SHELLFISH SCHEME

DISTRIBUTION SF013
SAMPLES SF0034 and SF0035

Distribution Date: 25 November 2002
Results Due: 06 December 2002
Report Date: 10 February 2003

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General Comments

The PHLS Shellfish EQA Scheme is now organised in collaboration with the Centre for Environment, Fisheries and Aquaculture (CEFAS), Weymouth, UK. The EU Council Decision of 29 April 1999 (1999/313/EC) defined the roles of both the European Community Reference Laboratory (CRL) and National Reference Laboratories (NRL) responsible for co-ordinating the requisite tests for the detection of bacterial and viral contamination of live bivalve molluscs, and designated the Centre for Environment, Fisheries and Aquaculture (CEFAS) Weymouth as the CRL. CEFAS Weymouth was subsequently also designated as the UK NRL.

Samples

PHLS/CEFAS Shellfish EQA samples consist of freeze-dried mixtures of fully characterised bacterial isolates. The proportions of organisms in the reconstituted samples are usually designed to mirror those that may be found in real shellfish. The samples simulate 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 usually performed in the PHLS 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 that 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. Copies of this publication are available to participants, free of charge, on request.

Participants' Results

Participants' results are assessed by comparison with those of other 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 participants using a five or three tube MPN method. The analyses have been amended since the scoring system was introduced. Each participant's reported MPN value is compared with the participants'

median MPN. 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 participant'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
Participants' results and the reference results for an *E.coli* enumeration are plotted on the same chart. The results charts are compiled from the tube combinations reported, not the final MPNs. A consequence of this is that participants who have used dilutions other than those indicated on the report form, or who have misread the MPN table, may find that the MPN on the results chart attributed to their laboratory differs from the MPN that they reported. The results charts also indicate the participants' median MPN value and the values calculated for 3 standard deviations and 5 standard deviations.

Results Analysis and Scoring System

***E.coli* MPN**
Participants' 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. In general, if the reported tube combination would result 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 standard deviations 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.

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

***Salmonella* spp.**
Participants' 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 *Salmonella*)	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 for *Salmonella* examinations is included with this report as Appendix 1; that for performance with *E.coli* MPNs is included as Appendix 2.

All participants who appear to be experiencing problems with these examinations will be offered advice. Participants who fail with *Salmonella* examinations in 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.

Repeat Samples
Participants who want to repeat any of the examinations are advised that additional sample vials are usually available on request. These are provided free of charge and will be dispatched with the next distribution of samples.

Advice and Comments
Participants who experience problems with any of the examinations requested are encouraged to contact one of the organisers for advice.
Comments regarding the samples and scheme in general should be directed to the organisers.
Comments and queries about the statistical analyses should be directed to the statistician.

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Web-sites	http://cphl.phls.org.uk/services/foodeqa.htm http://www.crlcefas.org

SF0034

Sample
Raw shellfish from a new shellfish bed

Contents
Escherichia coli and *Citrobacter freundii*

Examinations
Escherichia coli enumeration – in duplicate
Salmonella spp.

Results

***E.coli* MPN**

	Median MPN per 100g	Median MPN -3 standard deviations	Median MPN -5 standard deviations	Median MPN +3 standard deviations	Median MPN +5 standard deviations
Reference results	$3.1x10^2$	51	1	$1.9x10^3$	$6.2x10^3$
Participants' results	**$1.7x10^2$**	**28**	**1**	**$1.0x10^3$**	**$3.4x10^3$**

Comment
The duplicate results reported by participants and the reference results for *E.coli* MPNs for sample SF0034 are plotted in Fig 1.

The MPN value five standard deviations lower than the participants' median is lower than the lower limit of detection for the test (i.e. <20 per 100g). Therefore, all participants who reported an MPN value for *E.coli* in this sample that was less than 28 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 42 per 100g.

Two participants (Labs 595 and 600) reported one result that was three standard deviations lower than the participants' median MPN value.

One participant (Lab 599) reported one result that was three standard deviations lower than the participants' median MPN value; the other result was five standard deviations lower than the participants' median MPN value.

One participant (Lab 601) reported one MPN value that was not consistent with the tube result combination. Two points were deducted from the score because of the discrepancy between the tube combinations and MPN values reported.

All other participants results were within the expected range.

All 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 participants who undertook the examination reported correctly that *Salmonella* spp. were not detected.

SF0035

Sample
Raw shellfish from a new shellfish bed

Contents
Escherichia coli, Citrobacter freundii and *Salmonella lanka*

Examinations
Escherichia coli enumeration – in duplicate
Salmonella spp.

Results

***E.coli* MPN**

	Median MPN per 100g	Median MPN -3 standard deviations	Median MPN -5 standard deviations	Median MPN +3 standard deviations	Median MPN +5 standard deviations
Reference results	$3.5x10^4$	$5.8x10^3$	$1.8\ x10^3$	$2.1\ x10^5$	$7.0x10^5$
Participants' results	**$3.4x10^4$**	**$5.5x10^3$**	**$1.7\ x10^3$**	**$2.0x10^5$**	**$6.7x10^5$**

Comment
The duplicate results reported by participants and the reference results for *E.coli* MPNs for sample SF0035 are plotted in Fig 2.

One participant (Lab 595) reported one result that was three standard deviations higher than the participants' median MPN value.

All other participants results were within the expected range.

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

***Salmonella* spp.**
The intended result was '***Salmonella* spp. present in 25 g shellfish**'.

Salmonella sp. was detected in all the samples selected for QC testing before or during the distribution period. **All** participants who undertook the examination detected *Salmonella* sp. in the sample.

SF0034

Table 1: Results reported by participants and scores allocated – SF0034

Refer also to Fig 1 for scores for *E.coli* MPNs

Lab Number	*E.coli* (per 100g)			*Salmonella* sp.	
	Replicate 1	Replicate 2	SCORE	*Salmonella* sp.	SCORE
593	160	500	12	Not detected	2
594	230	330	12	Not detected	2
595	0	20	6	Not detected	2
596	290	220	12	Not detected	2
597	170	170	12	Not examined	
598	110	70	12	Not detected	2
599	1700	3500	4	Not detected	2
600	90	20	9	Not detected	2
601	0	200	6	Not examined	
602	220	300	12	Not detected	2
603	230	490	12	Not detected	2
604	110	110	12	Not detected	2
605	130		12	Not detected	2
608	130		12	Not detected	2

SF0035

Table 2: Results reported by participants and scores allocated – SF0035

Refer also to Fig 2 for scores for *E.coli* MPNs

Lab Number	*E.coli* (per 100g)			*Salmonella* sp.	
	Replicate 1	Replicate 2	SCORE	*Salmonella* sp.	SCORE
593	50000	22000	12	Present	2
594	33000	33000	12	Present	2
595	220000	49000	9	Present	2
596	35000	24000	12	Present	2
597	70000	24000	12	Not examined	
598	24000	91000	12	Present	2
599	18000	18000	12	Present	2
600	50000	70000	12	Present	2
601	34000	24000	12	Not examined	
602	30000	50000	12	Present	2
603	35000	92000	12	Present	2
604	11000	11000	12	Present	2
605	22000		12	Present	2
608	70000		12	Present	2

Fig 1: SF0034 MPN Results - NRLs

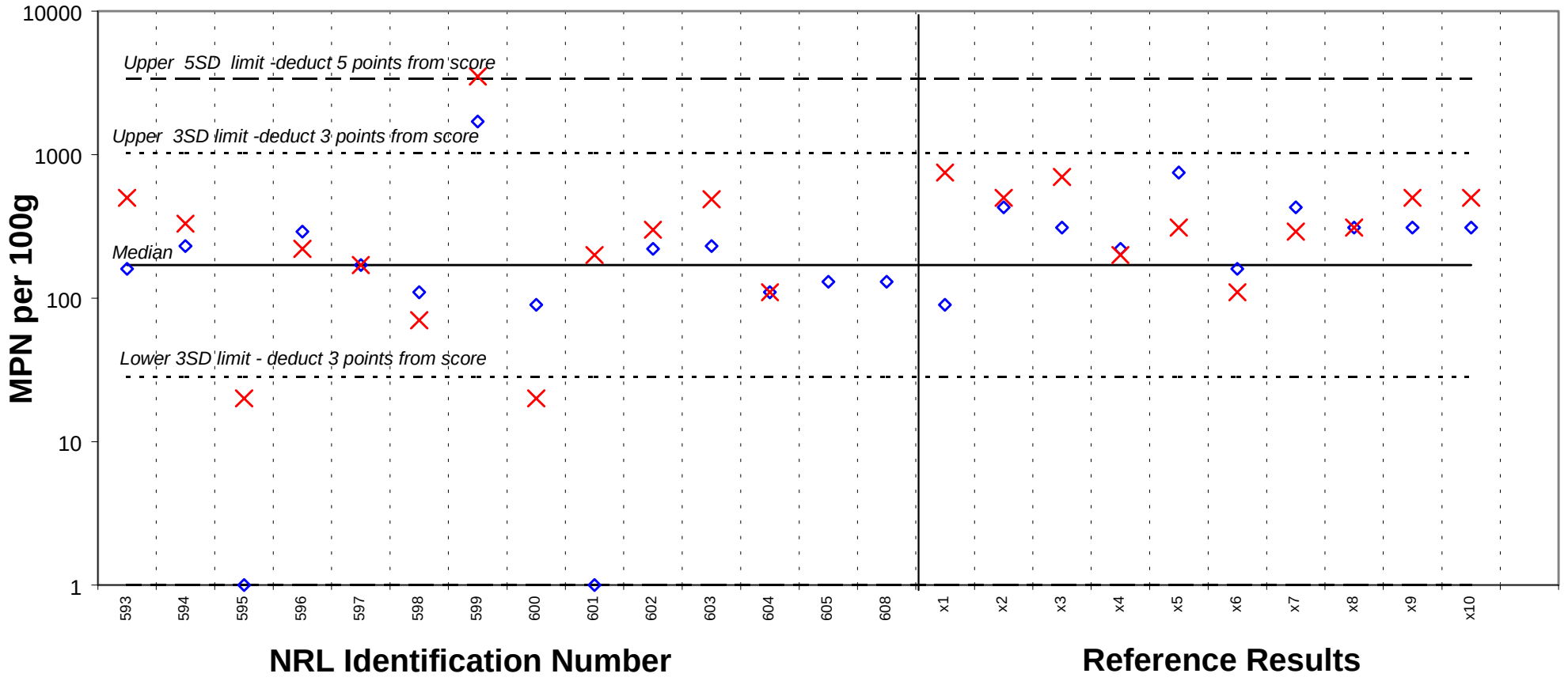

Fig 2: SF0035 MPN Results