

WS3/08



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



Report on *E.coli* / *Salmonella* EQA, 2004

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CRL ring trial reference : RT4 (*E. coli* / *Salmonella* EQA, 2004)

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Introduction

EU Council Decision of 29 April 1999 (1999/313/EC) designates the Centre for Environment, Fisheries and Aquaculture (CEFAS) Weymouth as the European Community Reference Laboratory (CRL) for monitoring bacteriological and viral contamination of bivalve molluscs. The CRL has collaborated with the UK Health Protection Agency on the development of a shellfish External Quality Assurance (EQA) Scheme for comparative (proficiency) testing among laboratories analysing shellfish. The Shellfish EQA scheme is primarily targeted at analysis of the statutory determinants *E. coli*/faecal coliforms and *Salmonella* spp. in shellfish.

At the 1st workshop of microbiological NRLs held in May 2002, NRLs supported the use of the CRL/HPA Shellfish EQA Scheme as the primary means of comparative bacteriological testing between NRLs. A pilot EQA distribution was performed during 2002 and the results were reported at the 2nd workshop and are available on the CRL website (www.crlcefafas.org).

At the 2nd workshop held in May 2003, it was agreed that NRLs would continue to participate in the CRL/HPA Shellfish EQA for *E. coli* and *Salmonella* testing with 3 distributions during the next year (until the next workshop). It was agreed that NRLs would cover the costs of their own participation in this scheme.

This report covers the two EQA distributions in which NRLs participated between the May 2003 workshop and the end of February 2004. For further details on the results of the EQA samples, please see the individual reports for distributions SF015 and SF016.

Samples

Health Protection Agency/CRL Shellfish EQA samples consist of freeze-dried mixtures of fully characterised bacterial isolates. The proportions of organisms in the reconstituted samples are designed to mirror those that may be found in real shellfish. The samples simulate the flora from raw bivalve molluscs from harvesting sites that should be examined in accordance with the EC Shellfish Hygiene Directive 91/492/EEC which relates to the enumeration of *Escherichia coli* and detection of *Salmonella* spp. The sample vials are accompanied by request forms, and instructions on handling and reconstitution. The date by which results must be returned is indicated at the foot of the request forms. Report forms for

the five tube most probable number (MPN) method with the modified confirmation method that requires the use of a chromogenic agar, are included with the samples.

Quality Control

The examinations required, as listed on the request form, are performed in the HPA Food EQA Laboratory on a minimum of 10 samples during the examination period. The results from these samples are the reference results. The method used to obtain the reference results is the CRL reference method described in ‘Modification of the standard method used in the United Kingdom for counting *Escherichia coli* in live bivalve molluscs’, Donovan T.J. *et al.*, Communicable Disease and Public Health 1998; 1 (3): 188-196. Full details of this method, with standard operating procedures, are available from the information center of the CRL website (www.crlcefas.org).

Participants’ Results

NRLs’ results are assessed by comparison with those of all Shellfish EQA participants, and also the reference results. Scores are allocated for a) the results reported for *E. coli* MPNs and b) results for examination for *Salmonella* spp.

***E.coli* MPNs**

Statistical Analyses

Statistical analyses are performed on results submitted for *E. coli* enumerations reported by NRLs using a five tube MPN method. The analyses have been amended since the scoring system was introduced. Each NRL’s reported MPN value is compared with the median MPN of all participants (due to the relatively small number of NRLs). The median is used rather than the mean because it is less affected by outlying results than the mean value.

All the analyses are based on the tube combinations reported, not the final MPNs and are as follows:

i) Within replicate variation

This analysis determines whether each tube combination reported by each participant is statistically acceptable.

ii) **Comparison with the participants' median MPN**

This analysis determines the participants' median and compares each NRL's MPN value, as calculated from the tube combination reported, with the median ± 3 and ± 5 standard deviations. The standard deviation is based on the expected inherent variability of the three by five tube MPN method, which on a \log_{10} scale has a value of 0.26.

iii) **Between sample variation**

This analysis is performed when two samples in a distribution are from the same batch. The analysis determines whether there is a significant difference between the results reported for the two samples.

Results Charts

NRLs' results and the reference results for an *E.coli* enumeration are plotted on the same chart. The results charts are compiled from MPNs reported by NRLs. The results charts also indicate the median MPN value for all participants (both NRLs and others) and the values calculated for 3 standard deviations and 5 standard deviations.

Results Analysis and Scoring System

***E.coli* MPN**

NRLs' MPN results reported for each sample are allocated scores up to a maximum of 12 points. Points are deducted if a tube combination reported shows significant within replicate variation (where applicable) and/or differs significantly from the participants' median value. If the reported tube combination results in an MPN value that falls outside the 5 standard deviation values then five points are deducted. If the reported tube combination would result in an MPN value that falls between the 5 and 3 standard deviations values then three points are deducted. A further two points may be deducted if the MPN value reported is inconsistent with the tube combination. **NRLs returning only a single MPN result for a sample will be allocated scores out of a maximum score of seven points.**

Normally scores are not allocated for high-censored results e.g. (>16000 per 100g etc).

All NRLs who return results will be allocated a minimum of two points regardless of the quality of the results reported.

***Salmonella* spp.**

NRLs' results for presence/absence examinations for *Salmonella* spp. are allocated scores as follows:

	Score
Fully correct result	2
Result partially misleading (e.g. incorrect serotype designation)	1
Grossly misleading result, e.g (failure to isolate <i>Salmonella</i>)	0

Performance Assessments

Performance assessments are undertaken after every distribution and take into account a participant's performance with the current and previous two distributions. A summary of the performance assessment with *E. coli* MPNs is included with this report as Table 5; that for performance for *Salmonella* examinations as Table 6.

All participants who appear to be experiencing problems with these examinations will be offered advice. Participants who fail with *Salmonella* isolation on two consecutive distributions will be contacted, in confidence, by the organisers.

Participants who achieve <40% of the maximum possible score with a single distribution, or <70% of the maximum possible score over three distributions, for *E.coli* MPNs will also be contacted.

As NRLs had only participated in two distributions by the time of this report, the cumulative performance was undertaken over two, rather than three, distributions. The cumulative performance assessments are therefore only provisional at this stage.

Participation (see Table 1)

Sweden has not registered to participate in the Shellfish EQA Scheme. The Netherlands has registered but, of the two distributions considered here, has not returned results for either for *E. coli* and only one for *Salmonella*. The NRL from Estonia, a candidate country, registered for the scheme after a meeting of candidate country NRLs hosted by the CRL.

Table 1. SUMMARY OF PARTICIPATION BY NRLS

NRL	Participation in Shellfish EQA Scheme for	
	<i>E. coli</i>	<i>Salmonella</i>
Austria	Yes	Yes
Belgium/Luxembourg	Yes	Yes
Denmark	Yes	Yes
Estonia ¹	Yes	Yes
Finland	Yes	Yes
France	Yes	Yes
Germany	Yes	Yes
Greece	Yes	Yes
Ireland	Yes	Yes
Italy	Yes	Yes
Netherlands	No ²	Yes ³
Portugal	Yes	Yes
Spain	Yes	Yes
Sweden	No	No
UK	Yes	Yes

COMMENTS

1. This NRL joined the EQA Scheme after distribution SF015 and therefore hasn't been included in this summary
2. Registered for the EQA scheme but no results returned over the 2 distributions SF015 and SF016 – therefore not included in the performance summaries.
3. Registered for the EQA scheme but results only returned for 1 out of the 2 distributions – therefore not included in the performance summaries.

General comments on performance

The assessment of performance is provisional as this is normally undertaken after three distributions. Table 5 summarises the performance of NRLs in the *E. coli* test over the two distributions. Only 3 NRLs scored 100% for the *E. coli* tests over this period. However, most of the other NRLs scored more than the 70% poor performance assessment limit set for

the EQA Scheme. Two NRLs scored below this and are advised to review their methods, albeit on the basis of the provisional assessment. Table 6 summarises the performance of NRLs in the *Salmonella* test over the two distributions. All NRLs apart from one scored 100% for this test.

DISTRIBUTION SF015: SAMPLE SF0038

Reference results

All samples

Escherichia coli <20 per 100g

Salmonella sp. present in 25 g shellfish

NRL results

E.coli MPN

E.coli was not detected from any of the samples selected randomly from the batch for QC testing.

All NRLs returning results correctly reported that *E.coli* concentration was less than their limit of detection. **One** NRL (Lab 601) reported *E.coli* MPNs of <180 per 100g. This limit of detection is too high for the examination requested. Four points were deducted from the score for that participant.

Salmonella spp.

Salmonella sp. was detected in all the samples selected for QC testing before or during the distribution period.

All NRLs who undertook the examination detected *Salmonella* sp. in the sample. It is unusual to detect *Salmonella* in samples that do not contain *E. coli*. However, such results do arise on occasion with shellfish samples.

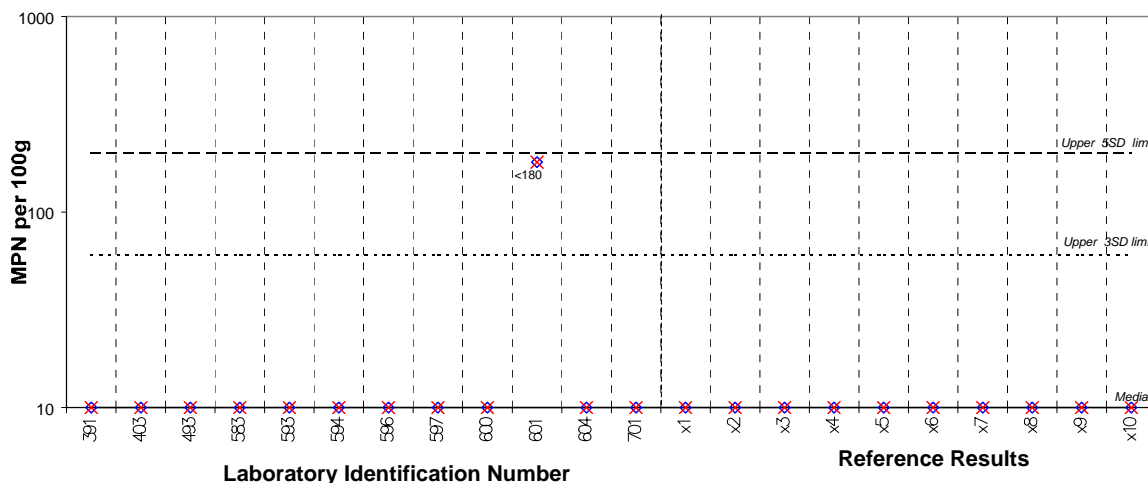
SF0038

Table 2: Results reported by participants and scores allocated – SF0038

Lab Number	<i>E.coli</i> (per 100g)			<i>Salmonella</i> sp.	
	Replicate 1	Replicate 2	SCORE	<i>Salmonella</i> sp.	SCORE
391	<20	<20	12	Present	2
403	<20	<20	12	Present	2
493	<20	<20	12	Present	2
583	<20	<20	12	Present	2
593	<20	<20	12	Present	2
594	<18	<18	12	Present	2
596	<20	<20	12	Present	2
597	<18	<18	12	Present	2
600	<20	<20	12	Present	2
601	<180	<180	8	Present	2
604	<20	<20	12	Present	2
701	<20	<20	12	Present	2

NR = not returned, ND = not detected, NE = not examined

Fig 1: SF0038 MPN Results



DISTRIBUTION SF015: SAMPLE SF0039

Reference results

E. coli Median MPN 3.5×10^5 per 100g

Salmonella sp. present in 25 g shellfish

NRL Results

E. coli MPN

No NRL reported results for either of the two replicates that were more than three standard deviations higher than the participants' median MPN value.

Two NRLs reported duplicate results that were both between three and five standard deviations lower than the participants' median MPN value.

Two NRLs reported results for one of the two replicates that were between three and five standard deviations lower than the participants' median MPN value.

One NRL reported duplicate results that were both more than five standard deviations lower than the participants' median MPN value.

One NRL reported duplicate results of >16000 per g. However those results were not compatible with the tube combination reported (541, 521). That participant was allocated a score of only two points.

Salmonella spp.

Salmonella sp. was detected in all the samples selected for QC testing before or during the distribution period.

All NRLs who undertook the examination detected *Salmonella* sp. in the sample.

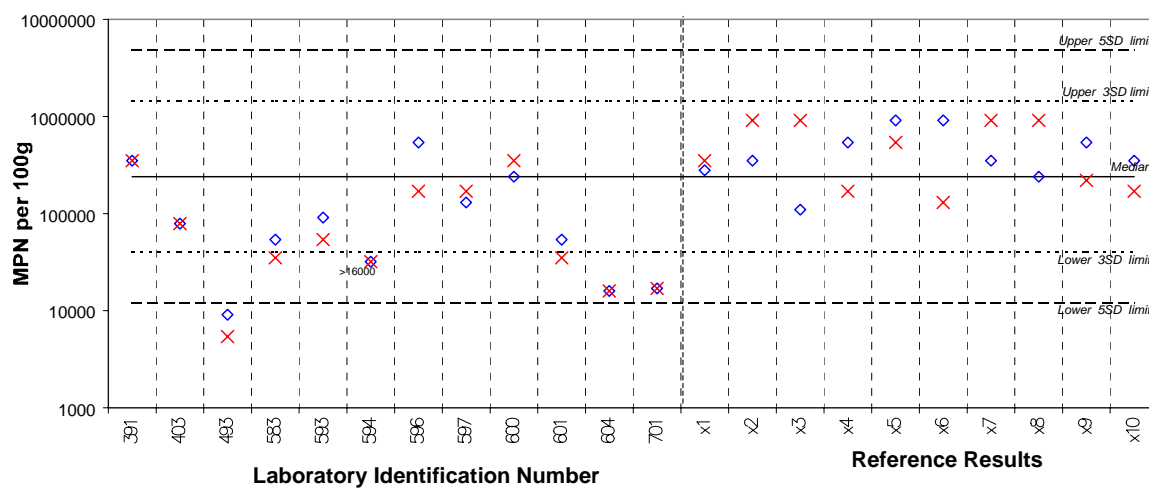
SF0039

Table 3: Results reported by participants and scores allocated – SF0039

Lab Number	<i>E.coli</i> (per 100g)			<i>Salmonella</i> sp.	
	Replicate 1	Replicate 2	SCORE	<i>Salmonella</i> sp.	SCORE
391	350000	350000	12	Present	2
403	7.9×10^4	7.9×10^4	12	Present	2
493	9.1×10^3	5.4×10^3	2	Present	2
583	54000	35000	9	Present	2
593	91000	54000	12	Present	2
594	>16000	>16000		Present	2
596	540000	170000	12	Present	2
597	130000	170000	12	Present	2
600	2.4×10^5	3.5×10^5	12	Present	2
601	5.4×10^4	3.5×10^4	9	Present	2
604	16000	16000	3	Present	2
701	17000	17000	6	Present	2

NR = not returned, ND = not detected, NE = not examined

Fig 2: SF0039 MPN Results



DISTRIBUTION SF016: SAMPLE SF0040

Reference results

E. coli Median MPN 2.4×10^4 per 100g

Salmonella sp. present in 25 g shellfish

NRL Results

E.coli MPN

No NRL reported results that were more than five standard deviations higher than the participants' median MPN value.

One NRL reported results for one of the two replicates that was between three and five standard deviations higher than the participants' median MPN value.

Four NRLs reported a result for one of the two replicates that was between three and five standard deviations lower than the participants' median MPN value.

One NRL reported a result for one of the two replicates that was between three and five standard deviations lower than the participants' median MPN value; the other result was more than five standard deviations lower than the participants' median value.

One NRL reported both results that were more than five standard deviations lower than the participants' median MPN value.

One NRL reported a single result for this sample and therefore five points were deducted.

Salmonella spp.

Salmonella sp. was detected in all the samples selected for QC testing before or during the distribution period. The HPA FEPTU laboratory examined the samples using Rappaport Vassiliadis soya peptone broth and selenite cystine broth as the selective enrichment media. The *Salmonella* was not isolated through the selenite cystine broth.

One NRL failed to detect *Salmonella* sp. in the sample. This laboratory is advised to audit their procedures for this examination. The failure rate seen with NRLs was much lower than that seen with other types of participant (for which the rate was unexpectedly high).

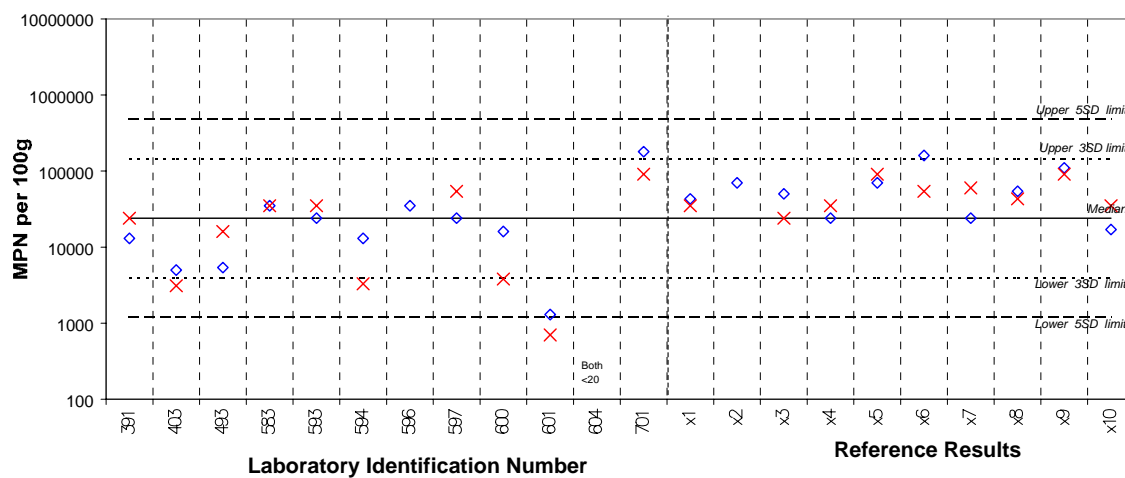
SF0040

Table 4: Results reported by participants and scores allocated – SF0040

Lab Number	<i>E.coli</i> (per 100g)			<i>Salmonella</i> sp.	
	Replicate 1	Replicate 2	SCORE	<i>Salmonella</i> sp.	SCORE
391	13000	24000	12	Present	2
403	5000	3100	9	Present	2
493	5400	16000	12	Present	2
583	35000	35000	12	Present	2
593	24000	35000	12	Present	2
594	13000	3300	7	Present	2
596	35000		7	Present	2
597	24000	54000	12	Present	2
600	16000	3800	9	Present	2
601	1300	700	4	Not detected	0
604	<20	<20	2	Present	2
701	>180000	91000	12	Present	2

NR = not returned, ND = not detected, NE = not examined

Fig 3: SF0040 MPN Results



DISTRIBUTION SF016: SAMPLE SF0041

Reference results

E. coli Median MPN 5.0×10^2 per 100g

Salmonella spp. not detected in 25 g shellfish

Results

E.coli MPN

The five standard deviation lower limit is less than the limit of detection for the test (i.e. <20 per 100g). Therefore, all NRLs that reported an MPN value for *E.coli* in this sample that was less than 83 per 100g, (i.e. the participants' median MPN value minus 3 standard deviations), had three points deducted from their score. Six points were deducted if both replicates were lower than 83 per 100g.

One NRL reported results for one replicate that was between three and five standard deviations higher than the participants' median MPN value.

One participant reported both results that were more than three standard deviations lower than the participants' median MPN value.

All of the reference results fell within the expected range calculated from the participants' results.

Salmonella spp.

The intended result was '***Salmonella* spp. not detected in 25 g shellfish**'.

Salmonella sp. was not detected in any of the samples selected for QC testing during the distribution period.

All NRLs who undertook the examination reported correctly that *Salmonella* spp. was not present.

SF0041

Table 4: Results reported by participants and scores allocated – SF0041

Lab Number	<i>E.coli</i> (per 100g)			<i>Salmonella</i> sp.	
	Replicate 1	Replicate 2	SCORE	<i>Salmonella</i> sp.	SCORE
391	330	330	12	Not detected	2
403	40	20	4	Not detected	2
493	500	310	12	Not detected	2
583	2200	3100	5	Not detected	2
593	500	1300	12	Not detected	2
594	790	330	8	Not detected	2
596	500	700	12	Not detected	2
597	230	220	12	Not detected	2
600	750	750	12	Not detected	2
601	230	230	12	Not detected	2
604	310	310	12	Not detected	2
701	500	500	12	Not detected	2

NR = not returned, ND = not detected, NE = not examined

Fig 4: SF0041 MPN Results

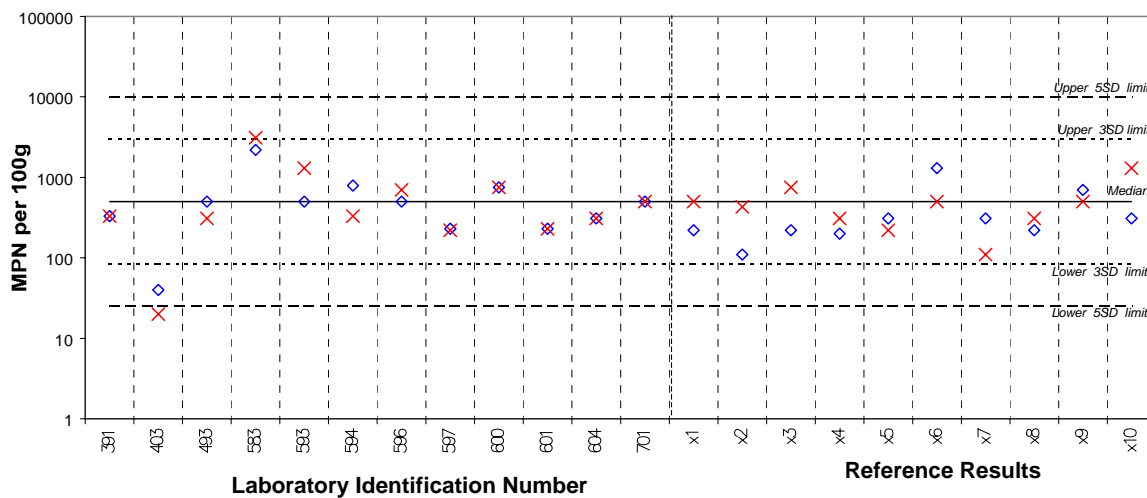


Table 5. *E. coli*: summary of performance of NRLs over two Shellfish EQA distributions, SF015 and SF016

Lab no.	Distribution SF015		Distribution SF016		Both distributions		
	SF0038	SF0039	SF0040	SF0041	Cumulative score	Maximum possible score	%
391	12	12	12	12	48	48	100
403	12	12	9	4	37	48	72
493	12	2	12	12	38	48	79
583	12	9	12	5	38	48	86
593	12	12	12	12	48	48	100
594	12		7	8	27	36	75
596	12	12	7	12	43	48	90
597	12	12	12	12	48	48	100
600	12	12	9	12	45	48	94
601	8	9	4	12	33	48	69
604	12	3	2	12	29	48	60
701	12	6	12	12	42	48	92

COMMENTS: NRL 601: Inappropriate limit of detection for *E.coli* for SF0038, Low MPN results for SF0039 & SF0040
NRL 604: Low MPN results for SF0039 & SF0040

Table 6: *Salmonella*: summary of performance of NRLs over two Shellfish EQA distributions, SF015 and SF016

Lab no.	Distribution SF015		Distribution SF016		Both distributions		
	SF0038	SF0039	SF0040	SF0041	Cumulative score	Maximum possible score	%
391	2	2	2	2	8	8	100
403	2	2	2	2	8	8	100
493	2	2	2	2	8	8	100
583	2	2	2	2	8	8	100
593	2	2	2	2	8	8	100
594	2	2	2	2	8	8	100
596	2	2	2	2	8	8	100
597	2	2	2	2	8	8	100
600	2	2	2	2	8	8	100
601	2	2	0	2	6	8	75
604	2	2	2	2	8	8	100
701	2	2	2	2	8	8	100

COMMENTS: None