



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

Escherichia coli and *Salmonella* spp. EQA

EURL PT reference number: PT 57

Report Final 1

20.03.15

19 pages

Contract Reference: Cefas ref (C6095)

Document approved by:	C6095 Project Manager – James Lowther	Review date:	Not applicable
Document checked by:	James Lowther	Classification:	Official
Document prepared by:	Louise Stockley	Location	EURL drive

Contents	Page number
Methodology	2
Reference results	2
Participants' analysis and scoring system	2
Participation in statutory determinands	2
Performance assessments	3
Reference	5
Appendix 1 - Distribution SF047: Sample SF0102 and SF0103	6
Appendix 2 - Distribution SF048: Sample SF0104 and SF0105	10
Appendix 3 - Distribution SF049: Sample SF0106 and SF0107	14
Appendix 4 - EURL PT 54 - NRL results and allocated scores	17
Appendix 5 - Scoring for the PHE/EQA and EURL matrix scheme	18
Appendix 6 - Troubleshooting advice	19

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

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

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

The EU reference method for enumeration of *E. coli* in raw bivalve molluscs is ISO TS 16649-3, Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide. EU approved alternative methods for the enumeration of *E. coli* are 'Enumeration of *Escherichia coli* in live bivalve molluscan shellfish by the direct impedance technique using the BacTrac 4300 series analyser' and 'Enumeration of *Escherichia coli* in bivalve molluscan shellfish by the colony-count technique'. Protocols for the application of these methods are available at www.eurlcefafas.org

The EU reference method for detection of *Salmonella* spp. in live bivalve molluscs is ISO 6579, Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Anon 2002).

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

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

If you are experiencing problems please contact the EURL (contact details below), or alternately refer to the troubleshooting guide included as Appendix III 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 - EQA

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

Sample preparation – EURL PT

Two shellfish samples comprising of Manila clams (*T. philippinarum*) and common mussels (*Mytilus edulis*) both originating from a UK commercial harvesting area were distributed in October 2014. Samples were packaged according to IATA regulations and distributed with instructions and report forms.

Reference results

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

Participants' analysis and scoring system

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

Participation in statutory determinands

In total 20 laboratories participated in one, two or all of the distributions offered within the scheme, comprising of 18 Member State NRLs and 2 EFTA country. 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 2014 with 80% (20 NRLs) participating in the EURL matrix distribution and 1 or more EQA distributions. Currently there is no active participation from a designated NRL in Malta, Cyprus or Estonia. NRLs in Bulgaria, The Czech Republic, Finland, Greece, Latvia, Lithuania and Slovakia did not participate in the mandatory number of EQA distributions per year agreed in Resolution 8 of the NRLs annual workshop 2012. The EURL recommends that NRLs not registered to the EQA scheme should join this scheme.

Table 1: Participation by NRLs in 2014 for *E. coli* and *Salmonella* spp. determinands.

Country	Austria	Belgium and Luxembourg	Bulgaria	Croatia	Denmark	France	Germany	Greece	Hungary	Iceland	Ireland	Italy	Italy	Latvia	Lithuania	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	United Kingdom
EURL PT 54	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Number of EQA distributions	0		✓					✓						✓	✓						✓				
	1								✓	✓			✓				✓		✓				✓		
	2				✓	✓	✓	✓																✓	
	3	✓	✓									✓	✓			✓		✓		✓		✓			✓

Performance Assessment

A cumulative performance assessment was undertaken on participant's results for both *E. coli* and *Salmonella* spp. from the EURL matrix distribution (PT 54) (Appendix 4) and 3 EQA distributions (March to November 2014). The allocated scores are summarised in Tables 2 and 3 respectively. Good performances is identified where a cumulative score of >70% is achieved. Participants who achieved <70% for *E. coli* enumeration and/or *Salmonella* spp. detection should in the first instance refer to the troubleshooting guide included as Appendix 6.

E. coli MPN assessment

Twenty-one laboratories participated in the EURL matrix scheme (Appendix 4) and 1 or more EQA distributions in 2014 and were therefore subject to a full performance assessment. Eighteen laboratories achieved a cumulative total of >70% for the two or more distributions analysed. Laboratories 121, 1498 and 1798 achieved a cumulative score <70%.

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

Lab no.	PT 54	Distribution SF047		Distribution SF048		Distribution SF049		All distributions		
	Sample 1	SF0102	SF0103	SF0104	SF0105	SF0106	SF0107	Cumulative score	Max score	%
121 [19]	3	12	12	7	7	8	8	57	84	68
391 [9]	8	12	12	-	-	-	-	32	36	89
403 [86]	8	12	12	-	-	5	8	45	60	75
413 [35]	5	12	12	12	12	8	8	69	84	82
493 [22] ^a	8	-	-	-	-	-	-	-	-	-
583 [32]	12	7	12	-	-	12	12	55	60	92
597 [41]	9	12	12	12	12	-	-	57	60	95
601 [17]	8	-	-	12	12	-	-	32	36	89
649 [68]	12	12	12	12	12	10	12	82	84	98
651 [43]	5	12	12	12	4	8	8	61	84	73
653 [39]	8	12	12	12	12	8	12	76	84	90
658 [23] ^a	5	-	-	-	-	-	-	-	-	-
660 [33] ^a	8	-	-	-	-	-	-	-	-	-
701 [10]	12	12	12	12	12	12	12	84	84	100
703 [3]	9	12	12	12	12	12	12	81	84	96
715 [21]	8	-	-	12	12	-	-	32	36	89
718 [7]	8	12	12	12	12	8	12	76	84	90
720 [90]	12	12	9	12	12	-	-	57	60	95
744 [47]	8	12	12	-	-	12	12	56	60	93
983 [44] ^a	8	-	-	-	-	-	-	-	-	-
1498 [27]	8	-	-	-	-	8	8	24	36	67
1527[170]	12	12	12	7	7	12	8	70	84	83
1578 [13]	8	12	12	-	-	-	-	32	36	89
1798 [147]	2	12	12	0	0	8	8	42	84	50
1859 [83]	2	-	-	12	12	-	-	26	36	72
[42] ^a	2	-	-	-	-	-	-	-	-	-
[212] ^a	12	-	-	-	-	-	-	-	-	-

^a full performance assessment was not carried as laboratories did not participate in PT 54 and 1 EQA distribution during 2014.

Salmonella spp. assessment

Twenty laboratories participated in the EURL matrix scheme (Appendix 5) and 1 or more EQA distributions in 2014 and were therefore subject to a full performance assessment. All laboratories achieved a cumulative total of >70% for the two or more distributions analysed.

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

Lab no.	PT 54	Distribution SF047		Distribution SF048		Distribution SF049		All distributions		
	Sample 1	SF0102	SF0103	SF0104	SF0105	SF0106	SF0107	Cumulative score	Max score	%
121 [19]	2	2	2	2	2	2	2	14	14	100
391 [9]	2	2	2	-	-	-	-	6	6	100
403 [86]	2	2	2	-	-	2	2	10	10	100
413 [35]	0	2	2	2	2	2	2	12	14	86
493 [22] ^a	2	-	-	-	-	-	-	-	-	-
583 [32]	0	2	2	-	-	2	2	8	10	80
597 [41]	2	2	2	2	2	-	-	10	10	100
601 [17]	2	-	-	2	2	-	-	6	6	100
649 [68]	2	2	2	2	2	2	2	14	14	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] ^a	2	-	-	-	-	-	-	-	-	-
660 [33] ^a	2	-	-	-	-	-	-	-	-	-
701 [10]	2	2	2	2	2	2	2	14	14	100
703 [3]	-	2	2	2	2	2	2	12	12	100
715 [21]	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]	0	2	2	-	-	2	2	8	10	80
983 [44] ^a	2	-	-	-	-	-	-	-	-	-
1498 [27]	2	-	-	-	-	2	2	6	6	100
1527 [170] ^a	2	-	-	-	-	-	-	-	-	-
1578 [13]	2	2	2	-	-	-	-	6	6	100
1798 [147]	2	2	2	0	0	2	2	10	14	71
1859 [83] ^a	2	-	-	2	2	-	-	6	6	100
[42] ^a	2	-	-	-	-	-	-	-	-	-
[212] ^a	-	-	-	-	-	-	-	-	-	-

^a full performance assessment was not carried as laboratories did not participate in PT 54 and 1 EQA distribution during 2014.

References

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

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

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

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

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

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

Appendix 1
Distribution SF047: Sample SF0102

Sample SF0102 contents – *Escherichia coli* (10^3), *Bacillus pumilus* (10^2), *Enterobacter cloacae* (10^4), *Pseudomonas fluorescens* (10^3)

Analysed February / March 2014 – Seventeen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 601, 715, 1498 and 1859 did not participate in this distribution.

Reference results

***E. coli* MPN** – 4.5×10^2 – 1.1×10^4 per 100g. ***Salmonella* spp.** – Not detected in 25g.

Table 4: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0102

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	2.4×10^3	4.8×10^2	2.2×10^2	1.2×10^4	2.6×10^4
Participants results	2.2×10^3	4.4×10^2	2.0×10^2	1.1×10^4	2.4×10^4

Participants results - SF0102 (Figure 1)

Table 5: Results reported by participants and scores allocated - SF0102

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	1100	3500	12	Not Detected	2
391	3500	5400	12	Not Detected	2
403	4900	1700	12	Not Detected	2
413	3500	3500	12	Not Detected	2
493	DNR	DNR	-	DNR	-
583	2200	NE	7	Not Detected	2
597	490	1100	12	Not Detected	2
601	DNP	DNP	-	DNP	-
649	1300	3500	12	Not Detected	2
651	2200	1100	12	Not Detected	2
653	1700	3500	12	Not Detected	2
658	DNR	DNR	-	DNR	-
660	DNR	DNR	-	DNR	-
701	5400	2200	12	Not Detected	2
703	2400	3500	12	Not Detected	2
715	DNP	DNP	-	DNP	-
718	1300	1300	12	Not Detected	2
720	9200	5400	12	Not Detected	2
744	790	1400	12	Not Detected	2
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	1620	1400	12	NE	-
1578	3300	1300	12	Not Detected	2
1798	2200	1300	12	Not Detected	2
1859	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

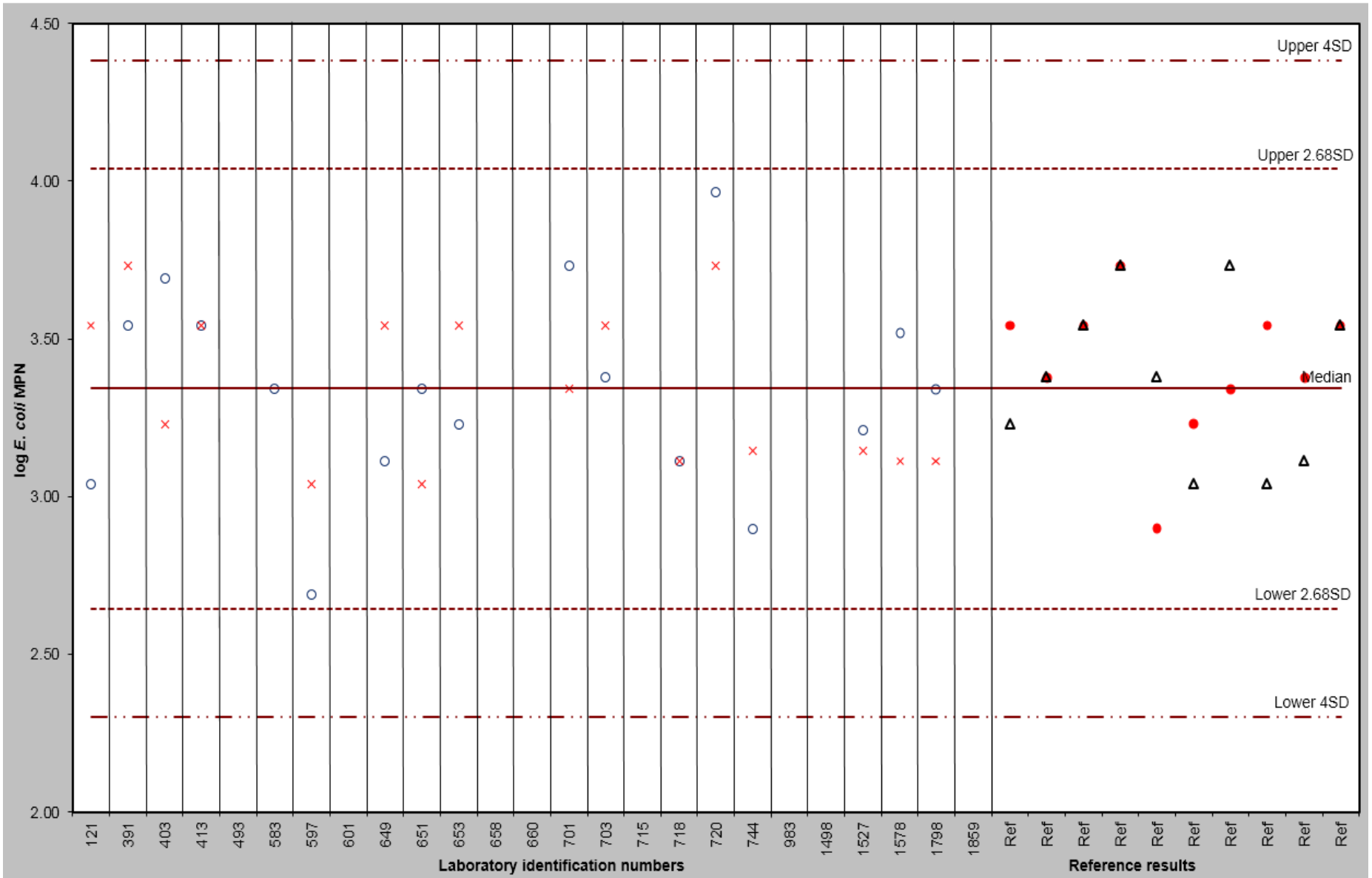
DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

***E. coli* MPN** – Sixteen laboratories reported replicate results within ± 2.68 SD of the participants' medium and received a maximum score of 12. Laboratory 583 reported one replicate result within ± 2.68 SD of the participants' medium and received a maximum score of 7.

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

Figure 1. Distribution SF047: Sample SF0102



Distribution SF047: Sample SF0103

Sample SF0103 contents – *Escherichia coli* (10^2), *Salmonella Typhimurium* (10), *Enterococcus faecalis* (10^3), *Pseudomonas putida* (10^3)

Analysed February / March 2014 – Seventeen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 601, 715, 1498 and 1859 did not participate in this distribution.

Reference results

***E. coli* MPN** – 2.7×10^1 - 6.4×10^2 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 6: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0103

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	1.7×10^2	3.4×10^1	1.6×10^1	8.5×10^2	1.9×10^3
Participants results	1.3×10^2	2.6×10^1	1.2×10^1	6.5×10^2	1.4×10^3

Participants results - SF0103 (Figure 2)

Table 7: Results reported by participants and scores allocated - SF0103

Lab Number	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	80	80	12	Detected	2
391	80	130	12	Detected	2
403	130	330	12	Detected	2
413	140	140	12	Detected	2
493	DNR	DNR	-	DNR	-
583	80	130	12	Detected	2
597	130	110	12	Detected	2
601	DNP	DNP	-	DNP	-
649	230	110	12	Detected	2
651	170	50	12	Detected	2
653	170	130	12	Detected	2
658	DNR	DNR	-	DNR	-
660	DNR	DNR	-	DNR	-
701	130	220	12	Detected	2
703	50	220	12	Detected	2
715	DNP	DNP	-	DNP	-
718	330	230	12	Detected	2
720	130	20	9	Detected	2
744	330	80	12	Detected	2
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	40	100	12	NE	-
1578	110	110	12	Detected	2
1798	130	110	12	Detected	2
1859	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

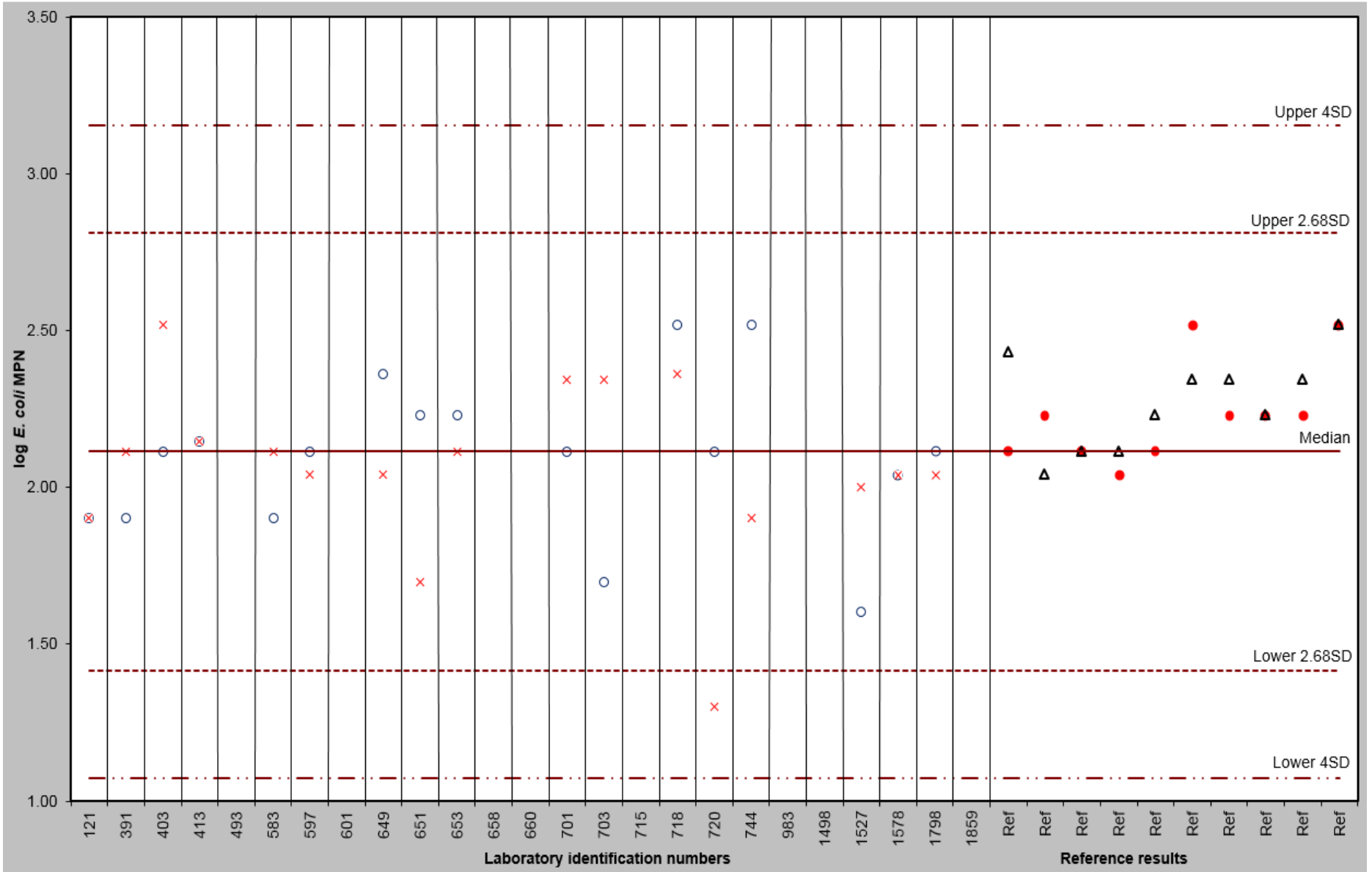
DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

***E. coli* MPN** – Sixteen laboratories reported replicate results within ± 2.68 SD of the participants' median and received a maximum score of 12. Laboratories 720 reported one replicate result between ± 2.68 and ± 4 SD of the participants' median and received a score of 9.

***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 2. Distribution SF047: Sample SF0103



Appendix 2

Distribution SF048: Sample SF0104

Sample SF0104 contents – *Escherichia coli* (10^2), *Salmonella Agona* (170), *Pantoea agglomerans* (10^3), *Pseudomonas fluorescens* (10^3)

Analysed June / July 2014 – Fifteen laboratories received material for examination with 14 laboratories returning results to be included in the assessment. Laboratory 744 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 403, 583, 1498 and 1578 did not take part in this distribution.

Reference results

***E. coli* MPN** – 1.0×10^2 – 2.4×10^3 per 100g. ***Salmonella* spp.** - Detected in 25g.

Table 8: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0104

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	7.4×10^2	1.5×10^2	6.8×10^1	3.7×10^3	8.2×10^3
Participants results	4.1×10^2	8.2×10^1	3.7×10^1	2.0×10^3	4.5×10^3

Participants results - SF0104 (Figure 3)

Table 9: Results reported by participants and scores allocated - SF0104

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	490	NE	7	Detected	2
391	DNP	DNP	-	DNP	-
403	DNP	DNP	-	DNP	-
413	700	700	12	Detected	2
493	DNR	DNR	-	DNR	-
583	DNP	DNP	-	DNP	-
597	170	330	12	Detected	2
601	330	210	12	Detected	2
649	230	490	12	Detected	2
651	1300	460	12	Detected	2
653	330	230	12	Detected	2
658	DNR	DNR	-	DNR	-
660	DNR	DNR	-	DNR	-
701	330	490	12	Detected	2
703	330	220	12	Detected	2
715	750	500	12	Detected	2
718	790	790	12	Detected	2
720	330	700	12	Detected	2
744	NE	NE	-	NE	-
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	360	NE	7	NE	-
1578	DNP	DNP	-	DNP	-
1798	NR	NR	0	NR	0
1859	490	220	12	Detected	2

DNR – NRL did not register for the scheme

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

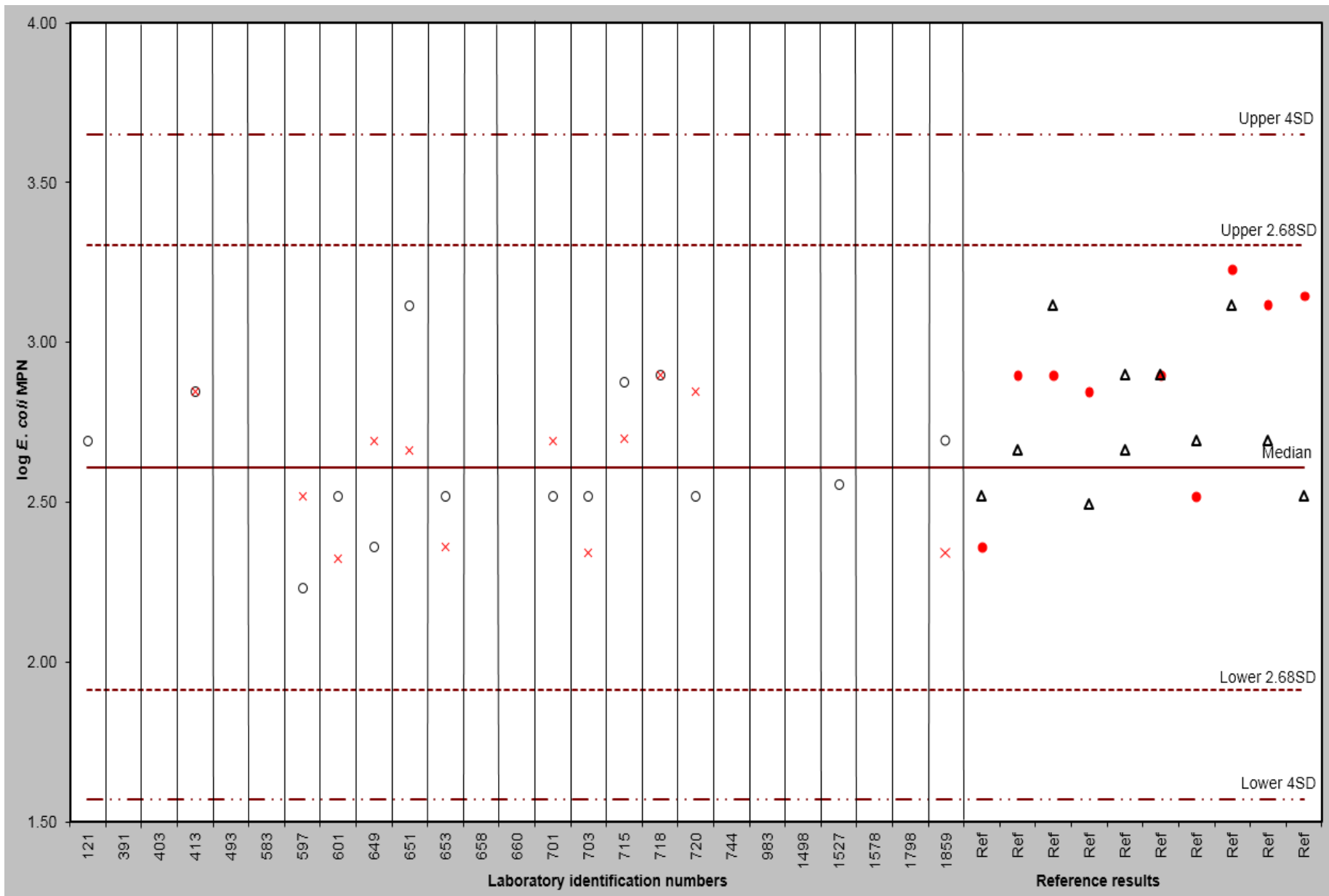
NR – No results returned

NE – Not examined

***E. coli* MPN** – Twelve laboratories reported replicate results within ± 2.68 SD of the participants' medium and received a maximum score of 12. Laboratories 121 and 1527 reported one replicate result within ± 2.68 SD of the participants' medium and received a score of 7.

***Salmonella* spp.** – All 13 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Figure 3. Distribution SF048: Sample SF0104



Distribution SF048: Sample SF0105

Sample SF0105 contents – *Escherichia coli* (50-230), *Pantoea agglomerans* (10³), *Enterococcus faecalis* (10²)

Analysed June / July 2014 – Fifteen laboratories received material for examination with 14 laboratories returning results to be included in the assessment. Laboratory 744 and 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 403, 583, 1498 and 1578 did not take part in this distribution.

Reference results

***E. coli* MPN** – 2.7 x 10¹ – 6.4 x 10² per 100g. ***Salmonella* spp.** – Not detected in 25g.

Table 10: Participants and reference results median, median ±2.68 and ±4 SD - SF0105

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	9.9 x 10 ¹	2.0 x 10 ¹	9.1 x 10 ⁰	5.0 x 10 ²	1.1 x 10 ³
Participants results	1.1 x 10 ²	2.2 x 10 ¹	1.0 x 10 ¹	5.5 x 10 ²	1.2 x 10 ³

Participants results - SF0105 (Figure 4)

Table 11: Results reported by participants and scores allocated - SF0105

Lab Number	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	230	NE	7	Not Detected	2
391	DNP	DNP	-	DNP	-
403	DNP	DNP	-	DNP	-
413	70	70	12	Not Detected	2
493	DNR	DNR	-	DNR	-
583	DNP	DNP	-	DNP	-
597	40	230	12	Not Detected	2
601	500	78	12	Not Detected	2
649	110	170	12	Not Detected	2
651	490	1300	4	Not Detected	2
653	170	230	12	Not Detected	2
658	DNR	DNR	-	DNR	-
660	DNR	DNR	-	DNR	-
701	40	110	12	Not Detected	2
703	80	80	12	Not Detected	2
715	310	200	12	Not Detected	2
718	110	230	12	Not Detected	2
720	93	78	12	Not Detected	2
744	NE	NE	-	NE	-
983	DNR	DNR	-	DNR	-
1498	DNP	DNP	-	DNP	-
1527	100	NE	7	NE	-
1578	DNP	DNP	-	DNP	-
1798	NR	NR	0	NR	0
1859	68	110	12	Not Detected	2

DNR – NRL did not register for the scheme

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

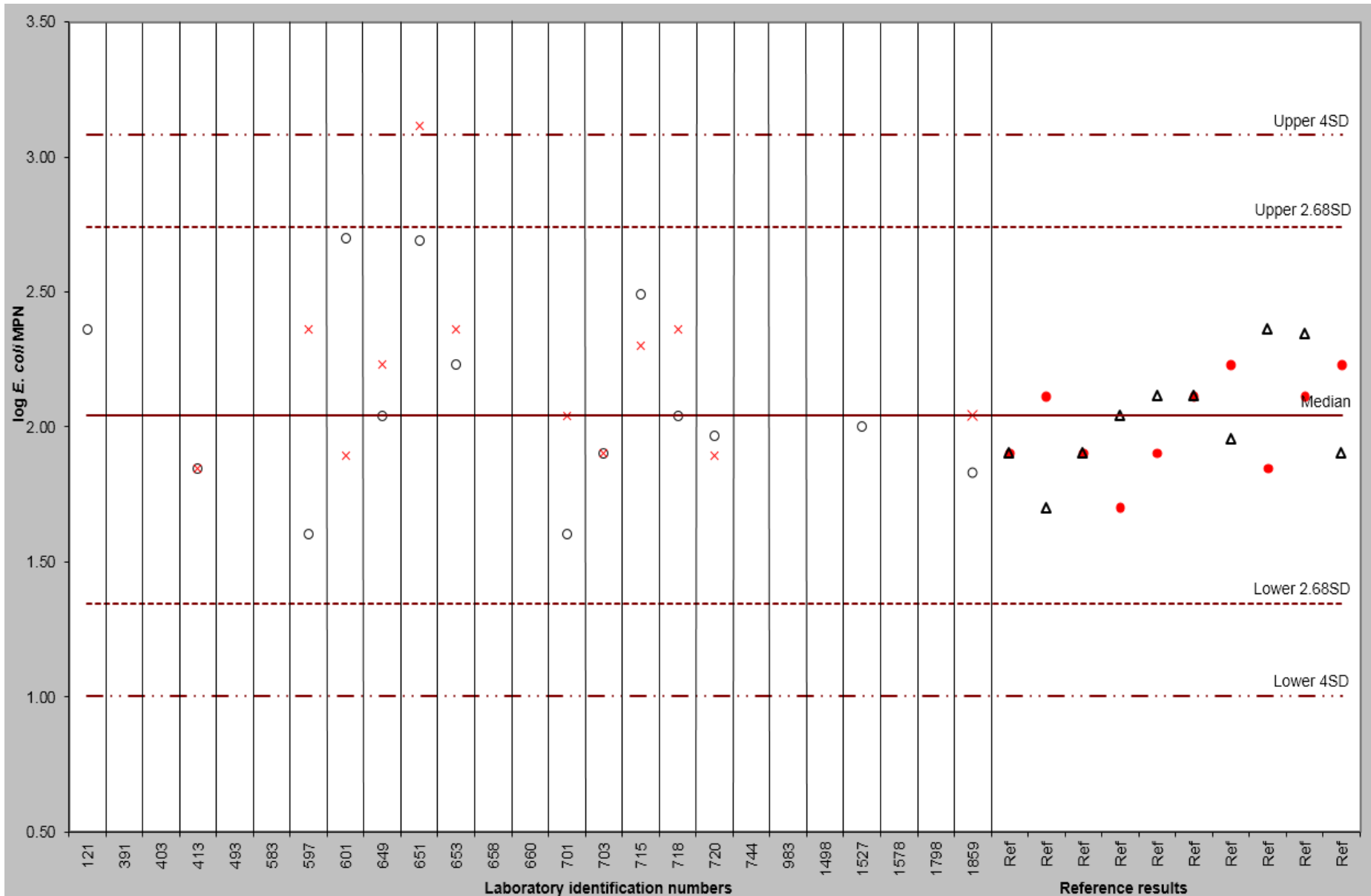
NR – No results returned

NE – Not examined

***E. coli* MPN** – Eleven laboratories reported replicate results within ±2.68 SD of the participants’ medium and received a maximum score of 12. Laboratories 121 and 1527 reported one replicate result within ±2.68 SD of the participants’ medium and received a score of 7. Laboratories 651 reported one replicate result outside ±4 SD of the participants’ medium and received a score of 4.

***Salmonella* spp.** – All 13 laboratories that reported a result for *Salmonella* spp. correctly reported that *Salmonella* spp. was not detected and received a maximum score of 2.

Figure 4. Distribution SF048: Sample SF0105



Appendix 3

Distribution SF049: Sample SF0106

Sample SF0106 contents – *Escherichia coli* (10^3 - 10^4), *Salmonella* Liverpool (10^2), *Klebsiella oxytoca* (10^4), *Micrococcus* sp. (10^3)

Analysed November / December 2014 – Fourteen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 597, 601, 715, 720, 1578 and 1859 did not take part in this distribution.

Reference results

***E. coli* MPN** – 2.1×10^3 – 5.0×10^4 per 100g. ***Salmonella* spp.** – Detected in 25g.

Table 12: Participants and reference results median, median ± 2.68 and ± 4 SD - SF0106

	Median MPN/100g	Median -2.68SD MPN/100g	Median -4SD MPN/100g	Median +2.68SD MPN/100g	Median +4SD MPN/100g
Reference results	8.5×10^3	1.7×10^3	7.8×10^2	4.2×10^4	9.3×10^4
Participants results	9.2×10^4	1.8×10^3	8.4×10^2	4.6×10^4	1.0×10^5

Participants results - SF0106 (Figure 5)

Table 10: Results reported by participants and scores allocated - SF0106

Lab Number	<i>E. coli</i> (per 100g)			<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2	Score	<i>Salmonella</i> spp.	Score
121	5400	3500	8	Detected	2
391	DNP	DNP	-	DNP	-
403	54000	16000	5	Detected	2
413	16000	24000	8	Detected	2
493	DNR	DNR	-	DNR	-
583	7900	7000	12	Detected	2
597	DNP	DNP	-	DNP	-
601	DNP	DNP	-	DNP	-
649	13000	24000	10	Detected	2
651	7000	9200	8	Detected	2
653	16000	16000	8	Detected	2
658	DNR	DNR	-	DNR	-
660	DNR	DNR	-	DNR	-
701	7900	13000	12	Detected	2
703	13000	3300	12	Detected	2
715	DNP	DNP	-	DNP	-
718	4900	3300	8	Detected	2
720	DNP	DNP	-	DNP	-
744	13000	17000	12	Detected	2
983	DNR	DNR	-	DNR	-
1498	11000	7000	8	Detected	2
1527	2740	2920	12	NE	-
1578	DNP	DNP	-	DNP	-
1798	3500	9200	8	Detected	2
1859	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

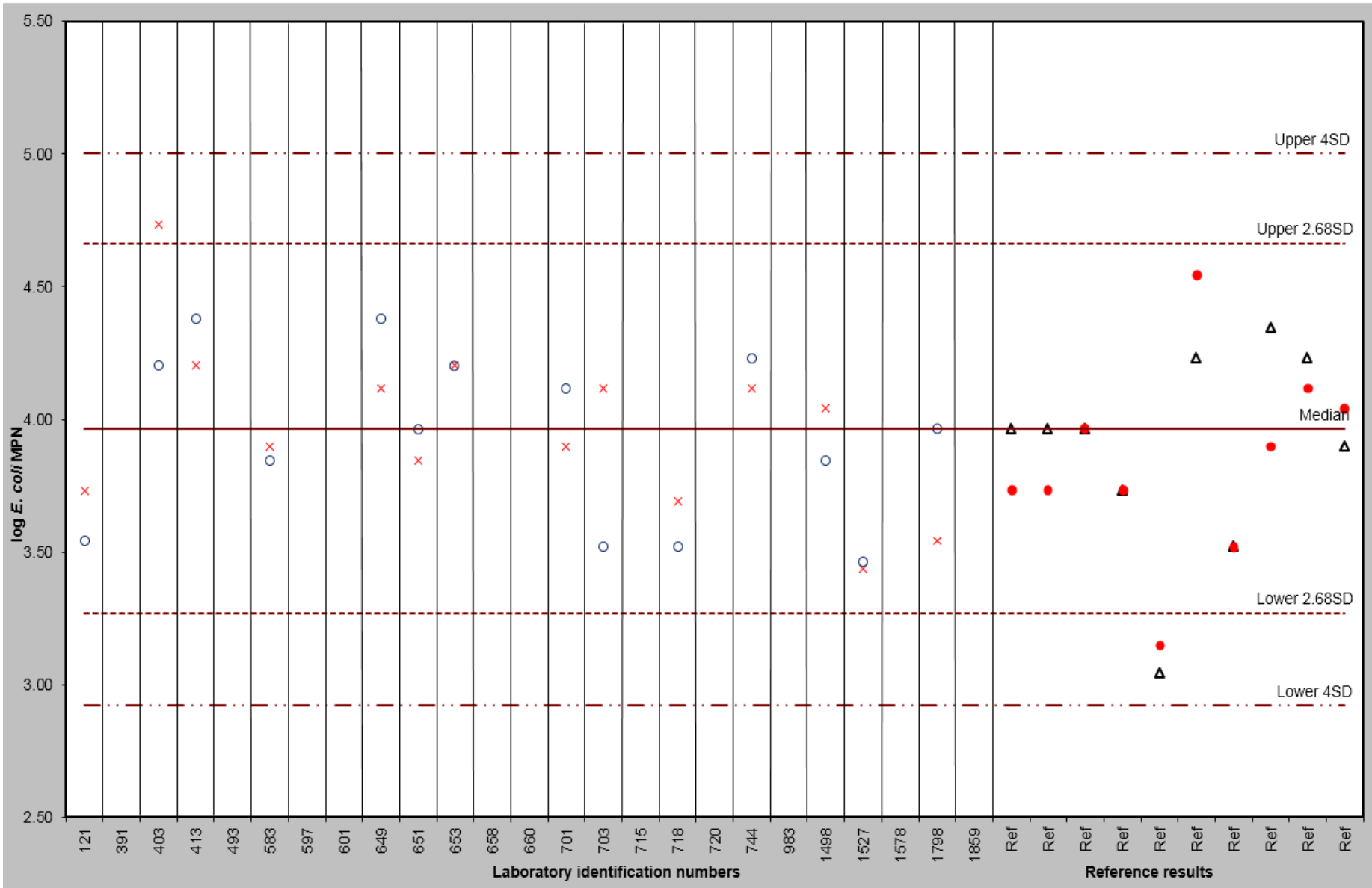
DNP – NRL registered for EQA scheme but did not participate in this distribution

NE – Not examined

***E. coli* MPN** – Thirteen laboratories reported replicate results within ± 2.68 SD of the participants' medium with 5 receiving a maximum score of 12. Laboratory 403 reported one replicate result between ± 2.68 and ± 4 SD of the participants' median. Laboratory 121, 403, 413, 649, 651, 653, 718, 1498 and 1798 had scores deducted as tube combinations reported were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

***Salmonella* spp.** – All 13 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Figure 5. Distribution SF049: Sample SF0106



Distribution SF049: Sample SF0107

Sample SF0107 contents – *Salmonella* Wentworth (40), *Aeromonas hydrophila* (<10), *Hafnia alvei* (10³)

Analysed November / December 2014 – Fourteen laboratories received material for examination with all laboratories returning results to be included in the assessment. Laboratory 1527 did not examine the sample for *Salmonella* spp.. Laboratories 391, 597, 601, 715, 720, 1578 and 1859 did not take part in this distribution.

Reference results

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

Participants results - SF0107
Table 13: Results reported by participants and scores allocated - SF0107

Lab Number	<i>E. coli</i> (per 100g)		Score	<i>Salmonella</i> spp. (per 25g)	
	Replicate 1	Replicate 2		<i>Salmonella</i> spp.	Score
121	<20	<20	8	Detected	2
391	DNP	DNP	-	DNP	-
403	<20	<20	8	Detected	2
413	0	0	8	Detected	2
493	DNR	DNR	-	DNR	-
583	<18	<18	12	Detected	2
597	DNP	DNP	-	DNP	-
601	DNP	DNP	-	DNP	-
649	<18	<18	12	Detected	2
651	<20	<20	8	Detected	2
653	<18	<18	12	Detected	2
658	DNR	DNR	-	DNR	-
660	DNR	DNR	-	DNR	-
701	<18	<18	12	Detected	2
703	<18	<18	12	Detected	2
715	DNP	DNP	-	DNP	-
718	<18	<18	12	Detected	2
720	DNP	DNP	-	DNP	-
744	<18	<18	12	Detected	2
983	DNR	DNR	-	DNR	-
1498	0	0	8	Detected	2
1527	<20	<20	8	NE	-
1578	DNP	DNP	-	DNP	-
1798	<20	<20	8	Detected	2
1859	DNP	DNP	-	DNP	-

DNR – NRL did not register for the scheme

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

NE – Not examined

***E. coli* MPN** – Fourteen laboratories reported the absence of *E. coli* in this sample with 7 receiving a maximum score of 12. Laboratory 121, 403, 413, 651, 1498, 1527 and 1798 had scores deducted as tube combinations reported were inconsistent with the guidance given in ISO 7218:2007/Amd 1:2013.

***Salmonella* spp.** – All 13 laboratories that reported a result for *Salmonella* spp. correctly reported the detection of *Salmonella* spp. and received a maximum score of 2.

Appendix 4:
EURL PT 54 - NRL results and allocated scores

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

Lab ID	Sample 1 – Manila clams				
	<i>E. coli</i> MPN/100g			<i>Salmonella</i> spp. in 25g	
	Replicate 1	Replicate 2	Score	Result	Score
3 [703]	13000	4600	9	NE	-
7 [718]	2800	4600	8	Not detected	2
9 [391]	790	1100	8	Not detected	2
10 [701]	780	2200	12	Not detected	2
13 [1578]	790	1300	8	Not detected	2
17 [601]	1300	1700	8	Not detected	2
19 [121]	2200	90	3	Not detected	2
21 [715]	790	1100	8	Not detected	2
22 [493]	1300	1300	8	Not detected	2
23 [658]	330	230	5	Not detected	2
27 [1498]	1300	2400	8	Not detected	2
32 [583]	780	780	12	Present	0
33 [660]	490	490	8	Not detected	2
35 [413]	5400	9200	5	Present	0
39 [653]	3500	3500	8	Not detected	2
41 [597]	7000	1700	9	Not detected	2
42	16000	16000	2	Not detected	2
43 [651]	7000	5400	5	Not detected	2
44 [983]	490	490	8	Not detected	2
47 [744]	490	330	8	Present	0
68 [649]	4600	1700	12	Not detected	2
83 [1859]	16000	9200	2	Not detected	2
86 [403]	1300	2400	8	Not detected	2
90 [720]	2300	4900	12	Not detected	2
147 [1798]	230	230	2	Not detected	2
170 [1527]	1300	1340	12	Not detected	2
212	1400	2800	12	NE	-

Appendix 5:
Scoring for the PHE/EQA and EURL matrix scheme
***E. coli* MPN scores allocated to participants returning 2 replicate results**

Result	Returning of results	Score allocated		Total score
		Replicate 1	Replicate 2	
Both replicate MPN results are within the expected range	2	5	5	12
One replicate MPN result is outside the expected range and falls between the median $\pm 3SD$ and median $\pm 5SD$ values	2	5	2	9
Both replicates MPN results are outside the expected range and fall between the median $\pm 3SD$ and median $\pm 5SD$ values	2	2	2	6
One replicate MPN result is outside the median $\pm 5SD$ value	2	5	0	7
Both replicates MPN results are outside the expected range. The first falls between the median $\pm 3SD$ and median $\pm 5SD$ values and the second falls outside the median $\pm 5SD$ values	2	2	0	4
Both replicates MPN results is outside the median $\pm 5SD$ value	2	0	0	2

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

Result	Returning of results	Score allocated	Total score
Single replicate MPN result is within the expected range	2	5	7
Single replicate MPN result is outside the expected range and falls between the median $\pm 3SD$ and median $\pm 5SD$ values	2	2	4
Single replicate MPN result is outside the median $\pm 5SD$ value	2	0	2

***E. coli* score deductions**

Result	Score deducted	
	Replicate 1	Replicate 2
Tube combination inconsistent with MPN reported and / or tube combination selected not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the EURL.	2	2
High censored result (e.g. MPN = >18000 per 100g)	2	2
Sample not examined or results returned late - no explanation received	12	

***Salmonella* spp. scoring**

Result	Score allocated
Fully correct results	2
Misleading result, e.g. failure to isolate <i>Salmonella</i>	0

Appendix 6:**Troubleshooting advice**

1. Methods – Ensure that the method used is appropriate for the examination of the sample.
 - a. Ensure that any dilutions have been calculated correctly.
 - b. Ensure that the dilutions analysed are as specified on the report form.
 - c. Ensure that MPN tables (if used) are interpreted correctly.

Interpretation of MPN tables

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

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

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

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

Further advice can be obtained from the EURL on request.

About us

Cefas is a multi-disciplinary scientific research and consultancy centre providing a comprehensive range of services in fisheries management, environmental monitoring and assessment, and aquaculture to a large number of clients worldwide.

We have more than 500 staff based in 2 laboratories, our own ocean-going research vessel, and over 100 years of fisheries experience.

We have a long and successful track record in delivering high-quality services to clients in a confidential and impartial manner.
(www.cefas.defra.gov.uk)

Cefas Technology Limited (CTL) is a wholly owned subsidiary of Cefas specialising in the application of Cefas technology to specific customer needs in a cost-effective and focussed manner.

CTL systems and services are developed by teams that are experienced in fisheries, environmental management and aquaculture, and in working closely with clients to ensure that their needs are fully met.
(www.cefastechnology.co.uk)

Head office

Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK

Tel +44 (0) 1502 56 2244
Fax +44 (0) 1502 51 3865
Web www.cefas.defra.gov.uk

Customer focus

With our unique facilities and our breadth of expertise in environmental and fisheries management, we can rapidly put together a multi-disciplinary team of experienced specialists, fully supported by our comprehensive in-house resources.

Our existing customers are drawn from a broad spectrum with wide ranging interests. Clients include:

- international and UK government departments
- the European Commission
- the World Bank
- Food and Agriculture Organisation of the United Nations (FAO)
- oil, water, chemical, pharmaceutical, agro-chemical, aggregate and marine industries
- non-governmental and environmental organisations
- regulators and enforcement agencies
- local authorities and other public bodies

We also work successfully in partnership with other organisations, operate in international consortia and have several joint ventures commercialising our intellectual property

Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601

