European Community Reference laboratory
for monitoring bacteriological and viral
contamination of bivalve molluscs

Report on the whole animal bivalve shellfish ring trial:

Enumeration of *E.coli* and the detection of S*almonella* spp..

CRL ring trial reference: RT 23 (*E.coli/Salmonella* 2007)

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Contents

Tables

Figures

Annex

1. Introduction

Regulation (EC) No 882/2004 designates the Centre for Environment, Fisheries and Aquaculture Science at Weymouth U.K. as the Community Reference Laboratory (CRL) for monitoring the viral and bacteriological contamination of bivalve molluscs. Under Article 32 the CRL laboratory is responsible for organising comparative testing by National Reference Laboratories.

At the 6th annual workshop of NRLs Galway 2007, several resolutions were agreed relating to proficiency testing for statutory determinants including:

Resolution 6. NRLs agreed that it was also important to periodically undertake proficiency testing using matrix samples (e.g. whole bivalve shellfish) in order to challenge aspects of the methodologies (e.g. initial sample preparation, preparation of dilutions) that would not be challenged by proficiency testing using laboratory constructed samples (as used in the CRL/HPA EQA shellfish scheme).

Resolution 7. Further to the above NRLs requested that the CRL organise a whole animal ring trial amongst NRLs. For Member States where there were a small number of Official Control laboratories the CRL would make participation available to in country testing laboratories.

In September 2007 the CRL organised a distribution of naturally contaminated common mussels (*Mytilus edulis*) for enumeration of *E. coli* and detection of *Salmonella* spp. amongst NRL's. In addition, in Member States with ≤3 official control (OC) laboratories were offered additional samples for OC laboratories. Further extension of the scheme was not logistically feasible at this point.

2. Proficiency testing samples

2.1 Sample preparation

One batch, consisting of approximately 1500 common mussels (*M. edulis*), was collected from a UK commercial producer classified under Regulation (EC) 854/2004 as Class B. Naturally contaminated material for *Salmonella* spp. was used as in previous ring trials bioaccumulation of whole shellfish at levels low enough to sufficiently challenge the test had proven to be difficult. On arrival at the Cefas laboratory sub-samples of approximately 35 mussels were selected at random and placed in clean plastic sample bags. Each sample bag was transferred to a 72-hour thermal control unit (DGP Biotherm 10 – www.dgpgroup.com) containing three ice packs. Forty-eight thermal control units were prepared in this way and stored for no more than 1h until dispatch. The remaining mussels were stored at 2-6˚C for quality control and reference testing.

2.2 Distribution of samples

The *E. coli*/*Salmonella* spp. whole animal ring trial for September 2007 was designated RT 23. Thermal control units were packaged according to IATA regulations, UN3373 as diagnostic specimens, division 6.2 under the packing instruction code 650. All participating laboratories received a single thermal control unit comprising of one sealed bag containing approximately 35 mussels, three cool packs, a temperature logger and the relevant documentation. Samples were distributed chilled by CitySprint courier services on 18th September 2007. On receipt, participants were requested to analyse the material immediately and to return the temperature logger to the CRL.

2.3 Quality control at dispatch

At the time of dispatch three sub-samples each comprising of 35 mussels were selected at random and tested to determine the level of *E. coli* and *Salmonella* spp. (Table 1). Samples were analysed using CRL SOPs based upon ISO TS 16649-3 for *E. coli* enumeration and EN

ISO 6579 for the detection of *Salmonella* spp. Standard operating procedures are available from the information centre of the CRL website (www.crlcefas.org).

Table 1. *E. coli* and *Salmonella* spp. prior to ring trial distribution

Sample description	*E. coli* MPN/100g			*Salmonella* spp. in 25g		
	A	B	C	A	B	C
M. edulis	$1.3x10_2$	7 $x10^1$	2.2 $x10^2$	Not detected	Detected	Not detected

2.4 Participation and method of analysis

Summary participation statistics are given in section 3.2. Details of the 48 laboratories participating in the ring trial are given in Table 3. Participants were asked to give their method of analysis for *E. coli* enumeration and *Salmonella* spp., this information is also included in Table 3.

2.5 Sample temperature

Automated temperature recorders (Thermotrack, Progress Plus) set to record internal sample at 5 minute intervals were included in each consignment. Participants were asked to return the recorder to the CRL and manually record the internal air and sample temperature on arrival. Sample storage temperatures, if relevant, were also requested. The temperature range (as determined from downloaded recorders), and participants' temperature measurements are given in Table 4.

2.6 Reference results

Twelve sub-samples were analysed using the CRL standard methods (ISO 6579). The reference results are summarised in Tables 2 and included, for *E. coli*, in Figure 1. In addition all reference samples were tested by a qualitative realtime PCR method for presence of *Salmonella* spp. (Annex I)

Table 2: The *E.coli* range and *Salmonella* spp. detected / not detected from the reference results

Sample description	*E. coli* MPN/100g	*Salmonella spp..*	
		Detected in 25g	Not detected in 25g
M. edulis	7.0 x 10^1 – 3.1 $x10^2$	6 out of 12	6 out of 12

Table 3: Participation in ring trial and analytical methods used

Country	NRL / OC / CC / EFTA	Participation (RT 23)	*E.coli*	*Salmonella* spp
Austria	NRL	Yes	Donovan	ISO 6579
Belgium	NRL	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579, iQ-Check™
Bulgaria	NRL	Yes	ISO 16649-3	ISO 6579
Czech Rep.	NRL	No	-	-
Croatia	CC	Yes	ISO 16649-3	ISO 6579
Croatia	CC	Yes	ISO 16649-3	ISO 6579
Denmark	OC	Yes	ISO 16649-3	NMKL 71
	OC	Yes	Donovan (modified)	NMKL 71
	OC	Yes	Cefas SOP	NMKL 71
	OC	Yes	Cefas SOP	NMKL 71

Table 3 cont.: Participation in ring trial and analytical methods used

Country	NRL / OC / CC / EFTA	Participation (RT 23)	*E.coli*	*Salmonella* spp
Estonia	NRL	Yes	ISO 16649-3	ISO 6579
Finland	NRL	Yes	ISO 16649-3	NCFA 71
France	NRL	Yes	ISO 16649-3	ISO 6579
Germany	NRL	Yes	ISO 16643-3	ISO 6579
	OC	Yes	ISO 16649-3	ASU $ 64LFGB
	OC	Yes	Cefas SOP	ISO 6579
	OC	Yes	Report form not returned	Report form not returned
Greece	NRL	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	Donovan	ISO 6579
Hungary	NRL	Yes	ISO 16649-3	ISO 6579
Iceland	EFTA	Yes	ISO 16649-3	NMKL 71
Ireland	NRL	Yes	ISO 16649-3	ISO 6579
Italy	NRL	Yes	ISO 16649-3	Decreto Ministero Sanita
Latvia	NRL	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
Netherlands	NRL	Yes	ISO 16649-3	ISO 6579
	OC	Yes	NPR- ISO 16649-3	ISO 6579
Norway	EFTA	Yes	Donovan, ISO 16649-3	NMKL 71
Norway	EFTA	Yes	HPA 2004, F16	NMKL 71, AFNOR 12
Portugal	NRL	Yes	No information provided	No information provided
Poland	NRL	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 7251	ISO 6579
	OC	Yes	PN-ISO 7251	ISO 6579
Romania	NRL	Yes	ISO 16649-3	ISO 6579
	OC	Yes	ISO 16649-3	ISO 6579
Spain	NRL	Yes	Donovan	ISO 6579
Slovakia	NRL	Yes	Donovan	ISO 6579
Slovenia	NRL	Yes	ISO 16649-3	ISO 6579
Sweden	NRL	Yes	ISO 16649-3	Cefas SOP, NMKL NR71
	OC	Yes	ISO 16649-3, ISO 6887 - 3, ISO 7218, NMKL 96	No information provided
UK	NRL	Yes	ISO 16649-3	ISO 6579

NRL – National Reference Laboratory, OC – Official Control laboratory, CC – Candidate Country, EFTA – European Free Trade Association

2.7 Confidentiality of results

Each laboratory participant was identified by a code to preserve anonymity.

Table 4: Sample temperatures

Country	Date of dispatch	Date of arrival	Range (°C)[1]	Internal air (°C)[2]	Sample (°C)[2]	Storage (°C)[2]	Date analysed
Austria	11.09.07	12.09.07	4.5 – 8.5	3.0	3.0		12.09.07
Belgium	11.09.07	12.09.07	0.5 – 4.0	3.0	2.0	4.0	13.09.07
	11.09.07	12.09.07	3.5 – 8	3.4	1.4	4.7	14.09.07
	11.09.07	12.09.07	5.0 – 8.5	4.4	5.7	3.9	12.09.07
Bulgaria	11.09.07	12.09.07				2-8	13.09.07
Croatia	11.09.07	18.09.07	4.0 – 11.0	22.0	12.0	2.9	18.09.07
	11.09.07	18.09.07	1.0 – 8.0	22.0	13.0		18.09.07
Denmark	11.09.07	12.09.07	3.0 – 8.5	3.7	1.6	2.5	13.09.07
	11.09.07	12.09.07		3.0		2.0	13.09.07
	11.09.07	12.09.07	2.0 – 6.0		7.0		13.09.07
	11.09.07	13.09.07	1.5 – 5.5	11.8	5.6	4.5	14.09.07
Estonia	11.09.07	13.09.07	4.5 – 7.0	8.6	6.9	4.0	13.09.07
Finland	11.09.07	12.09.07	1.5 – 6.0	6.0	5.0	5.0	13.09.07
France	11.09.07	13.09.07	4.5 – 5.5	6.0	6.6	5.3	13.09.07
Germany	11.09.07	13.09.07	4.5 – 7.5	6.8	6.8	2.5	13.09.07
	11.09.07	13.09.07	3.0 – 5.0	19.0	7.0	23.0	13.09.07
	11.09.07	13.09.07	3.0 – 6.0	5.1	3.8	2.0	13.09.07
Greece	11.09.07	12.09.07	2.0 – 5.5	3.8	3.8	5.0	13.09.07
	11.09.07	13.09.07	0.5 – 4.0			4.0	13.09.07
	11.09.07	14.09.07	2.5 – 16.5	11.0	6.5	4.0	14.09.07
	11.09.07	13.09.07	2.0 – 8.0	8.0	8.0	4.0	13.09.07
Hungary	11.09.07	12.09.07	2.5 – 5.0	11.0	3.5	6.0	13.09.07
Iceland	11.09.07	12.09.07	7.0 – 13.5			4.0	13.09.07
Ireland	11.09.07	12.09.07	3.5 – 6.0	7.7	3.5		12.09.07
Italy	11.09.07	13.09.07	3.0 – 8.0	7.5	8.7	4.0	14.09.07
Latvia	11.09.07	12.09.07				6.0	15.09.07
	11.09.07	12.09.07	3.0 – 6.5	5.8	5.4	5.0	13.09.07
	11.09.07	14.09.07	1.0 – 6.5	5.0	5.0	4.0	15.09.07
	11.09.07	13.09.07	0.0 – 4.0	5.0	5.0	4.0	13.09.07
	11.09.07	14.09.07	2.0 – 6.0	11.0	6.0	5.0	15.09.07
Netherlands	11.09.07	12.09.07	3.5 – 9.4			4.0	13.09.07
	11.09.07	12.09.07	2.8 – 6.1	6.1	6.9	4.0	12.09.07
Norway	11.09.07	12.09.07	2.5 – 4.0	3.3	7.7	6.9	13.09.07
	11.09.07	12.09.07	2.5 – 6.5	6.0	5.5	4.5	13.09.07
Poland	11.09.07	14.09.07	2.5 – 8.0	12.0	10.0		14.09.07
	11.09.07	12.09.07		13.0	9.0	2.0	13.09.07
Poland	11.09.07	14.09.07	2.4 – 9.8	14.0	12.0		14.09.07
	11.09.07	14.09.07	4.0 – 7.5	10.2		4.0	14.09.07
Portugal	11.09.07	13.09.07		8.0	4.0	5.0	13.09.07
Romania	11.09.07	13.09.07	4.0 – 11.0	10.0	10.0	4.0	14.09.07
	11.09.07	13.09.07	3.0 – 6.9	8.0	4.0	4.0	14.09.07
Slovakia	11.09.07	13.09.07	2.0 – 6.5	10.7	12.3	2.5	14.09.07
Slovenia	11.09.07	12.09.07	1.0 – 6.5			5.0	13.09.07
Spain	11.09.07	12.09.07	3.0 – 6.0			5.0	18.09.07
Sweden	11.09.07	12.09.07	2.0 – 4.5	11.0	6.0	4.5	12.09.07
	11.09.07	12.09.07	2.0 – 5.5	5.4	4.3	3.3	12.09.07
UK	11.09.07	12.09.07		6.1	4.2	2.4	12.09.07

[1] temperature range as indicated by sample temperature recorder, [2] temperature recordings by participants

3. Results

3.1 Analysis of results

Participants were asked to carry out *E. coli* and *Salmonella* analysis in duplicate. The results reported by participants for *E.coli* and *Salmonella* spp.. are shown in Table 6. The reported *E. coli* MPN values were compared to the median MPNs from all participants' results, omitting laboratory 42 because of the unusual MPN result reported. These values were omitted from the calculation and Figure 1. The results chart was compiled using the MPN values reported and then $\log_{10}$ transformed (Figure 1). The results chart indicates the median MPN value calculated from participants' results. Reference results were omitted from this calculation. Upper and lower limits were calculated as the participants' median ±3 standard deviations (SD) and ±5 SD. SD calculations were based on the expected inherent variability of the five tube MPN method which is 0.26 $\log_{10}$. Performance assessment was performed according to the procedures described in the CRL/HPA EQA shellfish scheme for a single distribution, with minor modifications to reflect replicate analyses of a single sample (Table 5). The participants' and reference results median and SD are shown in Table 7 and the expected range for *E.coli* obtained from participants' results in Table 8. Scoring for detection of *Salmonella* spp. was not undertaken.

Table 5: *E.coli* MPN scoring

Result	Points allocated
Return of results	2
All replicate MPN results within the expected range	10
Or	
One replicate MPN result reported is outside the expected range and falls between the median ±3SD and median ±5SD value	7
Or	
Both replicate MPN results are outside the expected range and fall between the median ±3SD and median ±5SD value	4
Or	
One replicate MPN result reported is outside the median ±5SD value	5
Or	
Both replicate MPN results reported is outside the median ±5SD value	0
Or	
Single MPN result reported only	5
Or	
Tube combination inconsistent with MPN reported (one replicate)	7
Or	
Tube combination inconsistent with MPN reported (both replicates)	5
Or	
Sample not examined or results returned late - no explanation received	0
Or	
High censored result (e.g. MPN = >18000 per 100g)	Score not allocated

3.2 Summary participation statistics for RT 23

Total participants reporting duplicate results for *E. coli* MPN	46
Number of laboratories reporting outlying results	1
Participants reporting single MPN	1
Participants reporting MPN results within the expected range[1]	35
Participants reporting MPN results outside the expected range for one replicate	4
Participants reporting MPN results outside the expected range for both replicates	2
Participants reporting tube combination inconsistent with reported MPN	7
Participants reporting censored values for both replicates	0
Participants reporting censored values for one replicate	0
Participants not returning results	1

[1]expected range = participants' median ± theorectical 3SD

Table 6: Results reported by participants for *E.coli* and *Salmonella* spp..

Lab ID	*E.coli* MPN/100g			*Salmonella* spp. in 25g	
	Replicate 1	Replicate 2	Score	Replicate 1	Replicate 2
3 *	160	200	12	Not detected	Not detected
7 *	170	460	12	Not detected	Not detected
9 *	230	340	12	Not detected	Not detected
10 *	220	160	12	Not detected	Detected
13 *	93	170	9	Not detected	Not detected
14 *	20	20	6	Not detected	Not detected
15 *	130	130	12	Not detected	Not detected
16	750	310	12	Not detected	Not detected
18	78	78	7	Not detected	Not detected
19 *	329	329	7	Not detected	Not detected
21 *	40	-	7	Not detected	Not detected
22 *	110	110	12	Not detected	Insufficient material
26	160	160	12	Not detected	Not detected
27 *	90	90	12	Not detected	Not detected
30	70	160	12	Not detected	Not detected
32 *	110	110	12	Not detected	Not detected
33 *	750	1300	9	Not detected	Not detected
35 *	250	160	12	Not detected	Not detected
36 *	160	110	12	Not detected	Not detected
38	20	70	9	Not detected	-
39 *	310	430	12	Not detected	-
41 *	330	170	12	Not detected	Not detected
42 *	0.045	0.078	2	Not detected	Not detected
43 *	500	600	12	Not detected	Not detected
44 *	78	110	12	Not detected	Not detected
47 *	310	220	12	Not detected	Not detected
50	40	40	12	Not detected	-
51	160	310	12	Not detected	Not detected
52	230	92	9	Not detected	Not detected
54	130	1700	9	Not detected	Not detected
58	130	20	9	Not detected	Not detected
59	170	130	12	Not detected	Not detected
64	110	80	12	Not detected	Not detected
65	330	330	12	Not detected	Not detected
68 *	230	130	12	Not detected	Not detected
69	220	160	12	Insufficient material	Insufficient material
73	110	170	12	Not detected	Not detected
76	147	66	7	Not detected	Not detected
81	70	40	12	Not detected	Not detected
83 *	130	230	12	Not detected	Not detected
86	40	50	12	Not detected	-
90	160	110	12	Not detected	Not detected
91	130	170	12	Not detected	Not detected
92	160	200	12	Not detected	Not detected
96	790	490	12	Not detected	Not detected
98	220	490	12	Not detected	Not detected
100	70	40	12	Not detected	Not detected

* - denotes NRL participant

Table 7. Participants and reference results for *E. coli* MPN – replicates combined

	Median MPN/100g	Standard deviation
Reference results	$2.2x10^2$	2.5×10^2
Participants results	1.6×10^2	8.9×10^1

Table 8. Expected range for *E.coli* obtained from participants' results

	99% confidence interval (median ±3 SD)
Participants results	$2.7 \times 10^2 – 9.6 \times 10^2$

4. Comments *E.coli* MPN/100g

4.1 Participants' results

Forty of the 47 participating laboratories returned replicate results between ±3 SD of the participants' median, one laboratory returned a single replicate within the expected range. Four laboratories returned one replicate result between ±3 SD and ±5 SD of the participants' median. One laboratory returned results both falling outside ±5 SD of the participants' median. Seven laboratories reported one or both replicate results that were inconsistent with the tube combination given on the report forms. Laboratory 48 did not return results for this distribution.

4.2 Methods of analysis- *E. coli*

Thirty-five from 47 laboratories returning results gave ISO TS 16649-3 as a reference for the analytical method for the enumeration of *E. coli.* Five cited Donovan *et al* (1998), and three laboratories stated that they used Cefas CRL Standard Operating Procedure (SOP) but did not provide information on the document issue number. One laboratory used the Health Protection Agency SOP F16 2005. Two laboratories used ISO 7251, an MPN determination method for *E. coli* based upon primary incubation in lauryl sulphate broth and confirmation by gas and indole production. One laboratory did not provide information on methodology.

5. Comments *Salmonella* spp detection

5.1 Participants' results

Only one laboratory detected *Salmonella* spp in a single replicate of the mussel sample using conventional cultural techniques (ISO 6759). No other laboratory reported positive results using conventional culture techniques. One laboratory reported detection following enrichment using a commercial real-time PCR system on overnight enrichment broths.

5.2 Methods of analysis-*Salmonella* spp

Thirty-four laboratories gave ISO 6579 as the analytical method for detection of *Salmonella* spp. Eight laboratories cited the use of NMKL 71 *Salmonella* detection in foods. Three laboratories referred to alternative procedures, details of which were not available. Two laboratories did not provide information of methodology. One laboratory used real-time iQ-Check™ *Salmonella* kit (Bio-Rad) in addition to the routine laboratory method (see above).

6. Transit temperature

The internal temperature of samples in transit as measured by the automatic sample temperature recorders ranged between 0 and 8.5 in all sample boxes except one Croatian, Greek, Romanian and the Icelandic laboratory. On these four occasions the maximum recorded temperatures were 11.0, 16.5, 11.0, and 13.5˚C respectively, the Croatian distribution was subject to extreme delivery delays and did not reach the participating laboratory until 7 days after dispatch. The elevated temperature, and in the case of the Croatian laboratory and the delay in analysis, did not appear to influence the results returned to the CRL relative to other participants. Six participants failed to return the temperature recorders. Two laboratory participants (in addition to the two Croatian laboratories) recorded sample temperatures on

arrival of >10˚C, in one case the internal sample recorder indicated a sample temperature range of 2.0 - 6.5˚C. The second laboratory that recorded a sample temperature of >10˚C (12˚C) did not return the temperature recorder therefore the temperature could not be verified. One laboratory that received the sample on the 12th October did not analyse the sample until the 18th October.

7. Conclusion and recommendations

7.1 General comments

This was the largest proficiency-testing programme for statutory determinants using "real" samples that has been organised to date by the CRL. Due to the number of participants (48 laboratories) and requirements for chilled distribution the operational costs were very high (distribution costs approximately 12,000 euros). Forty percent of samples arrived at their destinations one day after dispatch, 20% of samples arrived within 48 hr. In approximately half of cases analysis was initiated within 48hr of sample collection.

7.2 *E.coli* analysis and methodology

The majority of laboratories returned results within the anticipated range (83%).

One laboratory returned results for both replicates below 5SD from the participants' median, these data were not included in the median calculations or Figure 1. It is recommended that this laboratory examine their procedures for interpreting MPN tables.

Several laboratories returned MPN results that were inconsistent with the tube combinations given on the returned results forms. It is recommended that these laboratories examine their procedures for reading MPN tables.

Two Official Control laboratories were not using the *E. coli* reference method specified in EU Regulation 882/2005, or equivalent. It is recommended that these laboratories establish the *E. coli* reference method ISO TS 16646-3 with the assistance of their NRL.

7.3 *Salmonella* spp. analysis and methodology

Salmonella spp. was present in distributed samples at low levels and was not consistently detectable by participants using ISO 6579. At low levels *Salmonella* may have been absent in a proportion of samples and potentially below or at the limit of detection of the assay in others. Previous studies at the CRL have estimated that the limit of detection of ISO 6579 in seeded bivalve shellfish matrix is 4CFU/25g. However, these data were not established using naturally contaminated samples and the potential effects of sample transit were not considered.

It was interesting to note that laboratories using real time PCR approaches detected *Salmonella* spp. from enrichment broths, indicating the enhanced sensitivity of these newer methodologies.

Where the information was provided a majority of laboratories were using the specified reference method for *Salmonella* spp (ISO 6579). In Denmark, Sweden, Iceland and Norway NMKL 71: *Salmonella* detection in foods was cited as the method of analysis. It is stated that the observed difference in performance of the ISO and NMKL methods is not statistically significant. However, it is not apparent that validation by collaborative trial included bivalve shellfish matrices therefore laboratory verification is recommended.

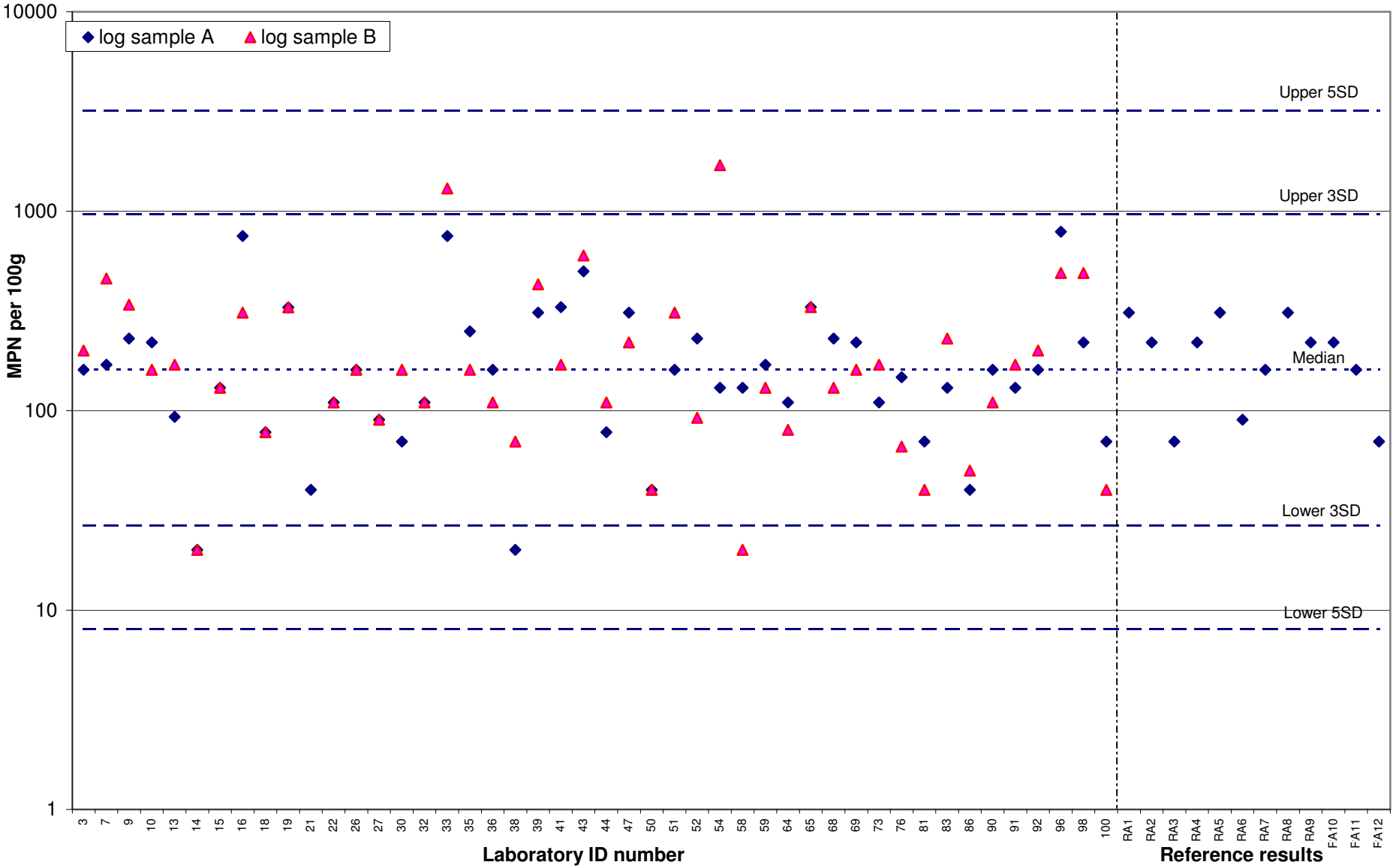
8.0 References

Anon 2004 ISO/TS 16649-3:2004. '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. 2002. ISO/TS 6579-2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp. ISO Copyright Office, Case Postale 56, CH-12111, Geneva 20, Switzerland.

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.

Figure 1: Participants duplicate results for *E.coli* (MPN) on whole animal bivalve shellfish



Annex I - RT 23 Reference testing for *Salmonella* spp. using TaqMan® *Salmonella enterica* Detection Kit (Applied Biosystems)

Introduction

The TaqMan® *Salmonella enterica* Detection Kit uses PCR to amplify the *Salmonella* spp. from 16±2 hour enrichment broths. Manufacturers validation data indicates 100% inclusivity (50 strains) and exclusivity (24 non-*Salmonella* strains), and a reported sensitivity of 1 colony forming unit (CFU) per 25g foodstuff.

Methods

DNA from the 12 reference test samples was extracted from 1 ml aliquots of 16-24 hour buffered peptone enrichments broths using PrepMan® Ultra (Applied BioSystems) following the manufacturers' instructions. Purified DNA was stored at 2 – 6˚C until PCR. TaqMan analysis was carried out using the thermal cycling parameters tabulated below:

Step	**AmpliTaq Gold® Enzyme Activation**	**PCR**	
	HOLD	Cycle (45 cycles)	
		Denature	Anneal/Extend
Time	10 min	15 sec	1 min
Temp	95ºC	95ºC	60ºC

For twelve reactions 196µl 2x environmental master mix was added to 39.6µl of 10x target assay mix (premix solution). Twelve µl of unknown sample was added to 18µl of premix in a 96 well plate, positive and negative controls were included throughout. Each reference sample was assayed in duplicate. The plate was sealed and run according to the cycling conditions above.

Results

Ct values for the 12 reference samples are given below.

CRL Sample no.	**Ct replicate 1**	**Ct replicate 2**	**Mean Ct[1,2]**
07/608	29.39	24.08	26.73
07/609	24.88	25.02	24.95
07/610	26.28	26.28	26.28
07/611	31.26	31.66	31.46
07/612	30.72	30.73	30.72
07/613	33.62	-	33.62
07/614	>45	35.43	42.71
07/615	37.03	-	37.03
07/616	31.17	31.58	31.37
07/617	35.98	>45	42.99
07/618	-	-	-
07/619	29.18	29.44	29.31

[1] samples giving mean Ct values <45 were considered positive provided control, FAM™ and VIC® dye signals were acceptable
[2] Ct values in excess of 45 were assigned a nominal value of 50 for calculation of mean values