected	2
2341	NE	NE	-	Not Detected	2

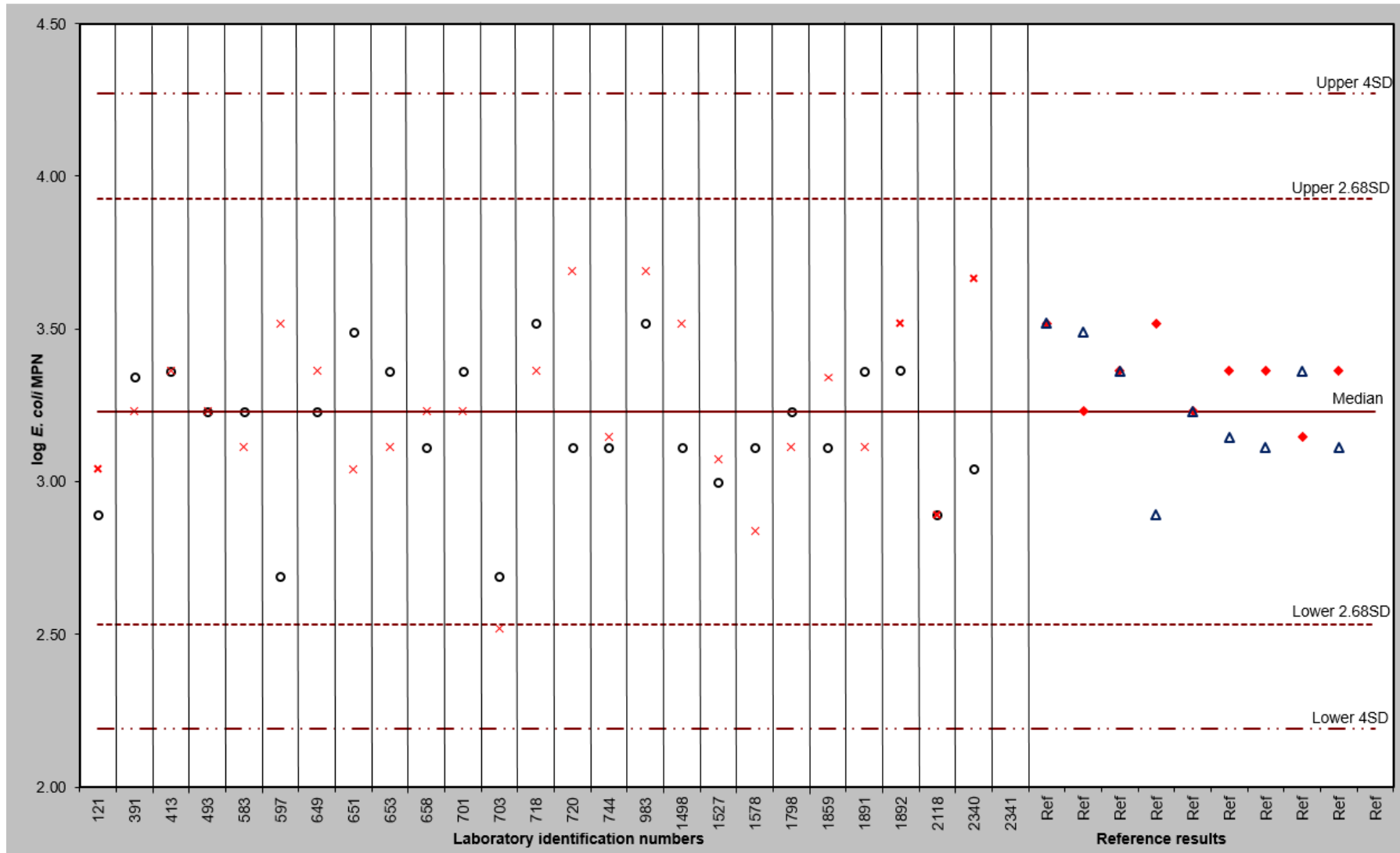
***E. coli* MPN** – Twenty-four laboratories reported replicate results within ± 2.68 SD of the participants' median with 23 laboratories receiving a maximum score. Laboratory 703 reported one replicate result between ± 2.68 and ± 4 SD of the participants median and scored 9. Laboratory 983 had points deducted as the tube combinations reported for both replicates were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

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

NE – Not examined

* Score calculated out of 8 rather than 12 as tube combination not reported using the alternative method used by this NRL.

Figure 1. Distribution SF059: Sample SF0126



Distribution SF059

Sample SF0127 contents – *Escherichia coli* 3.1×10^2 - 3.3×10^3 (wild strain), *Salmonella* Wentworth 11:Z₁₀:1,2 40 (wild strain), *Micrococcus* spp. 2.5×10^3 (wild strain), *Pseudomonas fluorescens* 3.9×10^3 (wild strain)

Analysed February / March 2018 – Twenty-six sets of results were returned for this distribution and were included in the assessment. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp..

Reference results - *E. coli* MPN – 160 – 3800 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 6: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0127

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	780	157	71	3881	8553
Participants' results	734	147	67	3650	8044

Participants' results - SF0127 (Figure 2)

Table 7: Results reported by participants and scores allocated - SF0127

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	490	310	12	Detected	2
391	2300	1300	12	Detected	2
413	330	330	12	Detected	2
493	490	780	12	Detected	2
583	1300	330	12	Detected	2
597	330	930	12	Detected	2
649	780	490	12	Detected	2
651	1300	1400	12	Detected	2
653	780	490	12	Detected	2
658	780	690	12	Detected	2
701	1300	2200	12	Detected	2
703	1300	780	12	NE	-
718	1100	1100	12	Detected	2
720	490	690	12	Detected	2
744	490	330	12	Detected	2
983	230	330	8	Detected	2
1498	780	330	12	Detected	2
1527*	660	540	8	NE	-
1578	1300	780	12	Detected	2
1798	490	330	12	Detected	2
1859	330	1300	12	Detected	2
1891	780	490	12	Not Detected	0
1892	1300	780	12	Detected	2
2118	450	1100	12	Detected	2
2340	490	330	12	Detected	2
2341	780	1300	12	Detected	2

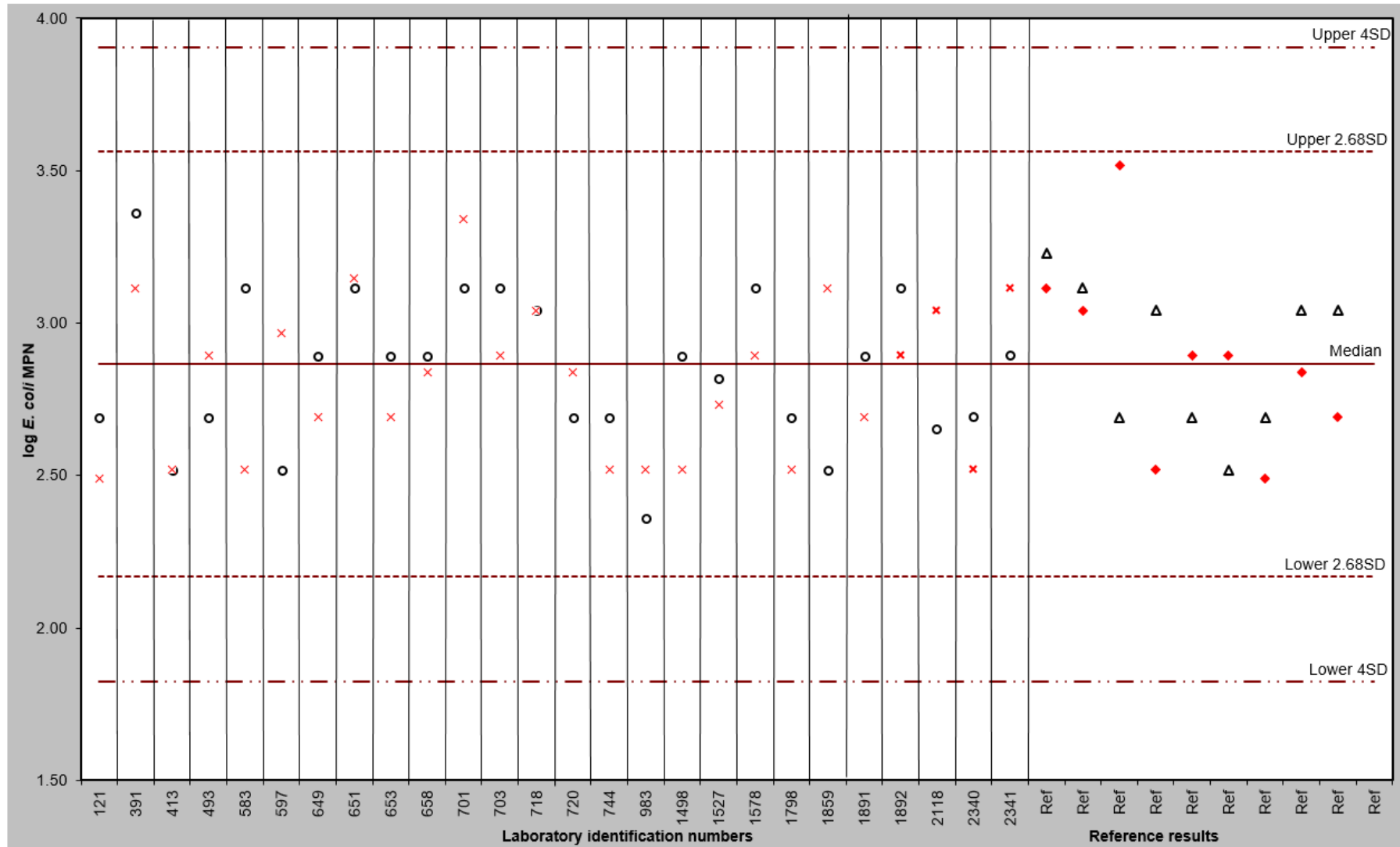
***E. coli* MPN** – Twenty-six laboratories reported replicate results within ± 2.68 SD of the participants' median with 25 receiving a maximum score. Laboratory 983 had points deducted as the tube combinations reported for both replicates were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

***Salmonella* spp.** – Twenty-three laboratories correctly reported the detection of *Salmonella* spp. and received a maximum score of 2. Laboratory 1891 incorrectly reported the absence of *Salmonella* spp. and received a scored 0.

NE – Not examined

* Score calculated out of 8 rather than 12 as tube combination not reported using the alternative method used by this NRL.

Figure 2. Distribution SF059: Sample SF0127



Appendix 2

Distribution SF060

Sample SF0128 contents – *Escherichia coli* 1.3×10^3 - 7.9×10^3 (wild strain), *Salmonella Typhimurium* 1,4,[5],12:i:1,2 (39 cfu per disc) (wild strain), *Klebsiella oxytoca* 7.1×10^3 (wild strain), *Staphylococcus epidermidis* 6.0×10^3 (wild strain)

Analysed June / July 2018 – Six sets of results were returned for this distribution and were included in the assessment. Laboratory 121 received samples but did not return results for this distribution. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 493, 583, 597, 649, 651, 653, 658, 718, 720, 744, 983, 1498, 1578, 1859, 1891, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – 450 - 11000 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 8: Participants' and reference results median, median ± 2.68 SD and ± 4 SD - SF0128

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	3300	663	301	16418	36184
Participants' results	1310	263	119	6517	14363

Participants' results - SF0128 (Figure 3)

Table 9: Results reported by participants and scores allocated - SF0128

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	NE	NE	-	NE	-
391	DNP	DNP	-	DNP	-
413	4900	4900	12	Detected	2
493	DNP	DNP	-	DNP	-
583	DNP	DNP	-	DNP	-
597	DNP	DNP	-	DNP	-
649	DNP	DNP	-	DNP	-
651	DNP	DNP	-	DNP	-
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	1100	2300	12	Detected	2
703	1300	780	12	Detected	2
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	DNP	DNP	-	DNP	-
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	1220	1320	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	1300	1700	12	Detected	2
1859	DNP	DNP	-	DNP	-
1891	DNP	DNP	-	DNP	-
1892	1100	2300	12	Detected	2
2118	DNP	DNP	-	DNP	-
2340	DNP	DNP	-	DNP	-
2341	DNP	DNP	-	DNP	-

***E. coli* MPN** – All laboratories returned replicate results within ± 2.68 SD of the participants' median and received a maximum score.

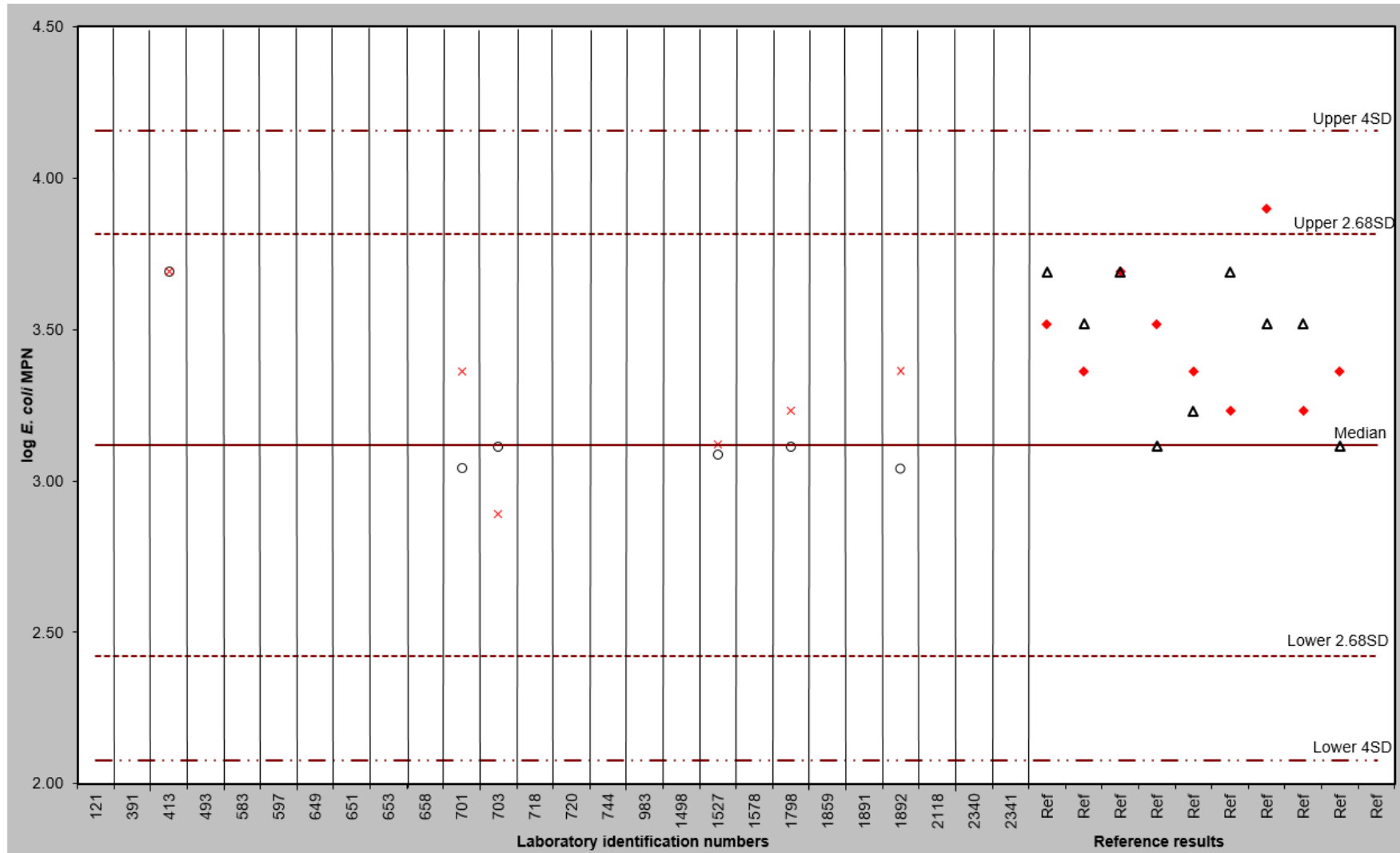
***Salmonella* spp.** – All laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

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 the alternative method used by this NRL.

Figure 3. Distribution SF060: Sample SF0128



Distribution SF060

Sample SF0129 contents – *Escherichia coli* 4.9×10^2 - 3.3×10^3 (wild strain), *Salmonella* Heidelberg 1,4,[5],12:r:1,2 (46 cfu per disc) (wild strain), *Aeromonas hydrophila* 4.0×10^2 (wild strain), *Enterococcus faecium* 1.1×10^2 (wild strain)

Analysed June / July 2018 – Six sets of results were returned for this distribution and were included in the assessment. Laboratory 121 received samples but did not return results for this distribution. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 493, 583, 597, 649, 651, 653, 658, 718, 720, 744, 983, 1498, 1578, 1859, 1891, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – 270 - 6400 per 100g. ***Salmonella* spp.** - Detected in 25g.

Table 10: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0129

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	1700	342	155	8458	18640
Participants' results	926	186	84	4608	10156

Participants' results - SF0129 (Figure 4)

Table 11: Results reported by participants and scores allocated - SF0129

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	NE	NE	-	NE	-
391	DNP	DNP	-	DNP	-
413	2300	2300	12	Detected	2
493	DNP	DNP	-	DNP	-
583	DNP	DNP	-	DNP	-
597	DNP	DNP	-	DNP	-
649	DNP	DNP	-	DNP	-
651	DNP	DNP	-	DNP	-
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	330	780	12	Detected	2
703	450	490	12	Not detected	0
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	DNP	DNP	-	DNP	-
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	780	760	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	1700	1700	12	Not detected	0
1859	DNP	DNP	-	DNP	-
1891	DNP	DNP	-	DNP	-
1892	1400	1100	12	Detected	2
2118	DNP	DNP	-	DNP	-
2340	DNP	DNP	-	DNP	-
2341	DNP	DNP	-	DNP	-

***E. coli* MPN** – All laboratories returned replicate results within ± 2.68 SD of the participants' median and received a maximum score.

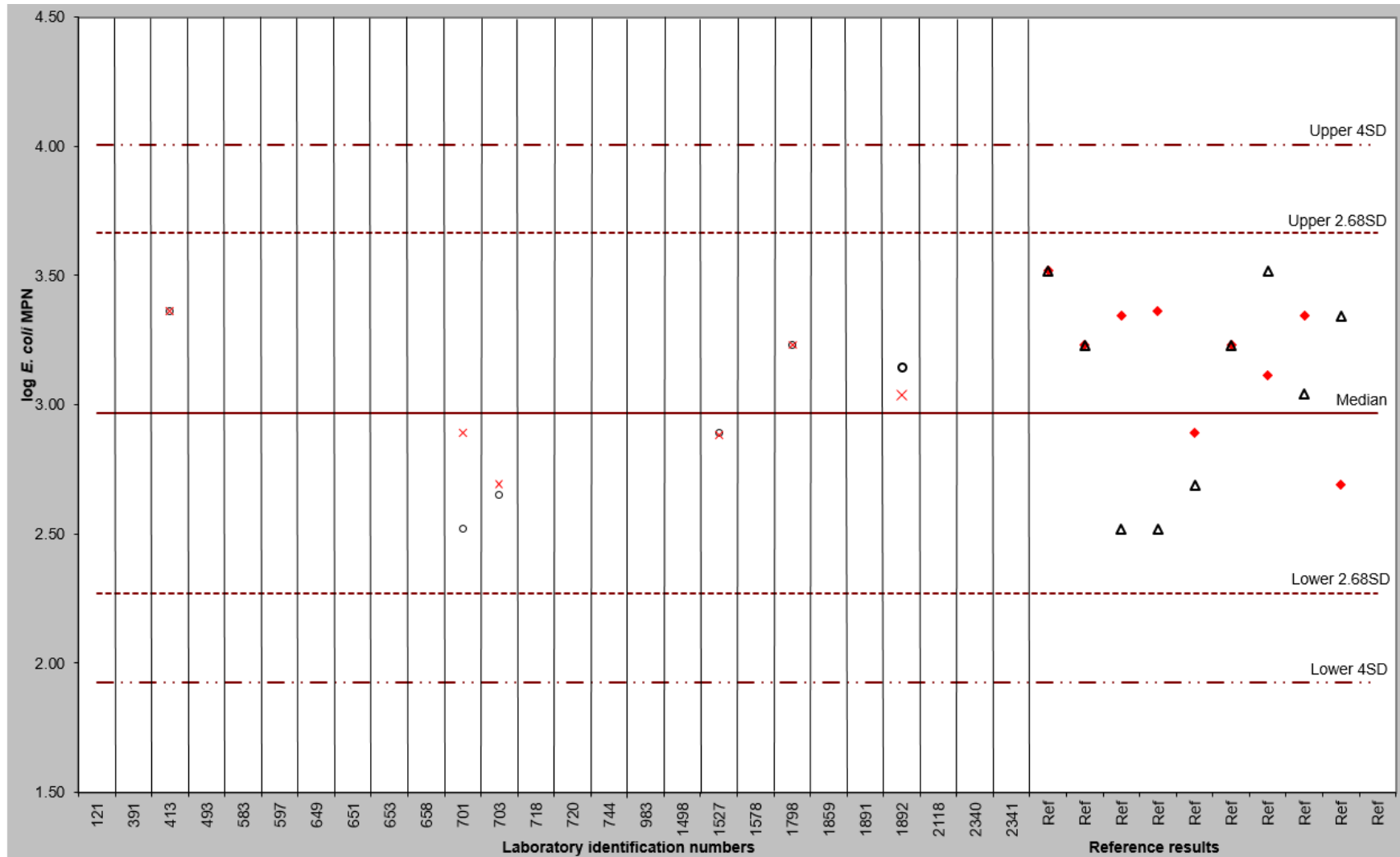
***Salmonella* spp.** – Three laboratories correctly reported the detection of *Salmonella* spp. and received a maximum score of 2. Laboratories 703 and 1798 incorrectly reported the absence of *Salmonella* spp. and received a scored 0.

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 the alternative method used by this NRL.

Figure 4. Distribution SF060: Sample SF0129



Appendix 3

Distribution SF061

Sample SF0130 contents – *Escherichia coli* 4.9×10^2 - 3.3×10^3 (wild strain), *Enterococcus faecalis* 4.9×10^4 (wild strain), *Staphylococcus hominis* 2.4×10^4 (wild strain)

Analysed November / December 2018 – Twelve sets of results were returned for this distribution and were included in the assessment. Laboratory 121 received samples but did not return results for this distribution. Laboratories 1527 did not examine the sample for *Salmonella* spp.. Laboratories 493, 597, 653, 658, 718, 720, 983, 1498, 1578, 1892, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – 270 - 6400 per 100g. ***Salmonella* spp.** – Not detected in 25g.

Table 12: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0130

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	1700	342	155	8458	18640
Participants' results	1100	221	100	5473	12061

Participants' results - SF0130 (Figure 5)

Table 13: Results reported by participants and scores allocated - SF0130

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	NE	NE	-	NE	-
391	1100	1100	12	Not Detected	2
413	690	1400	12	Not Detected	2
493	DNP	DNP	-	DNP	-
583	490	1700	12	Not Detected	2
597	DNP	DNP	-	DNP	-
649	3300	2300	12	Not Detected	2
651	1300	3300	12	Not Detected	2
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	690	1100	12	Not Detected	2
703	1300	490	12	Not Detected	2
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	2300	1300	12	Not Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	780	620	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	780	780	8	Not Detected	2
1859	2300	780	12	Not Detected	2
1891	1300	490	12	Not Detected	2
1892	DNP	DNP	-	DNP	-
2118	DNP	DNP	-	DNP	-
2340	DNP	DNP	-	DNP	-
2341	DNP	DNP	-	DNP	-

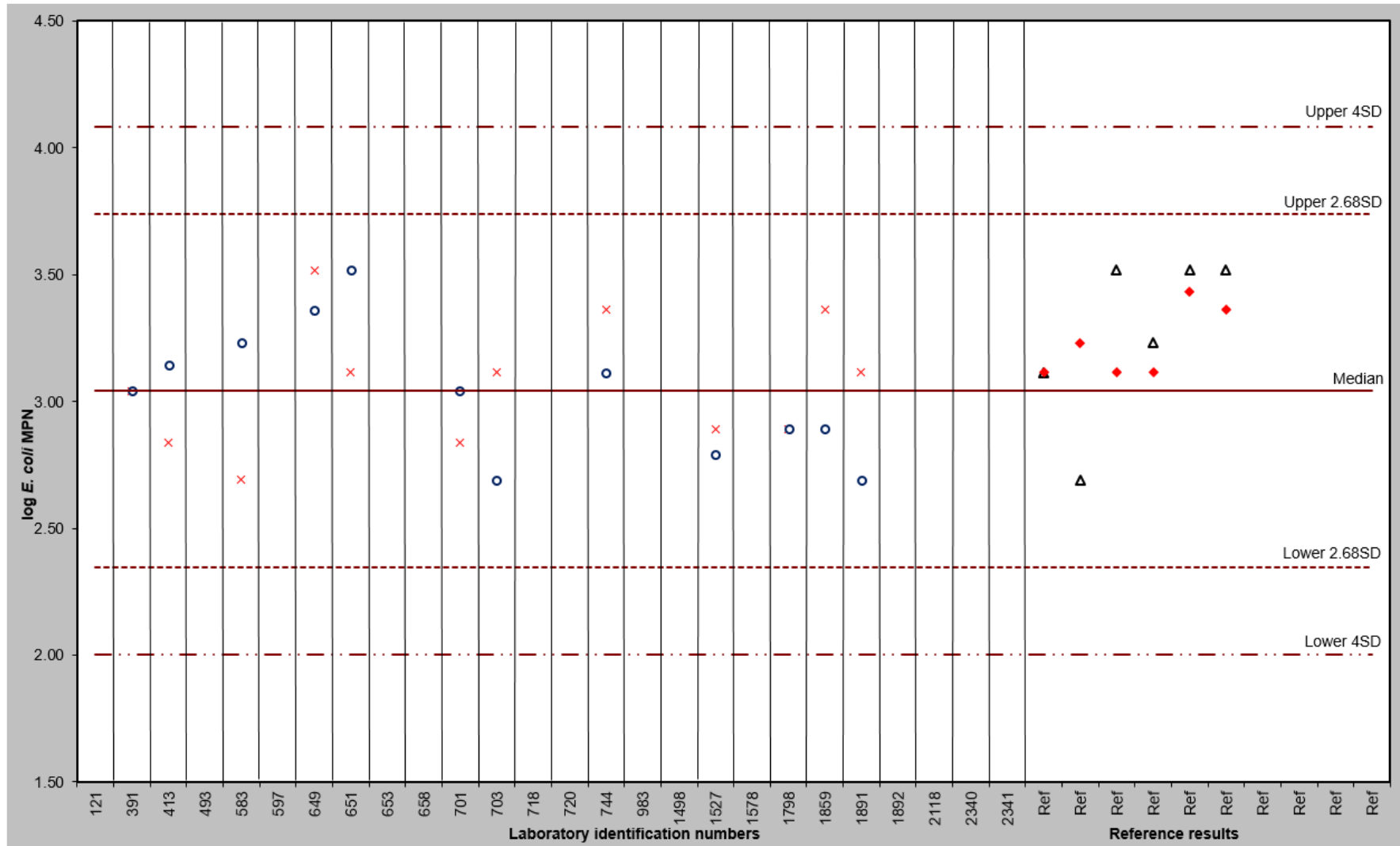
***E. coli* MPN** – All laboratories returned replicate results within ± 2.68 SD of the participants' median with 11 receiving a maximum score. Laboratory 1798 had points deducted as the tube combinations reported for both replicates were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

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

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 the alternative method used by this NRL.

Figure 5. Distribution SF061: Sample SF0130



Distribution SF061

Sample SF0131 contents – *Escherichia coli* 1.7×10^3 - 3.5×10^4 (wild strain), *Salmonella* Crewe 1.4×10^2 11:z:1,5 (wild strain), *Micrococcus* sp. 3.6×10^4 (wild strain), *Pseudomonas aeruginosa* 7.9×10^4 (wild strain)

Analysed November / December 2018 – Twelve sets of results were returned for this distribution and were included in the assessment. Laboratory 121 received samples but did not return results for this distribution. Laboratories 1527 did not examine the sample for *Salmonella* spp.. Laboratories 493, 597, 653, 658, 718, 720, 983, 1498, 1578, 1892, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – 1000 - 24000 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 14: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0130

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	4600	925	420	22885	50438
Participants' results	3749	754	342	18651	41106

Participants' results - SF0131

Table 15: Results reported by participants and scores allocated - SF0131

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	NE	NE	-	NE	-
391	3300	7000	12	Detected	2
413	4900	4900	12	Detected	2
493	DNP	DNP	-	DNP	-
583	13000	7900	12	Detected	2
597	DNP	DNP	-	DNP	-
649	17000	13000	12	Detected	2
651	2300	3300	12	Detected	2
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	3300	3300	12	Detected	2
703	4900	3300	12	Detected	2
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	490	1300	9	Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	3840	3660	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	2300	4900	8	Detected	2
1859	1300	7900	12	Detected	2
1891	4900	3300	12	Detected	2
1892	DNP	DNP	-	DNP	-
2118	DNP	DNP	-	DNP	-
2340	DNP	DNP	-	DNP	-
2341	DNP	DNP	-	DNP	-

***E. coli* MPN** – Eleven laboratories returned replicate results within ± 2.68 SD of the participants' median with 10 receiving a maximum score. Laboratory 744 reported one replicate result between ± 2.68 and ± 4 SD of the participants medium and scored 9. Laboratory 1798 had points deducted as the tube combinations reported for both replicates were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

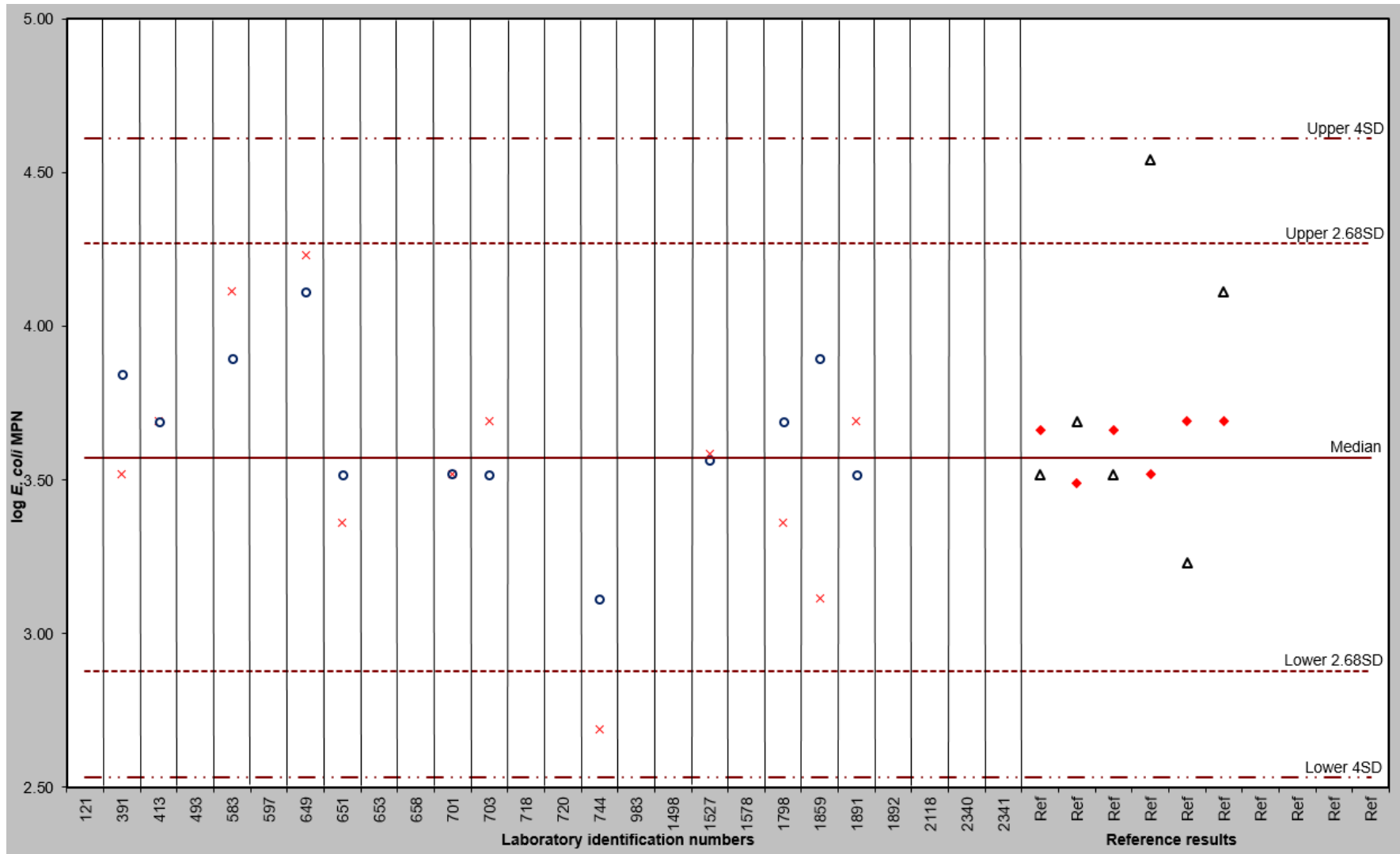
***Salmonella* spp.** – All laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

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 the alternative method used by this NRL.

Figure 5. Distribution SF061: Sample SF0130



Appendix 4:

EURL PT 76 - NRL results and allocated scores

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

Lab ID	Sample 1					Sample 2					Sample 3				
	E. coli MPN/100g			Sal. spp. in 25g		E. coli MPN/100g			Sal. spp. in 25g		E. coli MPN/100g			Sal. spp. in 25g	
	Rep 1	Rep 2	Score	Rep 1	Score	Rep 1	Rep 2	Score	Rep 1	Score	Rep 1	Rep 2	Score	Rep 1	Score
3 [703]	1300	490	9	ND	2	7000	35000	12	D	2	7900	11000	12	D	2
7 [718]	130	490	12	ND	2	24000	13000	12	D	2	4900	4900	12	D	2
9 [391]	490	330	12	ND	2	24000	11000	12	D	2	4600	11000	12	D	2
10 [701]	450	230	12	ND	2	24000	7900	12	D	2	3300	7900	12	D	2
13 [1578]	170	130	12	ND	2	17000	17000	12	D	2	13000	7900	12	D	2
19 [121]	490	230	12	ND	2	13000	22000	12	D	2	3300	4900	12	D	2
22 [493]	130	110	12	ND	2	28000	35000	12	D	2	2300	3300	12	D	2
23 [658]	690	490	12	D	0	35000	35000	12	D	2	11000	13000	12	D	2
27 [1498]	230	130	12	ND	2	24000	13000	12	D	2	3300	7000	12	D	2
32 [583]	130	130	12	ND	2	92000	54000	9	D	2	22000	24000	12	D	2
33 [2118]	93	780	12	ND	2	92000	17000	9	D	2	2700	13000	12	D	2
35 [413]	170	330	12	ND	2	35000	54000	12	D	2	3300	13000	12	D	2
39 [653]	330	330	12	ND	2	24000	24000	12	D	2	4900	13000	12	D	2
41 [597] ^a	230	780	8	ND	2	24000	13000	8	D	2	11000	3300	8	D	2
42 [2340]	450	3300	9	ND	2	11000	7900	12	D	2	2300	3300	12	D	2
43 [651]	170	210	12	ND	2	7000	3300	12	D	2	690	490	6	D	2
44 [983] ^a	230	230	8	ND	2	24000	24000	8	D	2	330	330	2	D	2
47 [744]	130	170	12	ND	2	160000	35000	9	D	2	35000	17000	9	D	2
68 [649]	110	93	12	ND	2	17000	24000	12	D	2	13000	11000	12	D	2
69 [1891]	NE	NE	-	NE	-	NE	NE	-	NE	-	NE	NE	-	NE	-
83 [1859]	490	690	12	ND	2	92000	92000	9	D	2	35000	17000	9	D	2
90 [720]	78	270	12	ND	2	17000	35000	12	D	2	4900	3300	12	D	2
102 [2341]	690	4900	7	ND	2	24000	NE	12	D	2	4900	NE	12	D	2
147 [1798]	20	170	9	ND	2	13000	17000	12	D	2	2100	4900	12	D	2
170 [1527] ^b	480	400	8	NE	-	13400	12340	8	NE	-	4140	3860	8	NE	-
212 ^b	<67	<67	8	NE	-	11000	15000	8	NE	-	580	2300	6	NE	-
245 [1892] ^a	78	230	8	ND	2	24000	13000	8	D	2	7900	4900	8	D	2

NE – Not examined; ND – Not detected; D – Detected.

^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).

Appendix 5:
***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 replicate 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 replicate MPN results are outside the expected range. The first falls between the median $\pm 3SD$ and median $\pm 5SD$ value and the second falls outside the median $\pm 5SD$ values.	2	2	0	4
Both replicate MPN results reported are outside the median $\pm 5SD$ 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 $\pm 3SD$ and median $\pm 5SD$ values.	2	2	4
Single replicate MPN result reported 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/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 <i>Salmonella</i>	0

Appendix 6:

Troubleshooting advice

1. **Methods** – Ensure that the method used is appropriate for the examination of the sample.
2. **Interpretation of MPN tables** – Ensure that all dilutions have been used to calculate the MPN value and the MPN tables (if used) are interpreted correctly.
 - Where three dilutions have been tested, record the number of TBGA/TBX positives for each dilution to give a 3 figure tube combination number. Use the MPN tables included in ISO 7218 and the EURL *E. coli* generic 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, use the Shellfish MPN calculator (available in the EURL *E. coli* generic protocol) or the MPN calculator available in ISO 7218 (<http://standards.iso.org/iso/7218/>)¹ to determine the MPN from all the dilutions tested. Only category 1 or 2 tube combinations should be reported.
 - If a tube combination result is an unacceptable combination (category 3), the result is reported as 'void'.
3. **Culture media** - Check the quality control data for media to ensure that they are within specifications and performing adequately.
4. **Equipment** - Check that the equipment used for the procedures (incubators, refrigerators, measuring instruments) are calibrated and performing adequately.
5. **Staff training** - Check that the staff performing the tests are fully trained and familiar with all the procedural steps.
6. **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.
7. **Accreditation**- Check that quality procedures are documented and adhered to at all times.
8. **Internal quality controls (IQC)** – Ensure adequate controls are in place and follow-up procedures are in place to deal with IQC failures.

¹ Note the value record by the Excel MPN calculator from ISO 7218 give an MPN/g. Multiply the value by 100 to obtain MPN/100g.



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