European Union Reference Laboratory (EURL)
Proficiency Testing Scheme

Enumeration of *Escherichia coli* and the detection of *Salmonella* spp. in Bivalve Molluscan Shellfish

EURL PT reference number: PT49

FINAL report version 4

18.10.2014

14 pages

Contract Reference: Cefas ref (C5836)

Document approved by:	**C5836 Project Manager – Rachel Hartnell**	**Review date:**	**Not applicable**
Document checked by:	**Rachel Hartnell**	**Classification:**	**Official**
Document prepared by:	**Louise Stockley**	**Location**	**PT CRL$**

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This scheme is intended to provide proficiency testing samples for laboratories undertaking examination of live bivalve molluscs from production areas in accordance with Regulation (EC) No. 854/2004 and from throughout the production chain in accordance with Regulation (EC) No. 2073/2005.

The scheme is organised by the European Union Reference laboratory (EURL) for monitoring bacteriological and viral contamination of bivalve molluscs. The EURL is designated by the European Union in accordance with Regulation (EC) No. 882/2004. The scheme is intended to compliment the EURL/PHE Shellfish Scheme (http://www.hpa.org.uk/ProductsServices/MicrobiologyPathology/ExternalQualityAssessmentProficiencyTesting/EQAPTForFoodWaterAndEnvironmentalMicrobiology/ShellfishScheme/ through examination of aspects of the methods not covered under the Shellfish Scheme (initial sample preparation and preparation of initial dilutions) and to provide additional data for laboratories for ISO 17025 accreditation purposes.

The EU stipulated reference method for enumeration of *E. coli* in live bivalve molluscs in 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 (Anon 2005). 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).

A scoring system is used to help assess participants' performance. Details of this system are included as Appendix I of this report. The purpose of scoring is to help the EURL, NRLs and other participating laboratories identify incorrect or outlying results. Further information on the use of scoring in proficiency testing and on recommended procedures for following up poor performance can be accessed via the EURL website (www.eurlcefas.org) or obtained by contacting the EURL. The European Union has produced a protocol for management of underperformance in comparative testing and/or lack of collaboration of NRLs with EURLs activities.

If you are experiencing problems with any aspects of these distributions please contact the EURL (contact details below), or alternately refer to the troubleshooting guide included as Appendix II of this report.

Further advice on microbiological testing of bivalve mollluscan shellfish can be obtained via the EURL website (www.eurlcefas.org)

Sample preparation

Sample 1

One batch consisting of approximately 1,400 (120kg) Pacific oysters (*Crassostrea gigas*) was collected from a UK commercial harvesting area. Approximately 120 oysters were evenly spread on individual trays and immersed in a small scale depuration unit that had been filled with 500 litres of filtered (50 micron filter) seawater and maintained at a temperature of 12°C (approximate temperature of seawater in harvesting area). Six trays were prepared for each tank. Seawater was re-circulated at 28 litres per min (with UV) for 96 hours to allow the shellfish to acclimatize. Screened raw sewage collected from a local sewage treatment works was analysed to determine the *E. coli* levels using membrane filtration (Anon 2000). All oyster trays were removed from the tanks. Two hundred ml of screened raw sewage (≈7 x 10^6 cfu/100ml) and 150 ml of an overnight culture of *Salmonella* Nottingham (NCTC 7832) (≈1.2 x 10^8 cfu/100ml) was added to each tank and thoroughly mixed. The oysters were re-immersed in the tank and the temperature of the seawater was increased to 16°C with constant re-circulation (without UV). After 3 hours of exposure the oysters were removed. Approximately 22 individual oysters were selected at random from both tanks and placed in sample bags.

Sample 2

Approximately 25kg of common mussels (*Mytilus edulis*) were collected from a UK commercial harvesting area. Sample 2 comprised of 35 randomly selected common mussels.

Sample distribution and examination

Samples were packed in accordance with Cefas protocol for packaging shellfish for transportation and distributed at 11:45am on 4th November 2013 to 50 participating laboratories. Participants were requested to analyse the material in duplicate immediately on receipt using their routine laboratory procedures for the enumeration of *E. coli* and the detection of *Salmonella* spp..

Sample temperature

Temperature recorders (Thermotrack, Progress Plus) were included in each consignment. Participants were requested to record the internal sample temperature on arrival and to return the temperature recorder. Temperatures recorded by participants are shown in Appendix I.

Results

Reference results - *E. coli*

Ten randomly selected sub-samples were analysed in duplicate on 2 consecutive days (05.11.13 and 06.11.13) for *E. coli* using EURL SOP No. 1175 (Table 1). Sample homogeneity was assessed following the procedure described in ISO 22117.

Table 1: *E. coli* MPN/100g reference results

Sample type	Analyses dates	Range	Median	GM	Median ±3*SD_T
Sample 1 - Oysters	05.11.13	$3.5 \times 10^3 – 9.2 \times 10^5$	1.3×10^4	1.3×10^4	2.4×10^3 - 6.6×10^4
	06.11.13	$3.5 \times 10^3 – 9.2 \times 10^5$	1.6×10^4	1.4×10^4	3.0×10^3 - 8.4×10^4
Sample 2 - Mussels	05.11.13	$5.0 \times 10^1 – 3.5 \times 10^3$	1.8×10^2	1.8×10^2	3.4×10^1 - 9.5×10^2
	06.11.13	$8.0 \times 10^1 – 3.5 \times 10^3$	4.8×10^2	4.7×10^2	9.3×10^1 - 2.6×10^3

GM - geometric mean, SD_T - theoretical standard deviation (0.24)

Reference results – *Salmonella* spp.

Ten randomly selected samples were analysed under repeatability conditions for *Salmonella* spp. Due to a laboratory quality control failure *Salmonella* reference results were considered void. Reference results for *Salmonella* spp. are not included in this report.

Note: Regulation (EC) No. 2073/2005 requires presence/absence testing for *Salmonella* spp. in live bivalve molluscs.

Participants' results

Performance assessment was according to the procedures described in the EURL/PHE EQA shellfish scheme for a single distribution, with minor modifications (Appendix II). Participants' results and scores allocated for PT 49 are shown in Tables 2, 3, 4 and Figure 1 and 2.

Note: The median and upper and lower limits (±3 SD and ±5 SD) were calculated from participants' results. SD_T calculations were based on the inherent variability of the 5 x 3 MPN method (0.24 log_{10}). Reference values were excluded from the calculation of participants' median.

Table 2: Participants results

Sample type	*E. coli* MPN/100g			
	Range	Median	GM	Median±3*SD_T
Sample 1 - Oysters	1.1×10^0 - $>1.8 \times 10^5$	9.2×10^3	8.5×10^3	1.8×10^3 – 4.8×10^4
Sample 2 - Mussels	$<2.0 \times 10^0$ - 2.4×10^3	2.2×10^2	1.7×10^2	4.2×10^1 – 1.6×10^3

GM - geometric mean, SD_T – theoretical standard deviation (0.24)

Table 3: Summary statistics of participants results

E. coli	Sample 1 - Oysters	Sample 2 - Mussels
Participants reporting duplicate results for *E. coli* MPN	49	49
Participants reporting a single MPN result	0	0
Participants reporting MPN results within the expected range for both replicates[1]	34	43
Participants reporting MPN results outside the expected range for one replicate	4	3
Participants reporting MPN results outside the expected range for both replicates	10	3
Participants reporting MPN results as censored results for one replicate	2	1
Participants reporting MPN results as censored results for both replicates	2	1
Participants reporting MPN results inconsistent with ISO 7218 (Anon 2007)[2]	18	3

Salmonella spp. summary statistics		
Participants reporting results for *Salmonella* spp.	48	44
Participants reporting the presence of *Salmonella* spp.	47	26
Participants reporting the absence of *Salmonella* spp.	0	17

Participants not returning results	1	1
Participants not receiving material due to problems at customs	1	1

[1] expected range = participants' median ± theoretical 3SD,
[2] points deducted from participants returning results inconsistent with ISO 7218 who reported using the reference method.

Table 4: Participants' results and allocated scores

Lab ID	Sample 1 - Oysters					Sample 2 - Mussels			
	E. coli MPN/100g			*Salmonella* spp. in 25g		*E. coli* MPN/100g			*Salmonella* spp. in 25g
	Replicate 1	Replicate 2	Score	Result	Score	Replicate 1	Replicate 2	Score	Result
3 * [b]	24000	11000	10	Present	2	80	110	12	Not detected
7 *	16000	16000	12	Present	2	1100	460	12	Present
8	5400	9200	12	Present	2	700	790	12	Present
9 *	2400	2400	12	Present	2	230	330	12	Present
10 *	5400	3500	12	Present	2	220	490	12	Not detected
13 *	3500	2400	12	Present	2	230	230	12	Present
17 * [b]	2800	4600	10	Present	2	80	170	12	Not detected
19 * [b]	9200	17000	10	Present	2	790	490	12	Present
20	35000	54000	9	Present	2	170	700	12	Present
21 * [b c]	4300	92000	7	Present	2	45	45	12	Not detected
22 *	22000	54000	9	Present	2	220	270	12	Present
23 *	16000	35000	12	Present	2	80	20	9	Present
27 *	11000	17000	12	Present	2	78	45	12	Not detected
32 *	160000	92000	4	Present	2	330	330	12	Present
33 * [b]	91000	91000	2	Present	2	70	220	8	Present
35 *	92000	160000	4	Present	2	230	220	12	Not detected
39 *	330	790	4	Present	2	230	170	12	Not detected
41 *	16000	16000	12	Present	2	68	45	12	Not detected
42 * [b]	>180000	>180000	2	Present	2	170	50	12	Not detected
43 *	160000	92000	4	Present	2	230	230	12	Present
44 * [a b]	3300	4900	8	Present	2	50	<20	7	Present
47 *	330	490	2	Present	2	130	790	12	Not detected
48	9200	5400	12	Present	2	330	220	12	Not detected
50	2400	2400	12	Present	2	110	110	12	Present
53	IP	IP	2	IP	-	IP	IP	2	IP
54 [b]	11000	7000	8	Present	2	170	130	12	Present
68 *	9200	5400	12	Present	2	230	130	12	Present

Lab ID	Sample 1 – Oysters					Sample 2 – Mussels			
	E. coli MPN/100g			*Salmonella* spp. in 25g		*E. coli* MPN/100g			*Salmonella* spp. in 25g
	Replicate 1	Replicate 2	Score	Result	Score	Replicate 1	Replicate 2	Score	Result
69 [b]	35000	16000	10	Present	2	490	220	12	Not detected
82	5400	3500	12	Present	2	230	330	12	Present
83 [b]	22000	24000	10	Present	2	70	80	12	Present
86 *	>180000	160000	2	Present	2	490	330	12	Not detected
90 *	35000	24000	12	Present	2	170	330	12	Present
92	16000	16000	12	Present	2	170	130	12	Present
95 [a b]	<3	1.1	2	Present	2	2.3	2.3	4	Present
96 [b]	5400	5400	12	Present	2	78	170	12	Not detected
104	1100	1300	6	Present	2	NE	NE	2	NE
111 [b]	490	330	2	Present	2	230	230	12	Present
132	5400	3500	12	Present	2	230	230	12	Present
141	3500	3500	12	Present	2	170	170	12	Present
147 * [b]	5400	7000	10	Present	2	130	130	12	Not detected
153 [b]	5400	5400	12	Present	2	220	500	8	Not detected
162 [b]	7900	4900	8	Present	2	80	170	12	Present
170 * [a]	>18000	>18000	12	NE	-	520	620	12	NE
175 [b c]	17000	7900	8	Present	2	400	170	10	Present
187	28000	35000	12	Present	2	2400	1700	6	Present
202 [b]	13000	11000	8	Present	2	NE	NE	2	NE
206	2400	2400	12	Present	2	330	490	12	Present
212 * [a]	1300	2600	9	NE	-	<67	<67	6	NE
245 [b]	4900	24000	10	Present	2	80	20	9	Not detected
248 [b]	3300	2300	8	Present	2	NE	NE	2	NE

[a] high or low censored values were halved or doubled to allow data assessment to be made.
[b] Scores deducted as inconsistent tube combination with ISO 7218 MPN table.
[c] Participant reported a category 3 MPN tube combination.
* Designated NRL's,
IP – Laboratory experienced import problems and was unable to receive material,
NE – Not examined,
NR – Not returned.

General comments
Fifty laboratories (26 NRL and 24 other laboratories) were sent material with only laboratory 53 not receiving the material due to incorrect or absence of import permissions required at customs and Laboratory 50 not returning their results. Information provided by laboratories on the temperature and arrival time of the material showed that 59% (28) of laboratories received the material the day after dispatch (05.11.13) with 36% (10) of these laboratories analysing the material on arrival. Twenty percent (10) of laboratories received material within 48 hours of dispatch with the remaining 22% (11) laboratories receiving their material between 72 hours and 9 days after dispatch. Delay during transportation were associated with incorrect or absence of import permissions. Temperature recorders stored in each consignment showed an in transit temperature range of 0.5 – 12.5°C. All temperature data, arrival and analysis dates and times recorded by participants are shown in Appendix I.

Methodology
Forty-seven laboratories provided information on the methodology used for *E. coli* and *Salmonella* spp. analyses and are shown in Tables 6 and 7.

E. coli
Eighty-six percent (21 NRLs and 21 other laboratories) of laboratories cited ISO TS 16649-3 (Anon 2005) as their laboratory method for the enumeration of *E. coli.* One laboratory cited use of FDA/BAM method. It is noted that this is not an approved method for the official control testing of live bivalve molluscs in the EU. The 5 x 3 MPN tables in Donovan *et al* (1998) and those contained in ISO 7251 (2005) differ slightly from those contained in ISO 7218. **Laboratories are reminded that for enumeration of *E. coli* in live bivalve molluscs for official control testing using ISO TS 16649-3 should use 5 x 3 MPN tables in ISO 7218 or those provided by the EURL.**

Table 6: *E. coli* methodology

E. coli methods	Number of laboratories
ISO TS 16649-3 (Anon 2005)	42
Donovan *et al* (1998)	2
FDA/BAM Chapter 4 (2002)	1
NF V08-106	1
ISO 16649-2 (Anon 2001)	1
No information provided	3

***Salmonella* spp.**
Seventy-three percent (20 NRLs and 16 other laboratories) of laboratories cited ISO 6579 (Anon 2005) as their laboratory method for the detection of *Salmonella* spp. with a further 2 laboratories citing ISO 6579 with supplementary confirmation tests as their laboratory method. **Laboratories are reminded that for official control testing of live bivalve molluscs for *Salmonella* spp the EU reference method is ISO 6579 (Anon 2002).**

Table 7: *Salmonella* spp. methodology

Salmonella spp. methods	Number of laboratories
EN ISO 6579 (Anon 2002)	38
NMKL 71	4
Biomerieux vidas	1
FDA/BAM Chapter 5 (2003)	1
BAX system (PCR)	1
Bio-Rad V10	1
iQ-Check Salmonella II	1
No information provided	5

Sample analyses
Forty-eight laboratories returned the report form for this PT distribution. Laboratory 50 did not return their results and Laboratory 53 experienced problems at customs. Laboratory 170 and 212 did not examine the material for *Salmonella* spp..

E. coli
Sample 1 – Oysters
Thirty-three laboratories returned duplicate *E. coli* MPN/100g results falling between ±3 SD of the participants' median with 18 laboratories obtaining a score of 12. Laboratories 20, 21, 22 and 212 reported one replicate result within ±3 SD of the participants' median. Laboratories 32, 33, 35, 39, 42, 43, 47, 86, 95, 104 and 111 reported both replicate results outside ±3 SD of the participants' median.

Eighteen laboratories (laboratories 3, 17, 19, 21, 33, 42, 44, 54, 69, 83, 95, 111, 147, 162, 175, 202, 245 and 248) were deducted points for reporting MPN results inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables.

Sample 2 – Mussels
Forty laboratories returned duplicate *E. coli* MPN/100g results falling between ±3 SD of the participants' median with 36 obtained a score of 12. Laboratory 23 and 245 reported one replicate result within ±3 SD of the participants' median. Laboratories 44, 95, 187 and 212 reported both replicate results outside ±3 SD of the participants' median. Laboratories 104, 202 and 248 did not examine sample 2.

Three laboratories (laboratories 33, 153 and 175) were deducted points for reporting MPN results inconsistent with the guidance given in ISO 7218 for interpretation of 5 x 3 MPN tables.

***Salmonella* spp.**
Sample 1 – Oysters
Forty-six laboratories returned results for *Salmonella* spp. and reported the presence of *Salmonella* spp in sample 1 as expected. Laboratories 170 and 212 did not examine this sample for *Salmonella* spp.. Laboratory 50 did not return any results.

Sample 2 – Mussels
Salmonella spp. reference results were considered void for this sample due to laboratory quality control failure. Results reported by participants indicated the sample contained a low level of naturally acquired contamination close to the limit of detection (LOD) or the levels were not consistent throughout the batch of sample. For this reason participants results were not allocated a score for this distribution.

References
Anon 2005. 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. Geneva, Switzerland.

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

Fravalo P, Hascoet Y, Le Fellic M, Queguiner S, Petton J and Salvat G. (2003). 'Convenient method for rapid and quantitative assessment of *Salmonella enterica* contamination: The mini-MSRV MPN technique.

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

Donovan TJ, Gallacher S, Andrews NJ, Greenwood MH, Graham J, Russel JE, Roberts D, Lee R. (1998). 'Modification of the standard method used in the united kingdom for counting *Escherichia coli* in live bivalve molluscs'. Communicable disease and public health 1: 188-96.

Anon 2001. ISO TS 16649-2. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* Part 2: Colony-count technique at 44˚C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. Geneva, Switzerland.

Anon 2005. ISO 7251. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive *Escherichia coli* – Most probable number technique.

Anon 2002, BAM: Enumeration of *Escherichia coli* and the Coliform Bacteria. Peter Feng, Stephen D. Weagant, Michael A. Grant, William Burkhardt. Bacteriological Analytical Manual, Chapter 4.

Anon 2010 ISO 22117:2010 Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison. Geneva, Switzerland.

Figure 1: Results chart sample 1 - Oysters

Note: The median and upper and lower limits (±3 SD and ±5 SD) were calculated from participants' results. SD calculations were based on the inherent variability of the 5 x 3 MPN method (0.24 $\log_{10}$). Reference values were excluded from the calculation of participants' median.

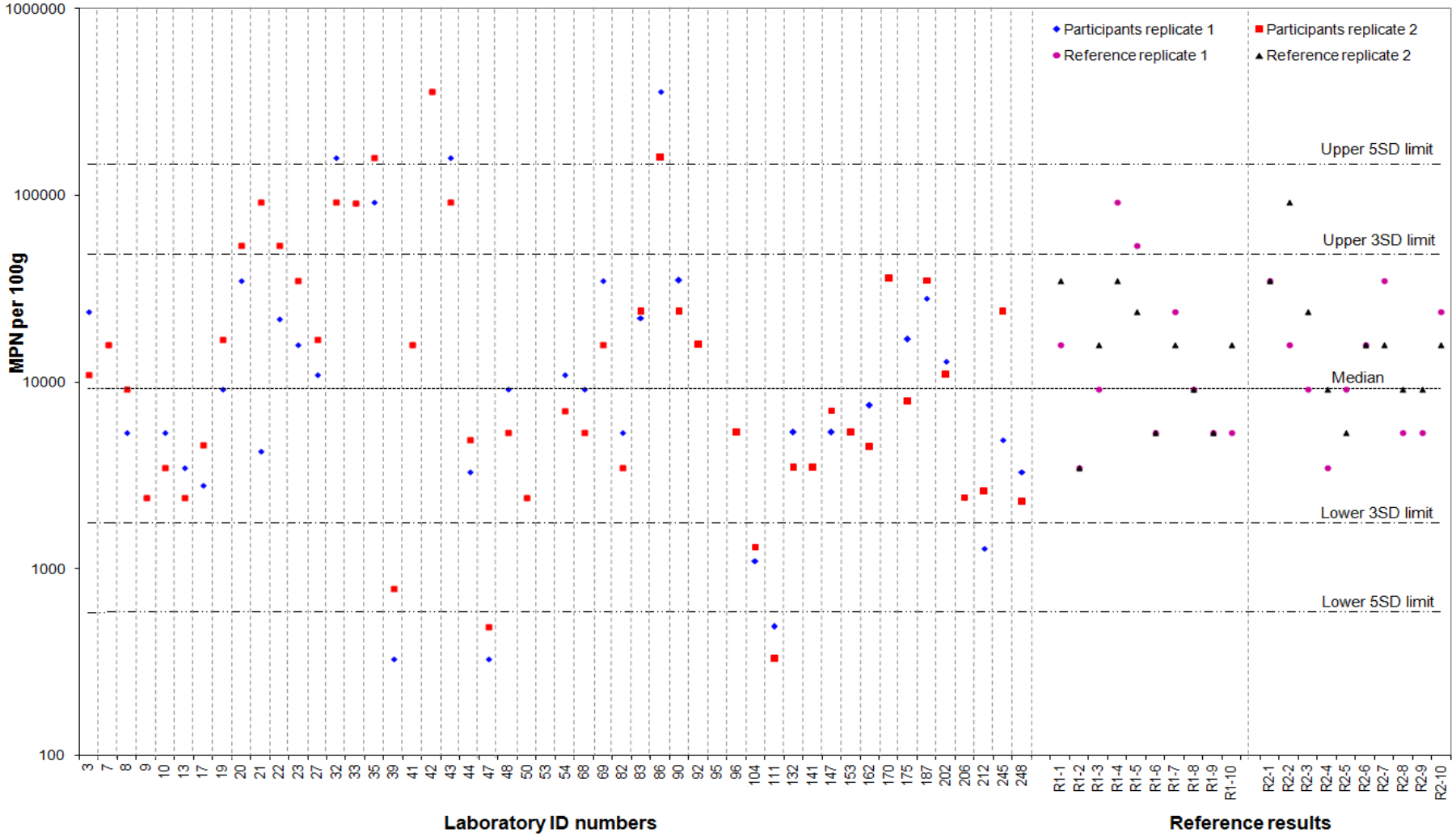
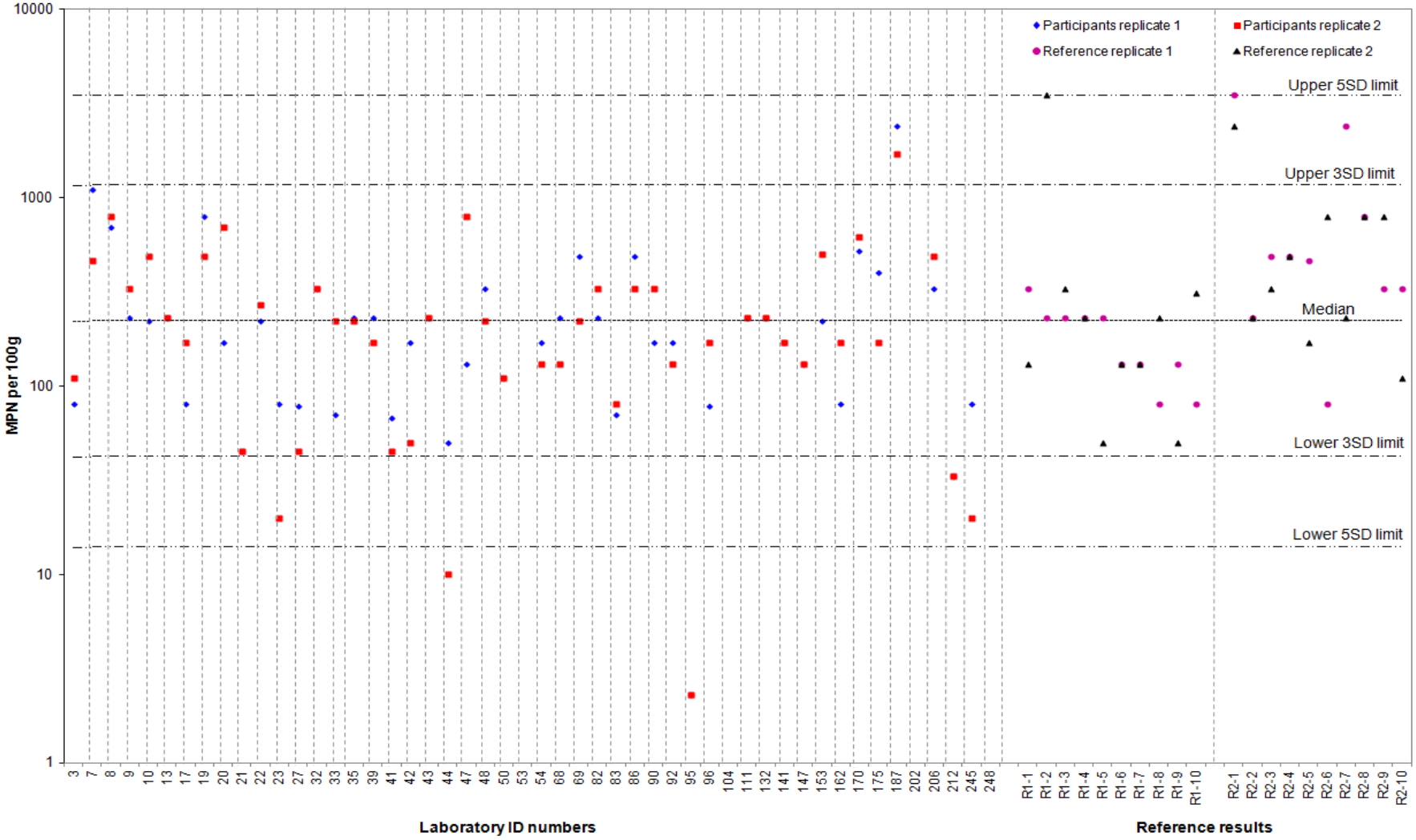


Figure 2: Results chart sample 2 - Mussels

Note: The median and upper and lower limits (±3 SD and ±5 SD) were calculated from participants' results. SD calculations were based on the inherent variability of the 5 x 3 MPN method (0.24 $\log_{10}$) Reference values were excluded from the calculation of participants' median.



Appendix I

Sample arrival and temperature

Lab ID	Date arrived	Time of arrival	Temp. recorder (°C)	Sample (°C)	Storage (°C)	Date analysed	Time of analysis
3 *	05.11.13	09:05	1.5 - 6	3.1	3 - 8	05.11.13	14:20
7 *	05.11.13	12:52	1 - 4.5	3		05.11.13	14:00
8	05.11.13	12:11	2.5 - 4	2.8	2	05.11.13	13:00
9 *	06.11.13	12:03		6	6	06.11.13	15:00
10 *	05.11.13	13:30	1 - 6.5	3		05.11.13	14:30
13 *	05.11.13	11:56	1 - 2.5	3.5	3.5	06.11.13	13:30
17 *	05.11.13	11:30	1.5 - 3.5		4	06.11.13	13:00
19 *	06.11.13	10:55	0.5 - 4.5	3.6	4	06.11.13	13:15
20	05.11.13	16:20	0.5 - 5	3	4	06.11.13	11:30
21 *	05.11.13	13:32	2 - 4.5	5.6	5	07.11.13	14:00
22 *	06.11.13	13:00	2.5 - 7	3.1	1 - 5	06.11.13	13:25
23 *	05.11.13	12:36	2 - 6	5	3 - 4	06.11.13	12:45
27 *	05.11.13	13:02	2.5 - 5	4	1 - 5	06.11.13	10:45
32 *	05.11.13	09:58	2 - 6	3.6		05.11.13	14:00
33 *	05.11.13	11:00	2 - 5	6.3	0 - 4	06.11.13	12:00
35 *	05.11.13	10:57	2 - 4.5			06.11.13	09:30
39 *	05.11.13	12:27	3 - 6.5	3.5	1 - 5	07.11.13	13:00
41 *	06.11.13	09:47	2 - 4	7.3	4	06.11.13	11:05
42 *	05.11.13	11:21	0.5 - 5	12	4	06.11.13	11:00
43 *	05.11.13	14:18	2 - 5.5	3.7	4	06.11.13	12:00
44 *	05.11.13	15:33	1 - 6.5	2.1	3	06.11.13	14:00
47 *	05.11.13	11:15	1.5 -	4	4	05.11.13	14:45
48	05.11.13	09:00	1.5 - 5.5	1.3	5	05.11.13	11:00
50	07.11.13	10:45	1.5 - 3.5	-	-	-	-
53	09.12.13	17:20					
54	06.11.13	10:56	1.5 - 7.0	4	4	07.11.13	11:00
68 *	05.11.13	11:08	1.5 - 4	2.6	4	06.11.13	10:30
69	05.11.13	12:59	1.5 - 7	4	4	05.11.13	13:40
82	05.11.13	10:55			4	06.11.13	
83	05.11.13	11:29	2 - 6.5	2.2	4.5	05.11.13	15:30
86 *	05.11.13	13:28	1.5 - 6	3.5	4.6	06.11.13	11:50
90 *	06.11.13	10:04	2 - 5	5.8	7	06.11.13	13:15
92	07.11.13	08:50	1.5 - 7	4.5	4.5	07.11.13	11:45
95	11.11.13	15:00		6	4	12.11.13	10:00
96	06.11.13	10:55	2.5 - 7	2.8		06.11.13	13:30
104	21.11.13	10:30		12.2	1 - 5	12.11.13	16:00
111	05.11.13	11:00	1 - 5.5		2	08.11.13	11:00
132	07.11.13	09:53		refrigerated		07.11.13	13:00

Sample arrival and temperature continued

Lab ID	Date arrived	Time of arrival	Temp. recorder (°C)	Sample (°C)	Storage (°C)	Date analysed	Time of analysis
141	06.11.13	12:59		5	4	11.11.13	
147 *	05.11.13	14:14	1.5 - 5.5	4	2.8	07.11.13	
153	05.11.13	13:37	2 - 4.5	<5		05.11.13	16:20
162	12.11.13	14:45	0.5 – 12.5	12		12.11.13	15:15
170 *	06.11.13	10:55	0.5 - 4.5	3.6	4	06.11.13	13:15
175	11.11.13	13:12		13	1 - 5	12.11.13	09:30
187	07.11.13	09:40	1.5 - 4.5	5.1		07.11.13	12:00
202	12.11.13	10:45	0.5 – 12.5	15.5	1 - 5	12.11.13	12:00
206	13.11.13	14:30		7	3	14.11.13	08:00
212 *	06.11.13	09:47	0.5 - 6				
245	05.11.13	12:21	2 - 5.5	2	2 - 4	07.11.13	09:30
248	13.11.13	18:00		12	1 - 5	14.11.13	21:30

* Designated NRL's

Appendix II:

***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 reported fall within the median ±3SD value	2	5	5	12
One replicate MPN result reported is outside the expected range and falls between the median ±3SD and median ±5SD value	2	5	2	9
Both replicates MPN results are outside the expected range and fall between the median ±3SD and median ±5SD value	2	2	2	6
One replicate MPN result reported is outside the median ±5SD value	2	5	0	7
Both replicates MPN results are outside the expected range. The first falls between the median ±3SD and median ±5SD value and the second falls outside the median ±5SD value.	2	2	0	4
Both replicates MPN results reported is outside the median ±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 reported is within the expected range	2	5	7
Single replicate MPN result reported only and falls between the median ±3SD and median ±5SD value	2	2	4
Single replicate MPN result reported is outside the median ±5SD value	2	0	2

***E. coli* score deductions**

Result	Score deducted	
	Replicate 1	Replicate 2
Tube combination inconsistent with MPN reported, ISO 7218 or 5 x 3 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 *Salmonella*	0

Appendix III:

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 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 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.

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.

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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

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

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