



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 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.

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 the 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 comprising of LENTICULE™ discs containing fully characterised bacterial isolates were distributed during February, June and October 2016. 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 Pacific oysters (*Crassostrea gigas*) originating from a UK commercial harvesting area were distributed in November 2016. 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 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. 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 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 2016; all 25 NRLs in the network participated in at least one distribution in 2016 (currently there are no NRLs designated in Malta, Cyprus, the Czech Republic or Estonia). Of those participating labs, 88% participated in both the EURL matrix distribution and 1 or more PHE EQA distributions as agreed in Resolution 8 of the NRLs annual workshop 2012. NRLs in Bulgaria, Finland and Norway did not participate in the required number of distributions. However, to assist NRLs to participate in the required number of distributions it was agreed at the 2016 annual workshop that the EURL would pay for NRL participation in a single PHE distribution per year (Resolution 8).

Table 1: Participation by NRLs in 2016 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 64	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PHE EQA distributions	0		✓			✓											✓								
	1				✓				✓					✓	✓								✓		
	2	✓			✓		✓	✓		✓	✓		✓								✓			✓	
	3		✓									✓				✓		✓	✓	✓		✓			✓

Performance Assessment

A cumulative performance assessment was undertaken on participants' results for both *E. coli* and *Salmonella* spp. from the EURL matrix distribution (PT 64) (Appendix 4) and 3 EQA distributions (March to November 2016). 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. NRLs Bulgaria, Finland and Norway did not participate in the mandatory number of EQA distributions (1) per year agreed in Resolution 8 of the NRLs annual workshop 2012 and were given a score of 0% as a result.

***E. coli* MPN assessment**

Twenty-two laboratories participated in the EURL matrix scheme (Appendix 4) and 1 or more EQA distributions in 2016 and were therefore subject to a full performance assessment. Of these, one laboratory participated using both the reference method and an approved alternative method for both EURL and PHE/EQA distributions. One laboratory participated using both the reference method and an approved alternative in the EURL distribution only (PHE/EQA LENTICULES are not suitable for analyses using this alternative method). In both cases different lab ID numbers are provided for each individual method used. Twenty-one laboratories 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-two laboratories participated in the EURL matrix scheme and 1 or more EQA distributions in 2016 and were therefore subject to a full performance assessment. All laboratories achieved a cumulative total of >70% for the distributions analysed.

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

Lab no. ^a	PT 64		Distribution SF053		Distribution SF054		Distribution SF055		All distributions		
	S - 1	S - 3	SF0114	SF0115	SF0116	SF0117	SF0118	SF0119	Cumulative score	Max score	%
121 [19]	12	12	9	12	12	12	12	12	93	96	97
391 [9]	12	12	12	12	12	12	12	12	96	96	100
413 [35]	12	12	12	12	12	12	-	-	72	72	100
493 [22]	12	12	-	-	-	-	12	12	48	48	100
583 [32]	8	12	12	12	-	-	12	12	68	72	95
597 [41]	12	12	9	12	12	12	0	0	69	96	72
649 [68]	12	12	12	12	-	-	12	9	69	72	96
651 [43]	12	12	12	12	12	9	12	12	93	96	97
653 [39]	12	12	12	12	12	12	12	12	96	96	100
658 [23]	12	12	-	-	-	-	12	10	46	48	96
701 [10]	12	12	12	12	12	12	12	12	96	96	100
703 [3]	12	12	12	12	12	12	12	12	96	96	100
718 [7]	9	12	12	12	12	12	12	12	93	96	97
720 [90]	12	12	12	12	12	12	-	-	72	72	100
744 [47]	12	12	12	12	-	-	12	12	72	72	100
983 [44]	8	8	-	-	8	8	-	-	32	48	67
1498 [27]	12	12	-	-	12	9	-	-	45	48	94
1527[170] ^b	8	8	8	8	8	8	8	8	64	64	100
1578 [13]	12	12	4	4	-	-	12	12	56	72	78
1798 [147]	12	12	12	12	12	12	12	12	96	96	100
1859 [83]	12	12	12	12	-	-	12	12	72	72	100
1892 [245]	12	12	-	-	12	12	-	-	48	48	100
2118 [33]	12	12	12	12	-	-	12	12	72	72	100
1891 [69] ^c	12	12	-	-	-	-	-	-	-	-	0
2340 [42] ^c	9	12	-	-	-	-	-	-	-	-	0
2341 [102] ^c	0	2	-	-	-	-	-	-	-	-	0
[212] ^d	8	8	-	-	-	-	-	-	-	-	-

^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 Full performance assessment was not carried out as the NRL did not register to the PHE EQA scheme during 2016.

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

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

Lab no. ^a	PT 64 ^b		Distribution SF053		Distribution SF054		Distribution SF055		All distributions		
	S - 1	S - 3	SF0114	SF0115	SF0116	SF0117	SF0118	SF0119	Cumulative score	Max score	%
121 [19]	-	2	2	2	-	-	-	-	6	6	100
391 [9]	-	2	2	2	2	2	2	2	14	14	100
413 [35]	-	2	2	2	2	2	-	-	10	10	100
493 [22]	-	2	-	-	-	-	2	2	6	6	100
583 [32]	-	2	2	2	-	-	2	2	10	10	100
597 [41]	-	2	2	2	2	2	0	0	10	14	72
649 [68]	-	2	2	2	-	-	2	2	10	10	100
651 [43]	-	2	2	2	2	2	2	2	14	14	100
653 [39]	-	2	2	2	2	2	2	2	14	14	100
658 [23]	-	2	-	-	-	-	2	2	6	6	100
701 [10]	-	2	2	2	2	2	2	2	14	14	100
703 [3]	-	2	-	-	-	-	2	2	6	6	100
718 [7]	-	2	2	2	2	2	2	2	14	14	100
720 [90]	-	2	2	2	2	2	-	-	10	10	100
744 [47]	-	2	2	2	-	-	2	2	10	10	100
983 [44]	-	2	-	-	2	2	-	-	6	6	100
1498 [27]	-	2	-	-	2	2	-	-	6	6	100
1527[170] ^c	-	-	-	-	-	-	-	-	-	-	-
1578 [13]	-	2	2	2	-	-	2	2	10	10	100
1798 [147]	-	2	2	2	2	2	2	2	14	14	100
1859 [83]	-	2	2	2	-	-	2	2	10	10	100
1892 [245]	-	2	-	-	2	2	-	-	6	6	100
2118 [33]	-	2	2	2	-	-	2	2	10	10	100
1891 [69] ^d	-	0	-	-	-	-	-	-	-	-	0
2340 [42] ^d	-	2	-	-	-	-	-	-	-	-	0
2341 [102] ^d	-	2	-	-	-	-	-	-	-	-	0
- [212] ^c	-	-	-	-	-	-	-	-	-	-	-

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

^b No scores were allocated for sample 1 as *Salmonella* spp. was detected 4/20 replicate reference samples

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

^d Full performance assessment was not carried out as the NRL did not register to the PHE EQA scheme during 2016.

References

Anon 2015. ISO 16649-3:2015. 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.

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

Appendix 1

Distribution SF053

Sample SF0114 contents – *E. coli* (1.3×10^2 - 1.7×10^3) (wild strain), *Salmonella Livingstone* 6,7,14:d:l,w (1.1×10^2) (wild strain), *Aerococcus viridans* (2.0×10^3) (NCTC 8251), *Staphylococcus epidermidis* (9.4×10^3) (wild strain)

Reference results - *E. coli* MPN – 74 – 1800 per 100g. *Salmonella spp.* – Detected in 25g.

Analysed February / March 2016 – Eighteen 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 493, 658, 983, 1498 and 1892 did not participate in this distribution.

Table 4: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0114

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	475	95	43	2362	5206
Participants results	450	90	41	2239	4934

Participants' results - SF01114 (Figure 1)

Table 5: Results reported by participants and scores allocated - SF0114

Lab No.	<i>E. coli</i> (per 100g)			<i>Salmonella spp.</i> (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella spp.</i>	Score
121	3300	780	9	Detected	2
391	780	1300	12	Detected	2
413	490	490	12	Detected	2
493	DNP	DNP	-	DNP	-
583	490	230	12	Detected	2
597	78	330	9	Detected	2
649	450	220	12	Detected	2
651	780	780	12	Detected	2
653	780	490	12	Detected	2
658	DNP	DNP	-	DNP	-
701	230	230	12	Detected	2
703	330	490	12	NE	-
718	450	1300	12	Detected	2
720	1100	230	12	Detected	2
744	490	230	12	Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527	180	200	8 *	NE	-
1578	<18	<18	4	Detected	2
1798	230	490	12	Detected	2
1859	310	230	12	Detected	2
1892	DNP	DNP	-	DNP	-
2118	230	490	12	Detected	2

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

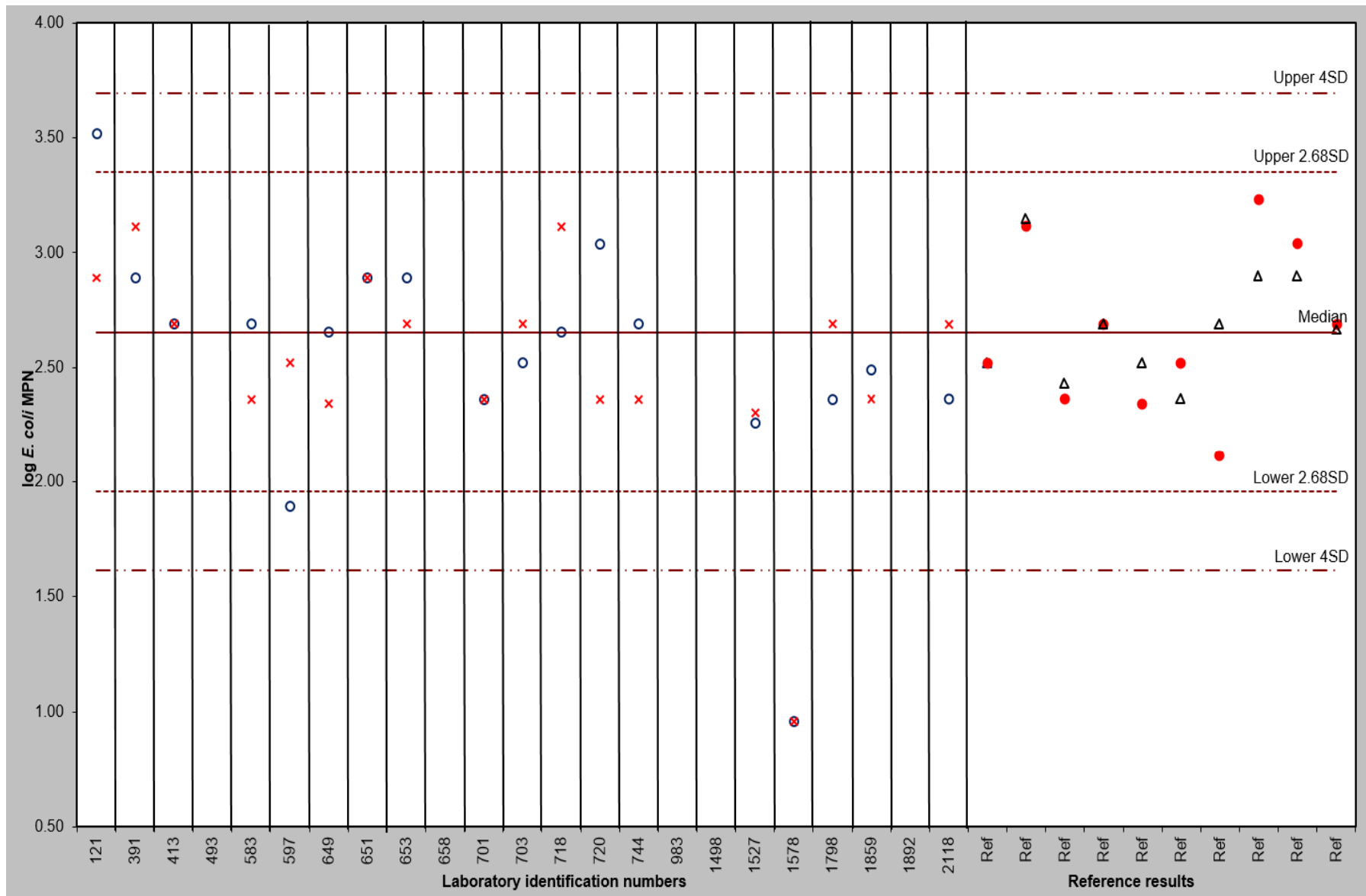
NE – Not examined

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

***E. coli* MPN** – Fifteen laboratories reported replicate results within ± 2.68 SD of the participants' median with all receiving a maximum score. Laboratory 121 and 597 reported one replicate result within ± 2.68 SD of the participants' median and received a score of 9 and laboratory 1578 reported both replicates outside ± 4 SD of the participants' median and received an overall score of 4.

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.

Figure 1. Distribution SF053: Sample SF0114



Distribution SF053

Sample SF0115 contents – *E. coli* (1.7×10^2 - 5.4×10^3) (wild strain), *Salmonella Enteritidis* 1,9,12:g,m (60) (wild strain), *Serratia liquefaciens* (1.9×10^4) (wild strain), *Streptococcus bovis* (1.9×10^3) (NCTC 8177)

Analysed February / March 2016 – Eighteen 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 493, 658, 983, 1498 and 1892 did not participate in this distribution.

Reference results - *E. coli* MPN – 290 - 6900 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 6: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0115

	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	1300	261	119	6468	14254

Participants results - SF0115 (Figure 2)

Table 7: Results reported by participants and scores allocated - SF0115

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	3300	1300	12	Detected	2
391	1700	1300	12	Detected	2
413	4900	4900	12	Detected	2
493	DNP	DNP	-	DNP	-
583	1300	1700	12	Detected	2
597	1100	330	12	Detected	2
649	2300	1300	12	Detected	2
651	3300	2300	12	Detected	2
653	1300	780	12	Detected	2
658	DNP	DNP	-	DNP	-
701	1300	780	12	Detected	2
703	780	1300	12	NE	-
718	2300	3300	12	Detected	2
720	1700	2300	12	Detected	2
744	3300	1700	12	Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527	1120	1160	8 *	NE	-
1578	<18	<18	4	Detected	2
1798	1100	930	12	Detected	2
1859	690	780	12	Detected	2
1892	DNP	DNP	-	DNP	-
2118	1700	1700	12	Detected	2

***E. coli* MPN** – Seventeen laboratories reported replicate results within ± 2.68 SD of the participants' median with all receiving a maximum score. Laboratory 1578 reported both replicate results outside ± 4 SD of the participants' median and received an overall score of 4.

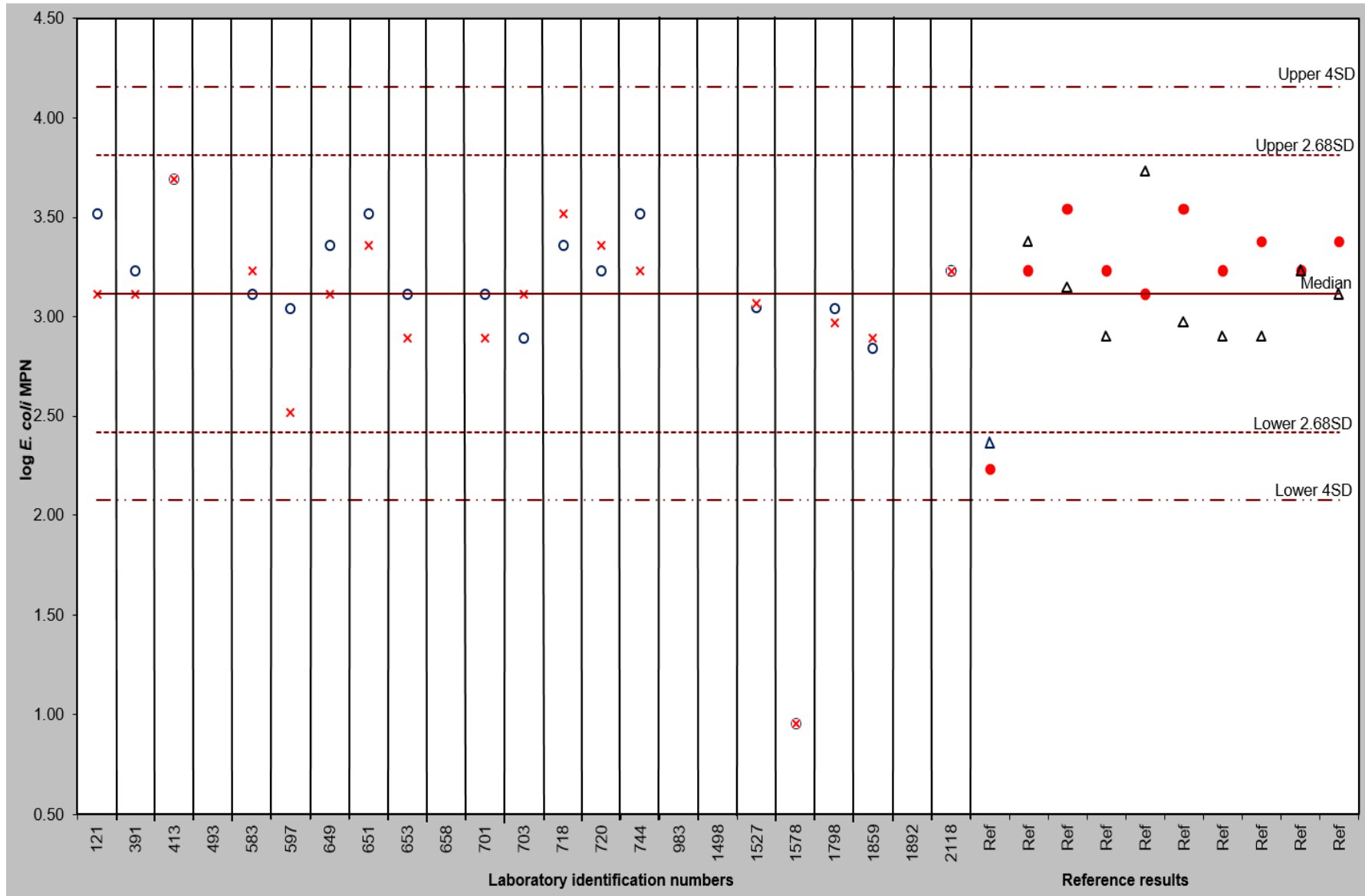
***Salmonella* spp.** – All 16 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

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 2. Distribution SF053: Sample SF0115



Appendix 2

Distribution SF054

Sample SF0116 contents - *Salmonella Agona* 1,4,[5],12:f,g,s: [1,2] [z₂₇],[z₄₅] (93) (wild strain), *Bacillus circulans* (<2.0x10²) (wild strain), *Enterococcus faecium* (1.2x10⁵) (wild strain)

Analysed June / July 2016 – Fifteen 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 493, 583, 649, 658, 744, 1578, 1859 and 2118 did not participate in this distribution.

Reference results - *E. coli* MPN – <18 per 100g. *Salmonella* spp. – Detected in 25g.

Participants results - SF0116

Table 8: Results reported by participants and scores allocated - SF0116

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	<18	<18	12	NE	-
391	<18	<18	12	Detected	2
413	<18	<18	12	Detected	2
493	DNP	DNP	-	DNP	-
583	DNP	DNP	-	DNP	-
597	<18	<18	12	Detected	2
649	DNP	DNP	-	DNP	-
651	<18	<18	12	Detected	2
653	<18	<18	12	Detected	2
658	DNP	DNP	-	DNP	-
701	<18	<18	12	Detected	2
703	<18	<18	12	NE	-
718	<18	<18	12	Detected	2
720	<18	<18	12	Detected	2
744	DNP	DNP	-	DNP	-
983	<1	<1	8	Detected	2
1498	<18	<18	12	Detected	2
1527	<200	<200	8 *	NE	-
1578	DNP	DNP	-	DNP	-
1798	<18	<18	12	Detected	2
1859	DNP	DNP	-	DNP	-
1892	<18	<18	12	Detected	2
2118	DNP	DNP	-	DNP	-

***E. coli* MPN** – Fourteen laboratories reported the absence of *E. coli* in this sample and received 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 and received an overall score of 8.

***Salmonella* spp.** – All 12 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.

Distribution SF054

Sample SF0117 contents – *E. coli* (4.6×10^2 - 5.4×10^3) (wild strain), *Salmonella Anatum* 3,_{{10}{15}{15,34}}:e,h:1,6 [z₆₄] (26) (wild strain), *Aeromonas hydrophila* (1.6×10^2) (wild strain), *Enterococcus faecium* (6.4×10^3) (wild strain)

Analysed June / July 2016 – Fifteen 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 493, 583, 649, 658, 744, 1578, 1859 and 2118 did not participate in this distribution.

Reference results - *E. coli* MPN – 350 - 8300 per 100g. ***Salmonella* spp.** - Detected in 25g.

Table 9: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0117

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	1300	261	119	6468	14254
Participants results	1700	342	155	8458	18640

Participants results - SF0117 (Figure 3)

Table 10: Results reported by participants and scores allocated - SF0117

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	1100	2300	12	NE	-
391	1300	2300	12	Detected	2
413	1700	1700	12	Detected	2
493	DNP	DNP	-	DNP	-
583	DNP	DNP	-	DNP	-
597	490	1100	12	Detected	2
649	DNP	DNP	-	DNP	-
651	13000	1300	9	Detected	2
653	1300	2200	12	Detected	2
658	DNP	DNP	-	DNP	-
701	1700	1700	12	Detected	2
703	3300	1300	12	NE	-
718	1400	780	12	Detected	2
720	2200	4900	12	Detected	2
744	DNP	DNP	-	DNP	-
983	7000	7900	8	Detected	2
1498	330	1400	9	Detected	2
1527	1700	1240	8 *	NE	-
1578	DNP	DNP	-	DNP	-
1798	4900	2300	12	Detected	2
1859	DNP	DNP	-	DNP	-
1892	1300	2200	12	Detected	2
2118	DNP	DNP	-	DNP	-

***E. coli* MPN** – Thirteen laboratories reported replicate results within ± 2.68 SD of the participants' median with 12 receiving a maximum score. Laboratories 651 and 1498 reported one replicate result between ± 2.68 and ± 4 SD of the participants' median and received a score of 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 and received an overall score of 8.

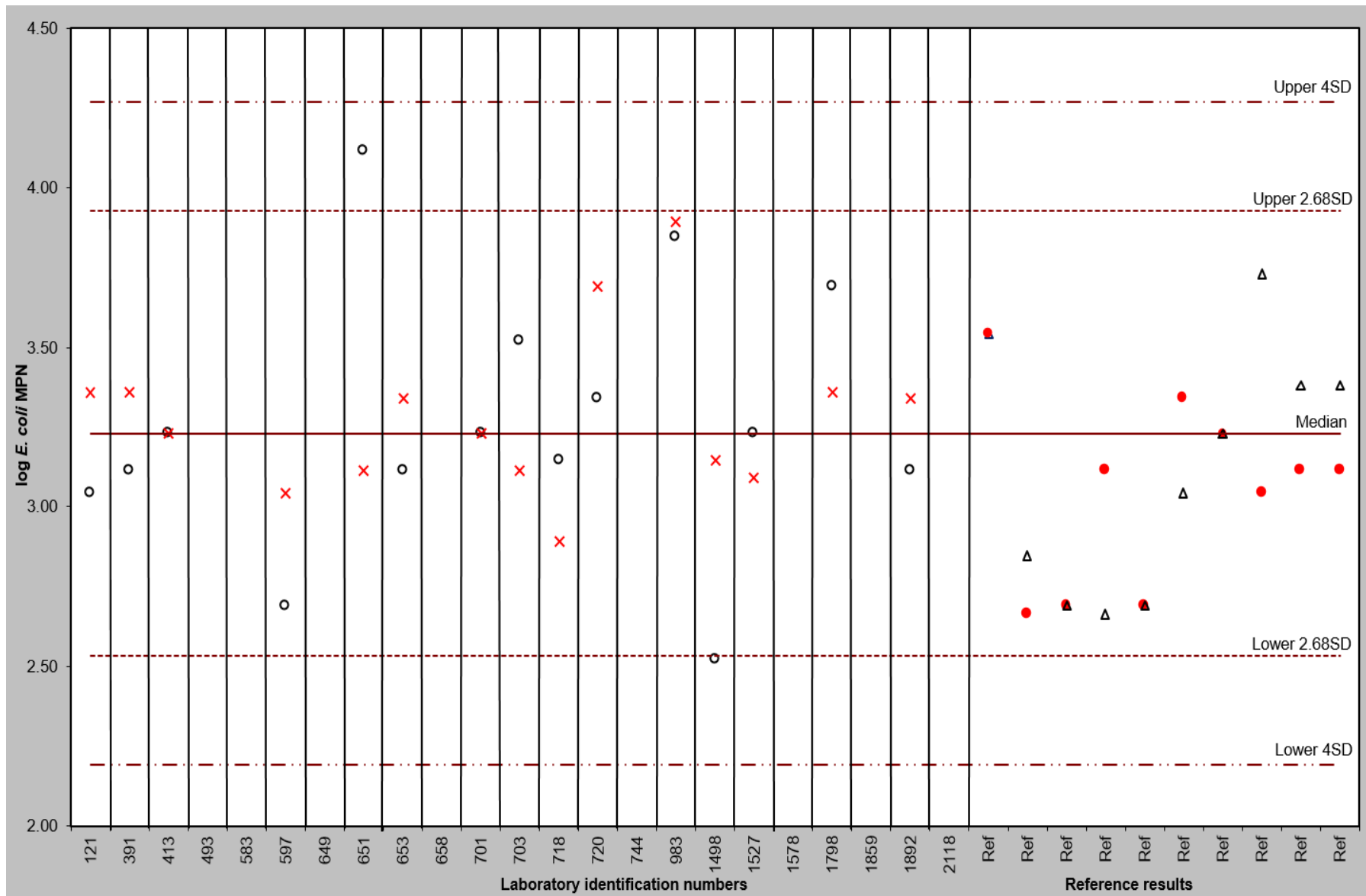
***Salmonella* spp.** – All 12 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

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 SF054: Sample SF0117



Appendix 3

Distribution SF055

Sample SF0118 contents – *E. coli* (1.3×10^3 - 1.6×10^4) (wild strain), *Pseudomonas putida* (1.5×10^4) (wild strain), *Streptococcus bovis* (2.6×10^3) (NCTC 8177)

Analysed November / December 2016 – Twenty laboratories received material for examination with 17 laboratories returning results to be included in the assessment. Laboratories 413, 597 and 720 did not return results for this distribution. Laboratories 121 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 983, 1498 and 1892 did not participate in this distribution.

Reference results - *E. coli* MPN – 1000 - 24000 per 100g. ***Salmonella* spp.** – Not detected in 25g.

Table 11: Participants and reference results median, median ± 2.68 SD and ± 4 SD - SF0118

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	4900	985	447	24378	53727
Participants results	3300	663	301	16418	36184

Participants results - SF0118 (Figure 4)

Table 12: Results reported by participants and scores allocated - SF0118

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	7000	4900	12	NE	-
391	3100	4900	12	Not detected	2
413	NE	NE	-	NE	-
493	7900	3300	12	Not detected	2
583	4600	7000	12	Not detected	2
597	NR	NR	0	NR	0
649	2200	1300	12	Not detected	2
651	4900	4900	12	Not detected	2
653	4900	7900	12	Not detected	2
658	3300	7000	12	Not detected	2
701	3300	3300	12	Not detected	2
703	3300	2700	12	Not detected	2
718	7900	2300	12	Not detected	2
720	NE	NE	-	NE	-
744	2300	1700	12	Not detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527	4040	4180	8 *	NE	-
1578	3300	3300	12	Not detected	2
1798	3300	3300	12	Not detected	2
1859	3100	2700	12	Not detected	2
1892	DNP	DNP	-	DNP	-
2118	7900	3300	12	Not detected	2

***E. coli* MPN** – Seventeen laboratories reported replicate results within ± 2.68 SD of the participants' median with all receiving a maximum score. Laboratory 597 did not provide a reason for not examining this distribution and scored 0.

***Salmonella* spp.** – All 15 laboratories that reported a result for *Salmonella* spp. correctly reported the absence of *Salmonella* spp. and received a maximum score of 2. Laboratory 597 did not provide a reason for not examining this distribution and scored 0.

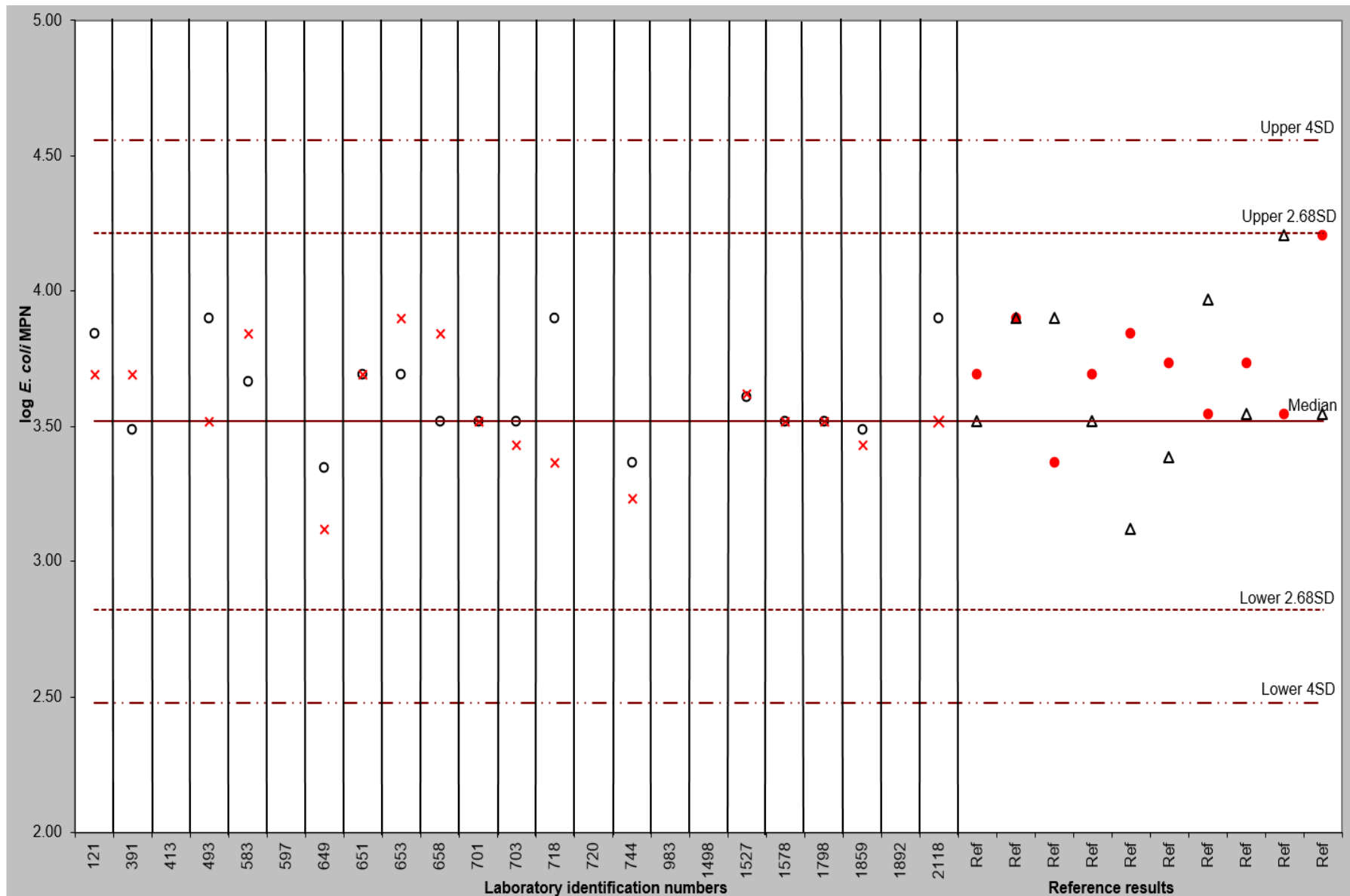
DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

NR – Not returned

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

Figure 4. Distribution SF055: Sample SF0118



Distribution SF055

Sample SF0119 contents – *E. coli* (3.1×10^2 - 1.3×10^3) (wild strain), *Salmonella* *Pensacola* 1,9,12:m,t:[1,2] (73 disc) (wild strain), *Bacillus pumilus* (1.4×10^3) (wild strain), *Pseudomonas putida* (3.3×10^3) (wild strain)

Analysed November / December 2016 – Twenty laboratories received material for examination with 17 laboratories returning results to be included in the assessment. Laboratories 413, 597 and 720 did not return results for this distribution. Laboratories 121 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 983, 1498 and 1892 did not participate in this distribution.

Reference results - *E. coli* MPN – 140 - 3400 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 13: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0119

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	490	98	45	2438	5373
Participants results	734	147	67	3650	8044

Participants results - SF0119

Table 14: Results reported by participants and scores allocated - SF0119

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	780	1300	12	NE	-
391	780	780	12	Detected	2
413	NE	NE	-	NE	-
493	690	1300	12	Detected	2
583	690	1300	12	Detected	2
597	NR	NR	0	NR	0
649	170	130	9	Detected	2
651	1100	780	12	Detected	2
653	330	780	12	Detected	2
658	780	680	10	Detected	2
701	1300	780	12	Detected	2
703	490	330	12	Detected	2
718	690	490	12	Detected	2
720	NE	NE	-	NE	-
744	330	230	12	Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527	680	880	12	NE	-
1578	1300	1100	12	Detected	2
1798	690	780	12	Detected	2
1859	490	490	12	Detected	2
1892	DNP	DNP	-	DNP	-
2118	1100	170	12	Detected	2

***E. coli* MPN** – Sixteen laboratories reported replicate results within ± 2.68 SD of the participants' median with 15 receiving a maximum score. Laboratory 649 reported one replicate result between ± 2.68 and ± 4 SD of the participants' median and received an overall scored 9. Laboratory 658 had points deducted as the tube combination reported for one replicate was inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013 and received an overall score of 10. Laboratory 597 did not provide a reason for not examining this distribution and scored 0.

***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. Laboratory 597 did not provide a reason for not examining this distribution and 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.

Appendix 4:

EURL PT 64 - NRL results and allocated scores

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

Lab ID	Sample 1			Sample 2 ^a		Sample 3				
	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
3 [703]	330	230	12	Not detected	-	3300	2300	12	Present	2
7 [718]	490	1300	9	Not detected	-	3300	1300	12	Present	2
9 [391]	130	130	12	Not detected	-	2300	1700	12	Present	2
10 [701]	490	490	12	Not detected	-	3300	2200	12	Present	2
13 [1578]	130	330	12	Not detected	-	7900	4900	12	Present	2
19 [121]	130	78	12	Not detected	-	3300	2300	12	Present	2
22 [493]	780	690	12	Not detected	-	1400	1400	12	Present	2
23 [658]	78	130	12	Not detected	-	1100	780	12	Present	2
27 [1498]	170	230	12	Not detected	-	2100	4900	12	Present	2
32 [583] ^b	490	45	8	Not detected	-	1300	2200	12	Present	2
33 [2118]	78	130	12	Present	-	3300	1300	12	Present	2
35 [413]	330	170	12	Not detected	-	3300	1300	12	Present	2
39 [653]	230	170	12	Not detected	-	1300	3300	12	Present	2
41 [597]	690	230	12	Not detected	-	3300	1700	12	Present	2
42 [2340]	68	20	9	Not detected	-	1300	690	12	Present	2
43 [651]	230	490	12	Not detected	-	1300	1300	12	Present	2
44 [983] ^b	130	78	8	Not detected	-	1300	3300	8	Present	2
47 [744]	78	45	12	Not detected	-	2200	2200	12	Present	2
68 [649]	210	130	12	Present	-	3300	4600	12	Present	2
69 [2342]	330	490	12	Not Detected	-	3500	2400	12	Not Detected	0
83 [1859]	230	230	12	Not detected	-	3300	4900	12	Present	2
90 [720]	490	330	12	Not detected	-	2300	4900	12	Present	2
102 [2341] ^{b c}	13	4.5	0	Not detected	-	330	170	2	Present	2
147 [1798]	140	110	12	Not detected	-	2300	4900	12	Present	2
170 [1527] ^d	<200	<200	8	NE	-	1620	1800	8	NE	-
212 ^d	230	440	8	NE	-	1300	1500	8	NE	-
245 [1892]	130	330	12	Not detected	-	1700	2200	12	Present	2

^a No scores allocated as *Salmonella* spp. was detected 4/20 replicate reference samples

^b Scores deducted as tube combination inconsistent with rules specified in ISO 7218.

^c Score deducted as incorrect MPN value given for recorded tube combination.

^d MPN tube combination is not required for the alternative method used by this NRL, the overall maximum score is reduced to reflect this (8).

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 $\pm 2.68SD$ and median $\pm 4SD$ values	2	5	2	9
Both replicate MPN results are outside the expected range and fall between the median $\pm 2.68SD$ and median $\pm 4SD$ values	2	2	2	6
One replicate MPN result is outside the median $\pm 4SD$ value	2	5	0	7
Both replicate MPN results are outside the expected range. The first falls between the median $\pm 2.68SD$ and median $\pm 4SD$ values and the second falls outside the median $\pm 4SD$ values	2	2	0	4
Both replicate MPN results are outside the median $\pm 4SD$ value	2	0	0	2

***E. coli* MPN scores allocated to participants returning a 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 $\pm 2.68SD$ and median $\pm 4SD$ values	2	2	4
Single replicate MPN result is outside the median $\pm 4SD$ 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 EURL.	2	2
High censored result (e.g. MPN = >18 000 per 100g)	2	2
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

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

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

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

2. **Culture media** - Check the quality control data for media to ensure that they are within specifications and performing adequately.
3. **Equipment** - Check that the equipment used for the procedures (incubators, refrigerators, measuring instruments) are calibrated and performing adequately.
4. **Staff training** - Check that the staff performing the tests are fully trained and familiar with all the procedural steps.
5. **Clerical procedures** - Check that the sample labeling, laboratory numbering and clerical procedures are adequate and that you have procedures for ensuring that test results are reported accurately and on time.
6. **Accreditation**- Check that quality procedures are documented and adhered to at all times.
7. **Internal quality controls (IQC)** – Ensure adequate controls are in place and follow-up procedures are in place to deal with IQC failures.

Further advice can be obtained from the EURL on request.

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