



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

***Escherichia coli* and *Salmonella* spp. EQA**

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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 32 of Regulation (EC) 882/2004 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'. Protocols for the application of these methods are available at www.eurlcefas.org

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

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.eurlcefas.org

Methodology

Sample preparation - PHE EQA

Samples comprising of LENTICULE™ discs containing fully characterised bacterial isolates were distributed during February, June and October 2017 (SF056, SF057 and SF058 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

Two shellfish samples comprising of whole Common Mussels (*Mytilus edulis*) and homogenised Pacific oysters (*Crassostrea gigas*) originating from a UK commercial harvesting area were distributed in November 2017 (EURL PT 73). 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 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 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 2017; 25 NRLs (NRL Denmark has nominated an OCL to take part in bacteriological PT schemes on behalf of the NRL 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 2017 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). Participation of NRLs and designated OCLs in EURL PT 73 and PHE EQA distribution SF056 was funded by the EURL; participation in the other PHE EQA distributions was self-funded.

Table 1: Participation by NRLs in 2017 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 73	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PHE EQA distribution	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 73) (Appendix 4) and 1 EQA distribution (SF056). The allocated scores are summarised in Tables 2 and 3 respectively. Scores for SF057 and SF058 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-five laboratories participated in the EURL matrix scheme (Appendix 4) and 1 or more PHE/EQA distributions in 2017. Of these, one NRL participated using 2 methods (the reference method and an approved alternative method) for both EURL and PHE/EQA distributions. One NRL participated using 2 methods (the reference method and an approved alternative) in the EURL 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 26 sets of reported results for PT 73 and SF056, 24 laboratories (93%) 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). Laboratories 983 and 2341 achieved a cumulative total of <70%.

***Salmonella* spp. assessment**

Twenty-five laboratories participated in the EURL matrix scheme and 1 or more EQA distributions in 2017 and were therefore subject to a full performance assessment with all laboratories achieving a cumulative total of >70% for the distributions analysed. Laboratory 648 did not examine PT 73 sample 1 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 ^e						
	PT 73		Distribution SF056		Cumulative score ^d	Max score	%	Distribution SF057		Distribution SF058		Cumulative score	Max score	%
	S - 1	S - 2	SF0120	SF0121				SF0122	SF0123	SF0124	SF0125			
121 [19]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
391 [9]	12	12	12	12	48	48	100	12	12	12	12	96	96	100
413 [35]	12	12	12	12	48	48	100	12	12	12	12	96	96	100
493 [22]	12	12	8	8	40	48	83	-	-	-	-	40	48	83
583 [32]	12	12	12	12	48	48	100	12	12	-	-	72	72	100
597 [41]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
649 [68]	12	12	12	12	48	48	100	-	-	12	12	72	72	100
651 [43]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
653 [39]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
658 [23]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
701 [10]	12	12	8	8	40	48	83	12	12	12	12	88	96	92
703 [3]	12	12	12	9	45	48	94	12	12	12	12	93	96	97
718 [7]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
720 [90]	12	10	12	12	46	48	96	-	-	-	-	46	48	96
744 [47]	12	9	8	8	37	48	77	-	-	12	12	61	72	85
983 [44]	8	8	8	8	32	48	67	-	-	-	-	32	48	67
1498 [27]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
1527[170] ^b	8	4	8	8	28	32	88	8	8	8	8	60	64	94
1578 [13]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
1798 [147]	12	12	12	12	48	48	100	12	12	12	12	96	96	100
1859 [83]	12	9	12	12	45	48	94	-	-	-	-	45	48	94
1891 [69]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
1892 [245]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
2118 [33]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
2340 [42]	12	10	8	8	38	48	79	-	-	-	-	38	48	79
2341 [102]	8	8	8	8	32	48	67	-	-	-	-	32	48	67
[212] ^{b,c}	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 EQA material cannot be analysed using the alternative method used by this laboratory, therefore a full assessment cannot be completed.

^d Cumulative score is based on scores from PT73 and distribution SF056 only.

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

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

Lab no. ^a	Performance assessment							Provided for information only ^d						
	PT 73		Distribution SF056		Cumulative score ^c	Max score	%	Distribution SF057		Distribution SF058		Cumulative score	Max score	%
	S - 1	S - 2	SF0120	SF0121				SF0122	SF0123	SF0124	SF0125			
121 [19]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
391 [9]	2	2	2	2	8	8	100	2	2	2	2	16	16	100
413 [35]	2	2	2	2	8	8	100	2	2	2	2	16	16	100
493 [22]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
583 [32]	2	2	2	2	8	8	100	2	2	-	-	12	12	100
597 [41]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
649 [68]	-	2	2	2	6	6	100	-	-	2	2	10	10	100
651 [43]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
653 [39]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
658 [23]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
701 [10]	2	2	2	2	8	8	100	2	2	2	2	16	16	100
703 [3]	0	2	2	2	6	8	75	2	2	-	-	10	12	83
718 [7]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
720 [90]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
744 [47]	2	2	2	2	8	8	100	-	-	2	2	12	12	100
983 [44]	0	2	2	2	6	8	75	-	-	-	-	6	8	75
1498 [27]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
1527[170] ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1578 [13]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
1798 [147]	2	2	2	2	8	8	100	2	0	2	2	14	16	88
1859 [83]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
1891 [69]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
1892 [245]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
2118 [33]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
2340 [42]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
2341 [102]	2	2	2	2	8	8	100	-	-	-	-	8	8	100
[212] ^{b,c}	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^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 Cumulative score is based on scores from PT 73 and distribution SF056 only.

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

References

Anon 2015. ISO 16649-3:2015. 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. Geneva, Switzerland.

Anon 2016. ISO 16649-3:2016 corrected. 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. Geneva, Switzerland.

Anon. 2017. ISO 6579:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* spp. Part 1: 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 SF056

Sample SF0120 contents – *Escherichia coli* (4.9×10^2 - 4.9×10^3) (wild strain), *Salmonella* Indiana 1,4,12: z: 1,7 (1.5×10^2) (wild strain), *Bacillus pumilus* (7.0×10^2) (wild strain), *Klebsiella oxytoca* (6.5×10^2) (wild strain)

Reference results - *E. coli* MPN – 220 – 5400 per 100g. *Salmonella* spp. – Detected in 25g.

Analysed February / March 2017 – Twenty-six sets of results were returned for this distribution and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp..

Table 4: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0120

	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	1100	221	100	5472	12061

Participants' results - SF0120 (Figure 1)

Table 5: Results reported by participants and scores allocated - SF0120

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	2300	12	Detected	2
391	780	780	12	Detected	2
413	1700	1700	12	Detected	2
493	690	1300	8	Detected	2
583	780	1100	12	Detected	2
597	1300	2300	12	Detected	2
649	490	690	12	Detected	2
651	690	1100	12	Detected	2
653	1100	780	12	Detected	2
658	780	1300	12	Detected	2
701	780	3300	8	Detected	2
703	780	230	12	Detected	2
718	490	3300	12	Detected	2
720	1700	3300	12	Detected	2
744	490	490	8	Detected	2
983	790	1100	8	Detected	2
1498	780	780	12	Detected	2
1527 *	620	620	8	Not Examined	NE
1578	2300	3300	12	Detected	2
1798	1100	3300	12	Detected	2
1859	2200	1300	12	Detected	2
1891	2300	1300	12	Detected	2
1892	1100	1700	12	Detected	2
2118	780	1100	12	Detected	2
2340	2300	1300	8	Detected	2
2341	1700	1700	8	Detected	2

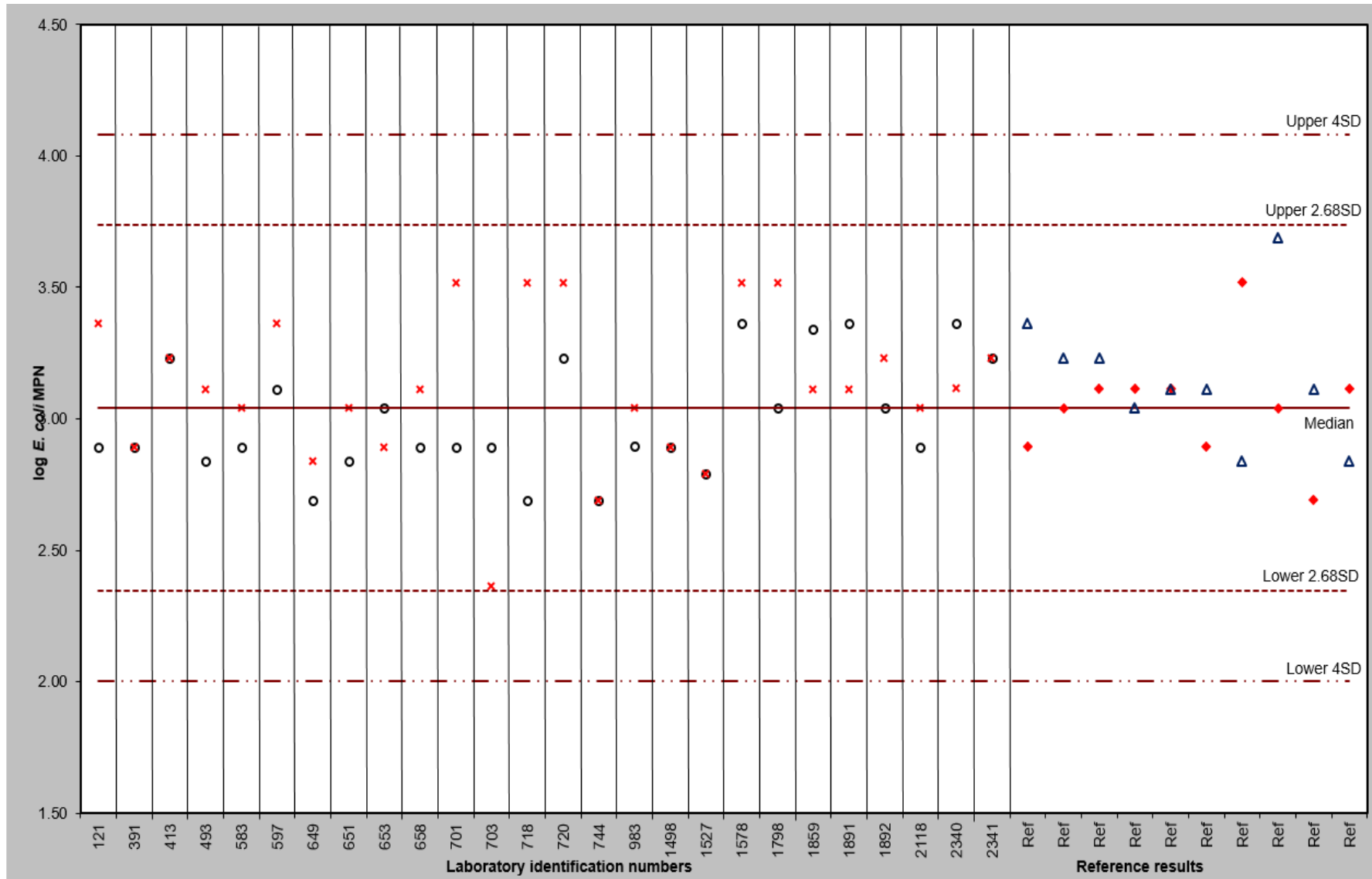
***E. coli* MPN** – All laboratories reported replicate results within ± 2.68 SD of the participants' median with 20 laboratories receiving a maximum score. Laboratories 493, 701, 744, 983, 2340 and 2341 had points deducted as the tube combinations reported for both replicates were inconsistent with 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.

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 SF056: Sample SF0120



Distribution SF056

Sample SF0121 contents – *Escherichia coli* (3.3×10^2 – 2.3×10^3) (wild strain), *Salmonella* London 3, {10}{15}: l,v: 1,6 (3.3×10^2) (wild strain), *Micrococcus* spp. (1.5×10^4) (wild strain), *Providencia rettgeri* (1.3×10^2) (NCTC 11801)

Analysed February / March 2017 – Twenty-six sets of results were returned for this distribution and were included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp..

Reference results - *E. coli* MPN – 190 - 4600 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 6: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0121

	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	1100	221	100	5472	12061

Participants' results - SF0121 (Figure 2)

Table 7: Results reported by participants and scores allocated - SF0121

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	230	1100	12	Detected	2
391	780	780	12	Detected	2
413	1700	1700	12	Detected	2
493	1300	2300	8	Detected	2
583	490	1300	12	Detected	2
597	1100	1300	12	Detected	2
649	780	490	12	Detected	2
651	1300	1400	12	Detected	2
653	2200	1400	12	Detected	2
658	3300	4900	12	Detected	2
701	1100	2300	8	Detected	2
703	170	490	9	Detected	2
718	490	1700	12	Detected	2
720	780	2300	12	Detected	2
744	490	1100	8	Detected	2
983	1700	2200	8	Detected	2
1498	330	490	12	Detected	2
1527*	740	860	8	Not Examined	NE
1578	1300	2300	12	Detected	2
1798	780	1100	12	Detected	2
1859	330	490	12	Detected	2
1891	3300	690	12	Detected	2
1892	690	490	12	Detected	2
2118	490	490	12	Detected	2
2340	1300	1700	8	Detected	2
2341	1700	950	8	Detected	2

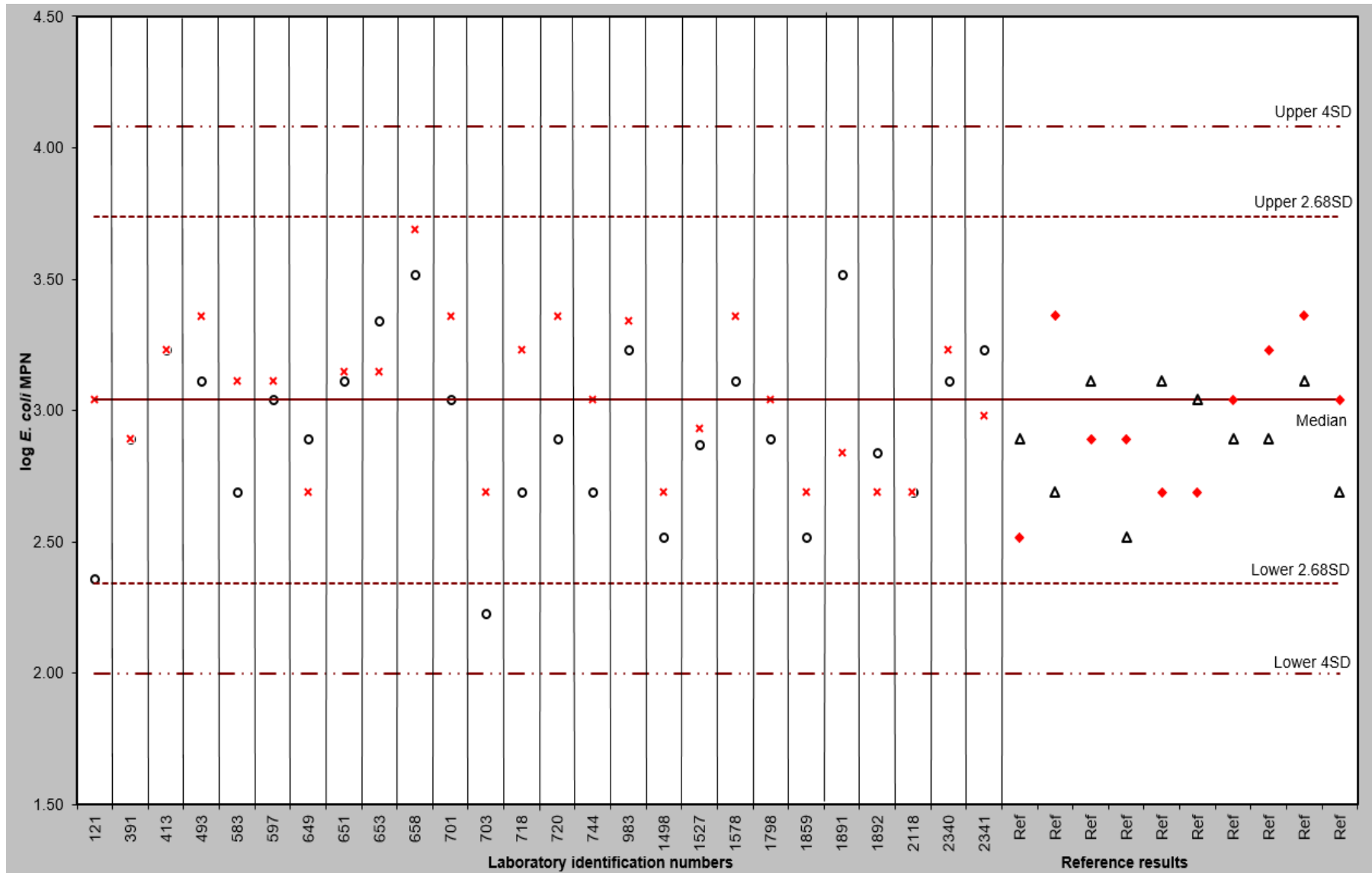
***E. coli* MPN** – Twenty-five laboratories reported replicate results within ± 2.68 SD of the participants' median with 19 receiving a maximum score. Laboratory 703 reported 1 replicate result between ± 2.68 SD and ± 4 SD of the participants' median and received a score of 9. Laboratories 493, 701, 744, 983, 2340 and 2341 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.

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 SF056: Sample SF0121



Appendix 2

Distribution SF057

Sample SF0122 contents – *Escherichia coli* (4.9×10^3 - 5.4×10^4) (wild strain), *Salmonella* Agona 1,4,[5],12:f,g,s:[1,2][z₂₇],[z₄₅] (10^3) (wild strain), *Salmonella* Typhimurium 1,4,[5],12:i:1,2 (<100 disc) (wild strain), *Klebsiella oxytoca* (1.2×10^4) (wild strain)

Analysed June / July 2017 – Seven sets of results were returned for this distribution and were included in the assessment. Laboratory 121 did not return results for this distribution. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 121, 493, 597, 649, 651, 653, 658, 718, 720, 744, 983, 1498, 1578, 1859, 1891, 1892, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – 2700 - 64000 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 8: Participants' and reference results median, median ± 2.68 SD and ± 4 SD - SF0122

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	13000	2613	1186	64676	142542
Participants' results	7900	1588	720	39303	86622

Participants' results - SF0122 (Figure 3)

Table 9: Results reported by participants and scores allocated - SF0122

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	DNP	DNP	-	DNP	-
391	7900	7900	12	Detected	2
413	13000	13000	12	Detected	2
493	DNP	DNP	-	DNP	-
583	7900	4900	12	Detected	2
597	DNP	DNP	-	DNP	-
649	DNP	DNP	-	DNP	-
651	DNP	DNP	-	DNP	-
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	3300	3300	12	Detected	2
703	1700	2200	12	Detected	2
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	DNP	DNP	-	DNP	-
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	11240	10080	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	35000	13000	12	Detected	2
1859	DNP	DNP	-	DNP	-
1891	DNP	DNP	-	DNP	-
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 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.

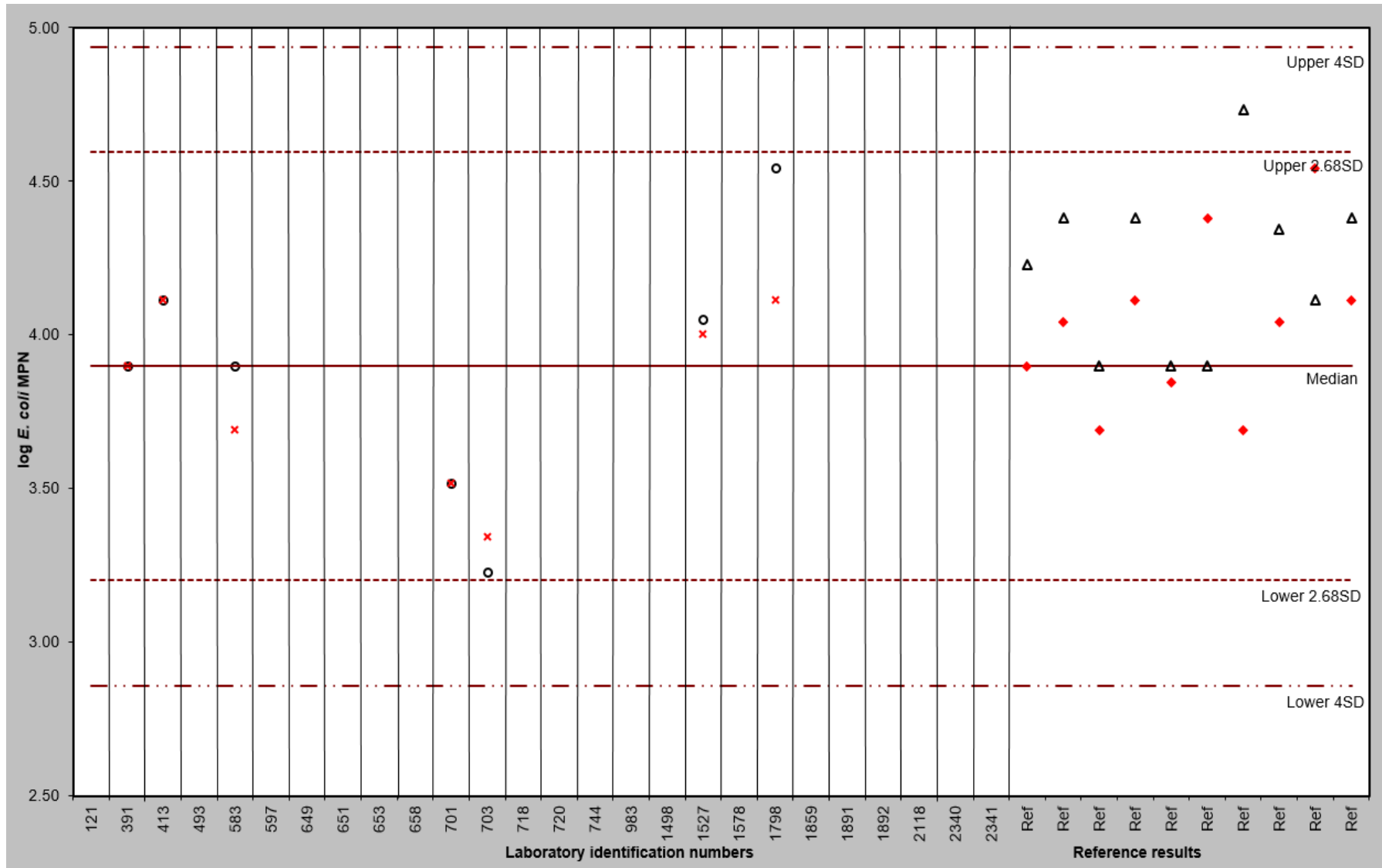
DNP – NRL registered for EQA scheme but did not participate in this distribution.

NR – Not returned

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 SF057: Sample SF0122



Distribution SF057

Sample SF0123 contents – *Escherichia coli* (4.9×10^2 - 2.7×10^3) (wild strain), *Salmonella Heidelberg* 1,4,[5],12:r:1,2 (46) (wild strain), *Aeromonas hydrophila* (4.0×10^2) (wild strain), *Enterococcus faecium* (1.1×10^2) (wild strain)

Analysed June / July 2017 – Seven sets of results were returned for this distribution and were included in the assessment. Laboratory 121 did not return results for this distribution. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 121, 493, 597, 649, 651, 653, 658, 718, 720, 744, 983, 1498, 1578, 1859, 1891, 1892, 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 - SF0123

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	1196	240	109	5949	13112
Participants' results	780	157	71	3881	8553

Participants' results - SF0123 (Figure 4)

Table 11: Results reported by participants and scores allocated - SF0123

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	DNP	DNP	-	DNP	-
391	780	780	12	Detected	2
413	1300	1300	12	Detected	2
493	DNP	DNP	-	DNP	-
583	2300	780	12	Detected	2
597	DNP	DNP	-	DNP	-
649	DNP	DNP	-	DNP	-
651	DNP	DNP	-	DNP	-
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	1700	780	12	Detected	2
703	780	270	12	Detected	2
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	DNP	DNP	-	DNP	-
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	800	760	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	780	1700	12	Not detected	0
1859	DNP	DNP	-	DNP	-
1891	DNP	DNP	-	DNP	-
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 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.

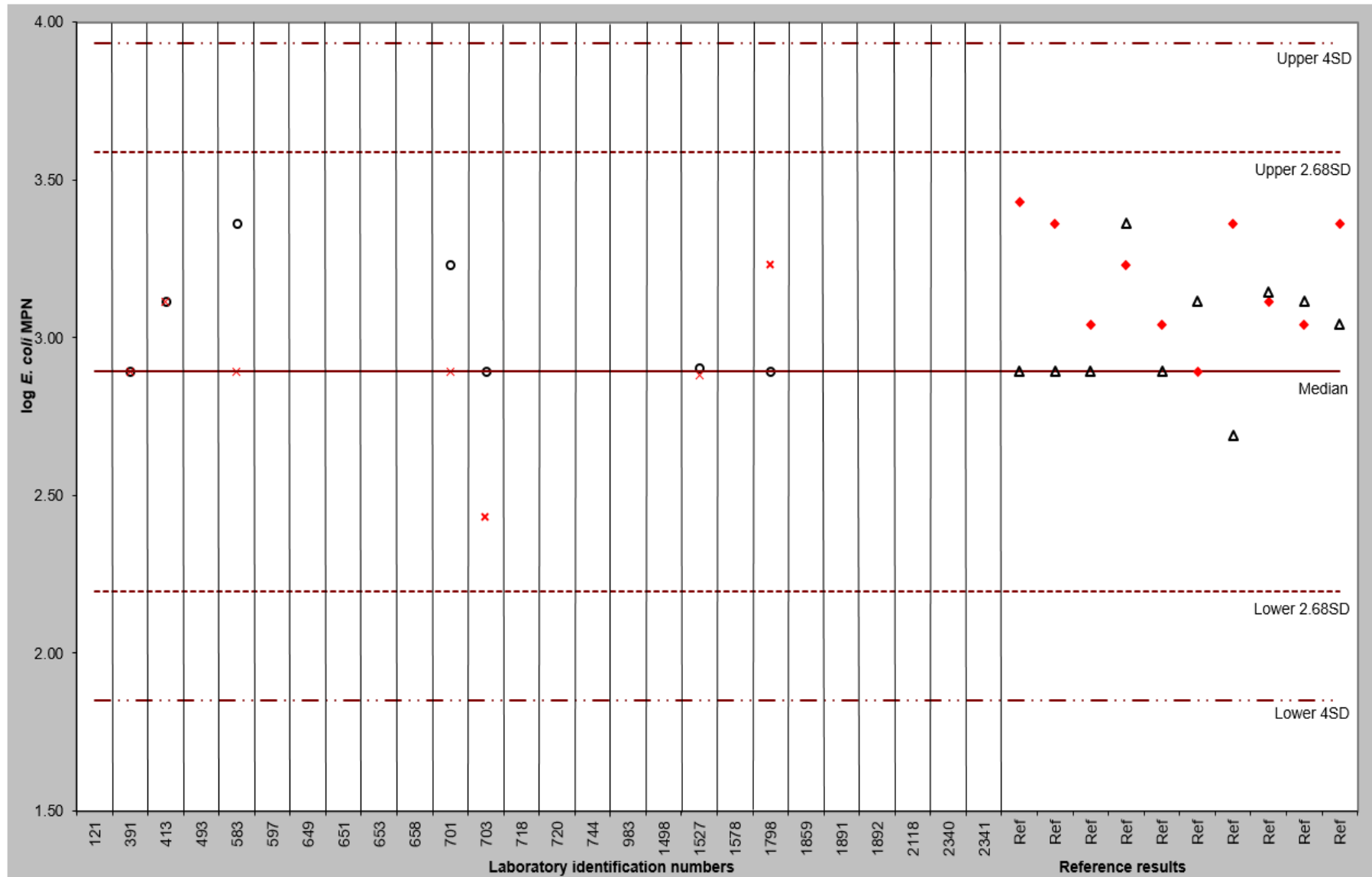
DNP – NRL registered for EQA scheme but did not participate in this distribution.

NR – Not returned

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 SF057: Sample SF0123



Appendix 3

Distribution SF058

Sample SF0124 contents – *Escherichia coli** (4.9×10^2 - 7.9×10^3) (wild strain), *Salmonella* Quiniela 6,8:c:e,n,z₁₅ (62) (wild strain), *Klebsiella oxytoca* 1.9×10^2 (wild strain), *Microbacterium luteolum* 16 (NCIMB 9568)

Analysed November / December 2017 – Eight sets of results were returned for this distribution and were included in the assessment. Laboratory 121 did not return results for this distribution. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 121, 493, 583, 597, 651, 653, 658, 718, 720, 983, 1498, 1578, 1859, 1891, 1892, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – 470 - 11000 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 12: Participants' and reference results median, median ± 2.68 and ± 4 SD - SF0124

	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	1977	397	180	9838	21683

Participants' results - SF0124 (Figure 5)

Table 13: Results reported by participants and scores allocated - SF0124

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	DNP	DNP	-	DNP	-
391	2300	2300	12	Detected	2
413	2200	2200	12	Detected	2
493	DNP	DNP	-	DNP	-
583	DNP	DNP	-	DNP	-
597	DNP	DNP	-	DNP	-
649	2300	3300	12	Detected	2
651	DNP	DNP	-	DNP	-
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	1300	3300	12	Detected	2
703	930	780	12	NE	-
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	1700	1300	12	Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	1880	2080	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	1700	1300	12	Detected	2
1859	DNP	DNP	-	DNP	-
1891	DNP	DNP	-	DNP	-
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 and received a maximum score.

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

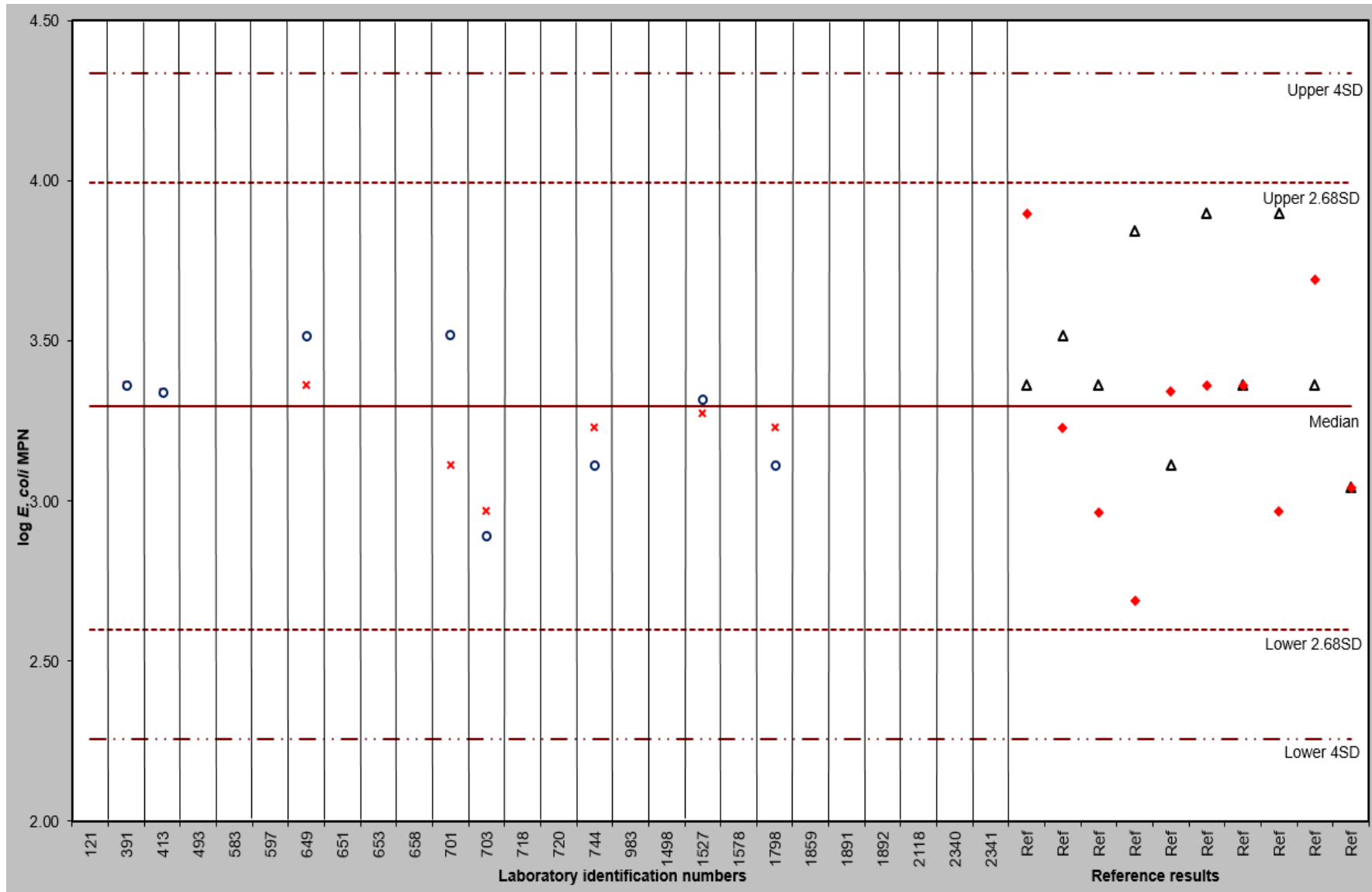
DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

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 5. Distribution SF058: Sample SF0124



Distribution SF058

Sample SF0125 contents – *Salmonella* Crewe 11:z:1,5 (19) (wild strain), *Bacillus subtilis* (7) (wild strain), *Pseudomonas putida* (1.4×10^2) (wild strain)

Analysed November / December 2017 – Eight sets of results were returned for this distribution and were included in the assessment. Laboratory 121 did not return results for this distribution. Laboratories 703 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 121, 493, 583, 597, 651, 653, 658, 718, 720, 983, 1498, 1578, 1859, 1891, 1892, 2118, 2340 and 2341 did not participate in this distribution.

Reference results - *E. coli* MPN – <18 – 220 per 100g. ***Salmonella* spp.** – Detected in 25g.

Participants' results - SF0125

Table 14: Results reported by participants and scores allocated - SF0125

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	DNP	DNP	-	DNP	-
391	<18	<18	12	Detected	2
413	<18	<18	12	Detected	2
493	DNP	DNP	-	DNP	-
583	DNP	DNP	-	DNP	-
597	DNP	DNP	-	DNP	-
649	45	45	12	Detected	2
651	DNP	DNP	-	DNP	-
653	DNP	DNP	-	DNP	-
658	DNP	DNP	-	DNP	-
701	<18	<18	12	Detected	2
703	<18	<18	12	NE	-
718	DNP	DNP	-	DNP	-
720	DNP	DNP	-	DNP	-
744	<18	<18	12	Detected	2
983	DNP	DNP	-	DNP	-
1498	DNP	DNP	-	DNP	-
1527*	<200	<200	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	<18	<18	12	Detected	2
1859	DNP	DNP	-	DNP	-
1891	DNP	DNP	-	DNP	-
1892	DNP	DNP	-	DNP	-
2118	DNP	DNP	-	DNP	-
2340	DNP	DNP	-	DNP	-
2341	DNP	DNP	-	DNP	-

***E. coli* MPN** – All laboratories returned replicate results reported the absence of *E. coli* or within the range determined by the PHE reference results 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

NR – Not returned

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 73 - NRL results and allocated scores

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

Lab ID	Sample 1					Sample 2				
	<i>E. coli</i> MPN/100g			<i>Sal. spp.</i> in 25g		<i>E. coli</i> MPN/100g			<i>Sal. spp.</i> in 25g	
	Rep 1	Rep 2	Score	Rep 1	Score	Rep 1	Rep 2	Score	Rep 1	Score
3 [703]	3100	1300	12	Detected	0	54000	11000	12	Detected	2
7 [718]	780	450	12	Not Detected	2	4900	13000	12	Detected	2
9 [391]	780	930	12	Not Detected	2	35000	17000	12	Detected	2
10 [701]	490	490	12	Not Detected	2	11000	24000	12	Detected	2
13 [1578]	1300	780	12	Not Detected	2	13000	7900	12	Detected	2
19 [121]	780	310	12	Not Detected	2	35000	7900	12	Detected	2
22 [493]	3300	4900	12	Not Detected	2	17000	24000	12	Detected	2
23 [658]	4900	3300	12	Not Detected	2	54000	54000	12	Detected	2
27 [1498]	490	490	12	Not Detected	2	13000	13000	12	Detected	2
32 [583]	1400	1100	12	Not Detected	2	7900	13000	12	Detected	2
33 [2118]	450	930	12	Not Detected	2	11000	3300	12	Detected	2
35 [413]	1700	1700	12	Not Detected	2	13000	35000	12	Detected	2
39 [653]	2300	4900	12	Not Detected	2	7900	7900	12	Detected	2
41 [597]	1300	1700	12	Not Detected	2	11000	7900	12	Detected	2
42 [2340] ^b	230	490	12	Not Detected	2	16000	35000	10	Detected	2
43 [651]	1700	1300	12	Not Detected	2	7000	4900	12	Detected	2
44 [983]	330	490	8	Detected	0	24000	11000	8	Detected	2
47 [744]	1400	2300	12	Not Detected	2	160000	54000	9	Detected	2
68 [649]	2300	2300	12	NE	-	13000	24000	12	Detected	2
69 [2342]	780	4900	12	Not Detected	2	13000	17000	12	Detected	2
83 [1859]	200	780	12	Not Detected	2	92000	24000	9	Detected	2
90 [720] ^b	230	1300	12	Not Detected	2	13000	24000	10	Detected	2
102 [2341] ^{a b}	1300	450	8	Not Detected	2	13000	22000	8	Detected	2
147 [1798]	930	620	12	Not Detected	2	35000	54000	12	Detected	2
170 [1527] ^c	<200	<200	8	NE	-	1200	1300	4	NE	-
212 ^c	5200	4200	8	NE	-	2600	3600	8	NE	-
245 [1892]	780	230	12	Not Detected	2	11000	13000	12	Detected	2

NE – Not examined.

NR – Not returned.

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

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

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

Appendix 5:
Scoring for the PHE/EQA 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

Note: For the EURL whole animal PT distribution the result ranges are based on participants' median ± 3 SD and ± 5 SD above and below the participants' median.

***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

Note: For the EURL whole animal PT distribution the result ranges are based on participants' median ± 3 SD and ± 5 SD above and below the participants' median.

***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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