Community Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs, CEFAS, Weymouth

Technical Report for Calendar Year 2002

Legal functions and duties

The functions and duties of the CRL are specified in Articles 3 and 4 of Council Decision 1999/313/EC (Official Journal of the European Communities No L 120 of 8.5.1999).

Introduction

2002 was the third complete year of operation for the CRL following designation by Council Decision 1993/313/EEC. The work plan for the CRL for 2002 was submitted to the European Commission in November 2001. This report details consequential activities of the CRL according to the agreed work plan, and to the tasks outlined in Council Decision 1993/313/EEC, for the calendar year 2002. Following agreement by the Commission this report will be placed in the public domain on the CRL website (www.crlcefas.org).

1. Scientific advice and support

To the European Commission

The CRL has provided scientific assistance to the European Commission on proposals for the development of European legislation on Microbiological Criteria for food operators in relation to molluscan shellfish. Dr D Lees attended several EU working groups on microbiological criteria for fishery products in March and October 2002. Dr Lees and Dr Lee have provide assistance via correspondence on a number of occasions. Officials from DG Sanco of the European Commission visited the CRL in April 2002 to discuss the activities of the CRL. The CRL has attended 2 coordination meetings of EU CRLs in Brussels in January 15-16, 2002 and December 3-4, 2002. The latter meeting included a co-ordination meeting with the JRC in Geel, Belgium regarding co-operation between CRLs and JRC on reference materials and ring trials.

The CRL has provided scientific assistance to the European Commission Food and Veterinary Office (FVO) in relation to mission on bivalve molluscs and fishery products. Dr R Lee attended an FVO mission to Germany, February 18 -28, 2002. The CRL provided technical advice to the FVO regarding potential missions to Canada and New Zealand and on the issue of equivalency between the US FDA monitoring approach and that of the EU. Dr Lees provide further assistance regarding sanitary control agreements in relation to imports from Peru.

The CRL has been involved in discussions on EU Candidate Countries and wrote to 13 CC NRLs introducing the CRL and the activities of the NRL network and requesting them to register their interests in ring-trials and other activities. Candidate Country NRL details are now included on the laboratory network page of the CRL web-site. A workshop for CC NRLs is planned for 2003.

The CRL participated in a Scientific Committee for veterinary measures relating to Public Health expert working group on Viruses in Food. Dr D Lees attended several SCVMPH working groups and drafted sections of the report. The report was submitted, and accepted, in January 2002 and is available on the Commission website (a copy is also available on the CRL website).

Dr R Lee acted as the representative of the Commission through the CRL for the ISO/CEN Food Microbiology Committees. As the representative he attended the 22nd meeting of the ISO/TC 34 SC9 and the 9th meeting of CEN/TC 275/WG 6, Bangkok, December 2-6, 2002.

Other scientific activities

Dr D Lees of the CRL was invited by the organisers of the 4th International Conference for Molluscan Shellfish Safety, Santiago de Compostela Spain, June 2002 to be on the international advisory panel, to attend the conference and to give a paper. Dr D Lees gave an oral presentation entitles : 'Viruses and bivalve shellfish: the development of sanitary controls.' D.N. Lees, K. Henshilwood, R. E. Rangdale and W. J. Dore (2002), which has also been submitted to the conference proceedings. Dr R Rangdale of the CRL participated at the 'Septieme conférence de microbiologie des denrées alimentaires' at the University of Liege, Belgium, June 20-21, 2002 and gave the following oral presentation : "Vibrios pathogenic for humans and their detection in bivalve molluscan shellfish." Dr R Lee participated in the expert working group on Vibrio spp. in seafood at the Joint FAO/WHO expert consultation on risk assessment for Vibrio spp. in seafoods. Bangkok, August 2002. Dr R Lee participated in a Nordic Council Workshop on Vibrios in Oslo, November 2002.

2. Co-ordination of activities of NRL network and provision of technical assistance and training (including to third countries)

Website

The CRL continues to develop, support and maintain its dedicated website (www.crlcefas.org) as the primary means of dissemination of information. The web-site provides technical information and guidance for interested parties while the activities page documents events taking place at the CRL and invites laboratories to participate. The website is well used and a successful and efficient means of disseminating information. User statistics for the CRL website for 2002 are given in the graph at appendix 5.

CRL Workshop May 14-16, 2002. CEFAS, Weymouth, UK.

The CRL and DG Sanco hosted the first workshop of European NRLs at the CRL in Weymouth May 14-16th 2002. Representatives were present from DG Sanco, the Food and Veterinary Office, Dublin, and the CRL. Private experts from 14 Member States attended and apologies were received from the Netherlands. A summary of the workshop and the resolutions are presented in Appendix 1.

Training

The CRL ran advanced training courses on FRNA bacteriophage methods and analysis on three occasions during September and October 2002. One third of the course involved presentations on the theory of FRNA, an introduction to the methods, accreditation issues and quality control. The remaining two-thirds of the course was spent carrying out practical sessions in the laboratories. All NRLs were invited to attend the two day courses run at the CRL, Weymouth. Thirteen delegates from eleven Member States attended the courses.

Feedback was received via course questionnaire's and delegates found the courses very useful.

Technical assistance to NRLs and Third Countries

Dr R Lee provided advice on sample size for Vibrio analysis to the Swedish National Food Administration. (December 2002). Dr D Lees and Bill Dore provided written advice on depuration and relaying to the Competent Authority of the New Zealand Government. (March 2002). Dr D Lees and Dr K Henshilwood liased with the Communicable Disease Group, ESR, New Zealand regarding viral samples and faecal material and participation in ring trials organised by the CRL. Dr D Lees advised the Embassy of India on bivalve controls. Dr R Lee provided advice on salmonella detection methods in foods to Sanita of the Latvian NRL. (September 2002). Contacts made through the website included staff from the Guangxi Entry-Exit Inspection and Quarantine Test Centre, Guangxi CIQ Bureau, located in south China. Currently they are establishing methods for investigation on the sanitary quality of shellfish. Various other ad-hoc queries were addressed by staff of the CRL. Further to the advanced training for NRLs on detection of FRNA bacteriophage several NRLs were helped with stocks of controls, media, biological reagents, etc, for this test.

3. Ring trials, comparative testing and quality assurance

E.coli pilot comparative test trial

The CRL has worked with the UK Public Health Laboratory Service (PHLS) to progress the CRL/PHLS collaborative shellfish EQA scheme as the primary means of comparative testing for E.coli and Salmonella in bivalve molluscs. The pilot ring trial for E.coli was distributed in November by the PHLS/CEFAS partnership for European Shellfish EQA. The dates for examination were between November 25 and December 2, 2002. The pilot distribution consisted of two samples of freeze-dried micro-organisms which were accompanied by instruction sheets for sample reconstitution and request/report forms. The NRLs were asked to examine the samples according to their routine methods. Three NRLs were unable to participate in this ring trial and nominated other laboratories within their countries to participate instead. The results and report for this ring trial are presented in Appendix 3.

Hepatitis A virus comparative test trial

A pilot ring trial for HAV was distributed by the CRL in November 2002. The samples sent out were tissue grown Hepatitis A virus (vaccine strain HM 174). The analysis required was nucleic acid extraction of a set volume in duplicate according to the usual methods used in each laboratory. Results were reported as positive or negative. The CRL also distributed stocks of shellfish contaminated with HAV (by normal filter-feeding mechanisms) for participants to store and use at a later date. Nine out of the fourteen NRLs participated in this ring trial. The results for this ring trial were analysed by the CRL and are presented in Appendix 2.

4. Confirmatory testing

During 2002 the CRL has maintained it's accreditation to ISO 17025 (EN 45000) for the following methods: 'Enumeration of male-specific RNA bacteriophages in molluscan bivalve shellfish', 'Examination of shellfish for Salmonella spp. other than Salmonella typhi', 'Enumeration of Escherichia coli in molluscan bivalve shellfish'.

The CRL has also maintained its laboratory testing ability for Norwalk-like virus and hepatitis A virus contamination of bivalve molluscs.

5. Development of analytical methods

The CRL has been progressing work relating to the adoption of the CRL method for E.coli and Salmonella for bivalve molluscs to the status of an ISO method as agreed at the 1st workshop of NRLs and in line with Commission policy. In line with this Dr R Lee liased with Betrand Lombard, Chair of the ISO/TC 34/SC 9, regarding the revision of the Standard for the enumeration of E.coli (MPN) and attended the 22nd meeting of the ISO/TC 34 SC9 and the 9th meeting of CEN/TC 275/WG 6, Bangkok, December 2-6, 2002. This activity has been on-going throughout the year. The CEN meeting also considered methods for detection of viruses in shellfish by PCR and Dr Lee also contributed to this discussion and provided feedback to NRLs and other interested parties.

The 1st NRLs workshop agreed a resolution on progressing a pilot ring trial for FRNA bacteriophage among NRLs. In preparation for this the CRL undertook laboratory work on suitable methods for preserving FRNA bacteriophage for such a ring trial and on the practicalities of distribution and analysis of the material. Material for the FRNA pilot ring trial will be distributed by the CRL in January 2003.

The 1st NRLs workshop also agreed a resolution on progressing a pilot ring trial for hepatitis A virus among NRLs. In preparation for this the CRL, with collaborators at the University of Santiago de Compostela, Spain, has undertaken growth and titration of suitable stocks of hepatitis A virus for such a ring trial. The work has also included investigation of procedures for artificial contamination of oysters with hepatitis A virus that could be used in a ring trial. This involved the use of natural bioaccumulation of tissue culture grown hepatitis A by oysters held in tanks of seawater within a containment isolator. Further work in preparation for conducting a ring trial for hepatitis A virus in bivalve molluscs has included generation of reference material, determination of recovery efficiencies, stability, transport of material, etc. HAV pilot ring trial distributions were conducted by the CRL in 2002 as described above.

The CRL has undertaken preliminary work towards conducting a ring trial for Norwalk-like virus in bivalve molluscs. Source material has been obtained and titrated and initial stability studies performed. Experience shows that this will be a difficult target organism to conduct a ring trial against. However, preparations are on-track for conducting a preliminary ring trial during 2003 as agreed by resolution at the 1st NRLs workshop.

Dr Angelo DePaola from the Gulf Coast Sea Food Laboratory, U.S. Food and Drug Administration, Dauphin Is. Alabama, USA visited the CRL on 25-26th November 2002. Discussions were held with Dr R Rangdale and Dr R Lee on research being undertaken by the FDA and the CRL on Vibrios. Dr DePaola spent some time in the laboratory viewing the practical methods especially with regard to vibrio's in oysters. These links and discussions are beneficial to the CRL in the context of developing expertise, and methods, for vibrio’s.

Following invitation by the European Commission Dr D Lees submitted the document 'Proposals for research in support of EU policy in the area of microbiological contaminants of bivalve molluscs - 2003' to the Commission, in December 10, 2002.

Dr David Lees, CRL Director

April 2003

CEFAS Weymouth Laboratory,
Barrack Road,
The Nothe,
Weymouth, Dorset, DT4 8UB, UK
Telephone : +44 (0) 1305 206600
Direct line : +44 (0) 1305 206635
Fax : +44 (0) 1305 206601
E-mail : d.n.lees@cefas.co.uk
General e-mail : fsq@cefas.co.uk

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

APPENDIX 1

Report and Resolutions of CRL Workshop May 14-16, 2002. CEFAS, Weymouth, UK.

1 **Work of the NRLs :** each NRL described their institutions and work to date. For many of the participants this was limited due to their only being recently designated.

2 Julie Younger gave the following presentation : 'United Kingdom NRL, The Centre for Environment, Fisheries and Aquaculture Science.'

3 **Responsibilities of CRL/NRLs :** the CRL presented the contents of the Council Decision 1999/313/EC relating to the reference laboratory network including the detailed duties of the CRL and the NRLs.

4 **FVO inspections :** Paulo Luciano, an inspector with the Food and Veterinary Office, gave a presentation of the main points that had been found on the two rounds of inspections on live bivalve molluscs.

5 **Microbiological criteria :** Paolo Caricato gave a brief introduction to the work on the microbiological criteria for foods.

6 **Methods for *E.coli*/faecal coliforms :** Dr R Lee gave the following presentation : 'Microbiological Criteria for Foodstuffs - Draft Revision May 2002.'

Most participants gave presentations on the methods they use for the *E.coli* analysis with Dr R Rangdale giving a presentation on the methods in use at the CRL. There was a discussion and general agreement of the need to provide a reference method for future use in conjunction with European standards/guidelines.

7 **Shellfish EQA :** Dr R Lee gave a presentation on the joint CEFAS/PHLS shellfish external quality assurance scheme. It was proposed that this be used for comparative testing between NRLs instead of using ring trials.

8 **Accreditation :** each NRL gave a presentation on its state of accreditation (and some on the other official labs testing shellfish samples). Some laboratories were accredited to ISO 17025 for their specific shellfish methods whereas others had general laboratory accreditation which did not include specific methods.

9 **Sampling plans and monitoring arrangements:** each NRL that had experience with the sampling plans for the monitoring of harvesting areas gave a presentation on the approach taken. Andy Younger gave the following presentation : 'Sampling plans for shellfish harvesting areas in the UK.'
There was a discussion on the differences in approach between member states on the harmonisation of monitoring arrangements. There was general agreement that there should be more detail in the European Legislation.

10 Viruses : presentations were undertaken by the UK, Germany, France and Italy on viruses and viral indicators. Dr K Henshilwood gave the following presentations for the UK : 'Bivalve shellfish and viruses, setting the scene' and 'Molecular methods for the detection of viruses in shellfish.'
It was generally agreed that the technology was not at a stage where viral standards could be proposed. The CRL undertook to prepare and distribute positive material in ring trials in the coming year.

11 Alternative viral indicators : there was a discussion on F+ bacteriophage as an alternative viral indicator.
Dr R Rangdale gave the following presentation : 'Use of alternative viral indicators' and Bill Dore presented the following : 'Research into viral elimination during depuration.'
The CRL agreed to progress a ring trial using F+ bacteriophage.

12 Microbiological criteria for food : there was discussion on the proposals of the microbiological criteria for food on the virus and viral indicator aspects. Bill Dore gave the following presentation for the CRL : 'Microbiological criteria for shellfish (viral aspects).' Most NRLs supported the contention that identifying a three year period in which to consider criteria was appropriate in order to drive developments forward. There was general agreement to support the introduction of F+ bacteriophage as a process criterion.

13 Marine vibrios : the CRL proposed that total *V.parahaemolyticus* be used as a process criterion for cooked crustacea and molluscs. Dr R Lee gave the following presentation: 'Vibrios pathogenic for humans.' Due to time constraints the discussion was suspended.

14 Resolutions : resolutions had been identified during each part of the meeting and twenty-one were finally agreed as follows:-

14.1 There was recognition by the NRLs of the progress in establishing the CRL/NRL network but there was need to develop and implement work programmes to ensure compliance with requirements of Council Decision 1999/313/EEC as soon as possible.

14.2 The NRLs agreed to build on the scientific and technical liaison between members of the reference laboratory network initiated at the workshop.

14.3 It was agreed that the method for shellfish examination as recommended by the CRL (Donovan, *et al*, 1998*) should be the reference method for *E.coli* in live bivalve molluscs. However it was identified that formal validation of this method would need to be carried out.

14.4 The NRLs agreed that the CRL *E.coli* method should be proposed to ISO/CEN, by the CRL, as a method to cover the requirements of a 5x3 MPN test satisfactory for live bivalve molluscs.

14.5 The NRLs agreed that rapid methods for the detection of *E. coli* in shellfish should be discussed at the next meeting (May 2003).

14.6 The NRLs agreed with the proposal in the draft microbiological criteria proposals that the faecal coliform standard be deleted and *E.coli* be used as the sole faecal indicator bacterium for live bivalve molluscs.

14.7 The NRLs supported the use of the CRL Collaborative Shellfish EQA Scheme as the primary means of comparative bacteriological testing between NRLs and also between National Laboratories within each member state.

14.8 Further to Resolution 7 the NRLs recommended a pilot EQA distribution to the reference laboratory network and it was agreed that this would be organised by the EQA scheme collaborators within six months of this meeting (by November 2002).

14.9 The NRLs agreed that specific ring trials would be undertaken in addition to EQA participation but that any such trials should be deferred until the EQA has been implemented for NRLs.

14.10 The NRLs endorsed the need for compliance with Article 4(g) in Council Decision 1999/313/EEC in that the reference laboratories should have an appropriate system of quality assurance.

14.11 The NRLs agreed that a working group should be established to produce a guidance document for the microbiological monitoring of shellfish harvesting areas.

14.12 Further to the preparation of the guidance document the NRLs recommended that certain criteria relating to the microbiological monitoring of shellfish harvesting areas inter alia, sample size, sample frequency, conditions of transport, should be specified in the relevant Community legislation.

14.13 Those laboratories which do not currently have the ability to carry out analysis of shellfish for FRNA bacteriophage undertook to seek training and advice from the CRL and/or other competent NRLs within a period of one year of this meeting (May 2003).

14.14 It was agreed that there would be a target for NRLs who do not currently have the ability to carry out analysis of shellfish for FRNA bacteriophage to achieve competence within a period of one year of this meeting (May 2003).

14.15 It was agreed that a pilot ring trial for FRNA bacteriophage would be organised by the CRL, within eight months of this meeting, for those NRLs able to undertake this analysis (by January 2003).

14.16 Those laboratories which do not currently have the ability to carry out analysis of shellfish for viruses undertook to seek training and advice from the CRL and/or other competent NRLs within a period of one year of this meeting (May 2003).

14.17 It was agreed that there would be a target for NRLs who do not currently have the ability to carry out analysis of shellfish for viruses to achieve competence within a period of two years of this meeting (May 2004).

14.18 It was agreed that a pilot ring trial for Hepatitis A virus would be organised by the CRL, within six months of this meeting, for those NRLs able to undertake this analysis (by November 2002).

14.19 It was agreed that a pilot ring trial for Norwalk-like virus would be organised by the CRL, within one year of this meeting, for those NRLs able to undertake this analysis (by May 2003).

14.20 There was general agreement by NRLs that the process performance criterion for depuration using FRNA bacteriophage as given in the draft microbiological criteria document dated 26 March 2002 was acceptable.

14.21 It was agreed that future workshops of the laboratory network should be held on an annual basis and that the next meeting would be on May 6-8 2003.

*Donovan T D, Gallacher S, Andrews N J, Greenwood M H, Graham J, Russell, J E, Roberts D and Lee R (1998) Modification of the standard UK method for the enumeration of *Escherichia coli* in live bivalve molluscs. *Communicable Disease and Public Health* **1,** 188-196.

Glossary for resolutions

CRL - Community Reference Laboratory
CEN - Comité Européen Normalisation (European Committee for Standardization)
EQA - External Quality Assessment
FRNA bacteriophage - male-specific ribonucleic acid bacteriophage
ISO - International Organisation for Standardisation
MPN - Most Probable Number
NRL - National Reference Laboratory

APPENDIX 2

Results of the Pilot Ring Trial among NRLs for detection of Hepatitis A Virus, 2002

Lab ID	1. A	2. B	3. C	4. D	5. A	6. B	7. C	8. D	Comments
NRL001	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	
NRL002	x	x	x	x	x	x	x	x	
NRL003	**-ve**	**+ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**	**-ve**	**Inconsistency found between the B samples**
NRL004	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**Nested PCR B pos. after PCR 1 and 2 D pos. after PCR 2**
NRL005	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	
NRL006*	--	--	--	--	--	--	--	--	
NRL007	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	
NRL008	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	
NRL009	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**B gave stronger bands than D**
NRL010*	--	--	--	--	--	--	--	--	
NRL011*	--	--	--	--	--	--	--	--	
NRL012*	--	--	--	--	--	--	--	--	
NRL013*	--	--	--	--	--	--	--	--	
NRL014	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**B more intense than D on agarose gel**

*These laboratories did not take part in the ring trial.

1 Comments

1.1 NRL001 - the samples were not frozen on arrival at the laboratory,

1.2 NRL002 - the samples were not frozen on arrival at the laboratory. The CRL did not receive any results for this lab.

1.3 NRL004 - the samples were not frozen on arrival at the laboratory.

2 Intended results

2.1 The four culture sample contents were as follows:
2.2 VIAL A - negative material, VIAL B - positive material: neat,
2.3 VIAL C - negative material, VIAL D - positive material: -4 dilution

3 Summary

3.1 Six of the nine participating NRLs analysed the material correctly.
3.2 One of the laboratories (NRL007) reported positives in the samples, which contained virus, and also in two samples to which no virus had been added.
3.3 One of the laboratories (NRL003) did not detect virus in the sample with the lower dilution of virus.
3.4 Primers from various regions of the HAV genome were used. One NRL (NRL009) reports less sensitive results with primers from the VP1 region compared to primers from the NCR region.

APPENDIX 3

Results of the Pilot Ring Trial (EQA) among NRLs for Detection of E.coli and Salmonella, 2002

SHELLFISH SCHEME DISTRIBUTION SF013

SAMPLES SF0034 and SF0035

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

Data analysed by: Heena Shah, Nick Andrews and Julie E. Russell
Report compiled by: Julie E. Russell and Dr Ron Lee

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.

Contact Details

Tel: 020 8200 4400, Int + 44 20 8200 4400
Fax: 020 8200 8264, Int + 44 20 8200 8264
e-mail: foodeqa@phls.nhs.uk

PHLS

Julie Russell	Ext. 4119	Scheme Co-ordinator
Prof. Eric Bolton	Ext. 4117	Scheme Consultant
Nick Andrews	Ext. 4419	Statistician

CEFAS

Dr Ron Lee	01305 206656

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

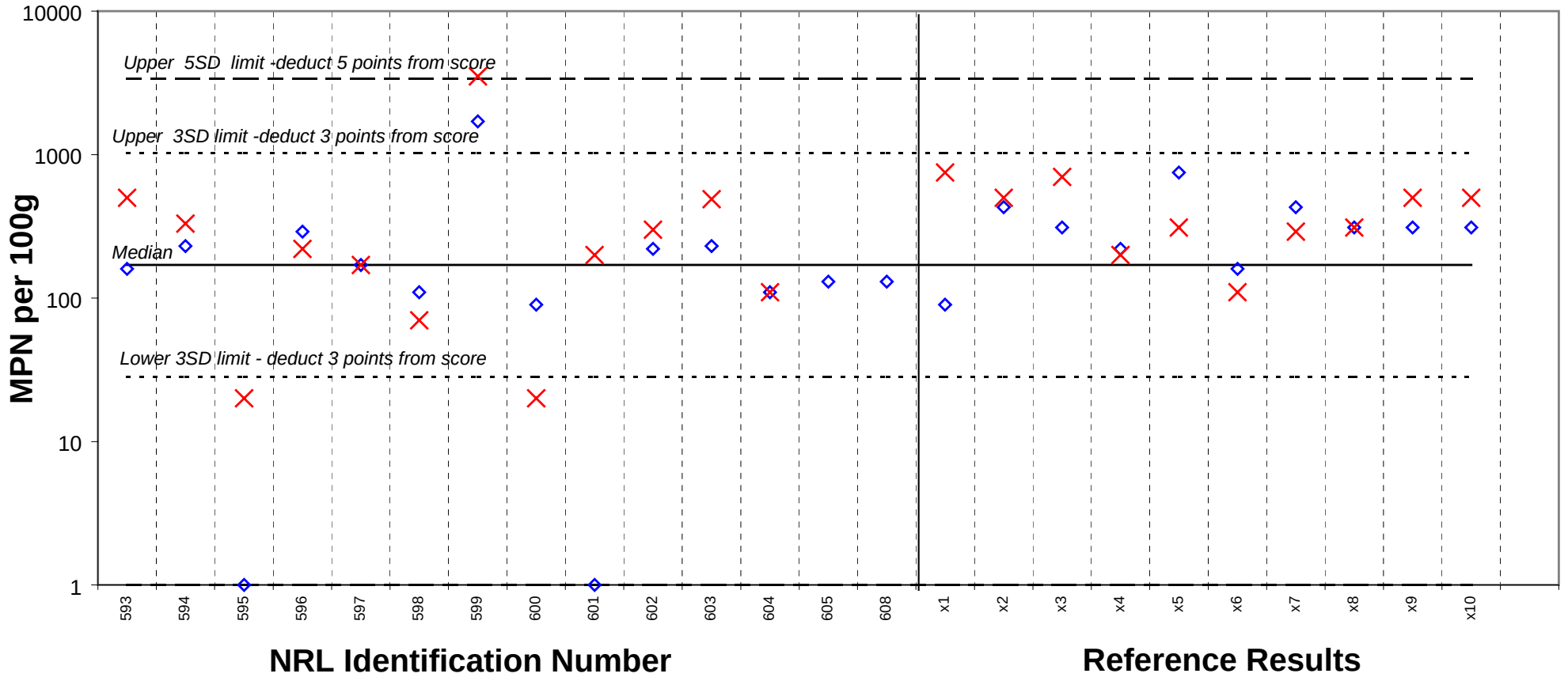
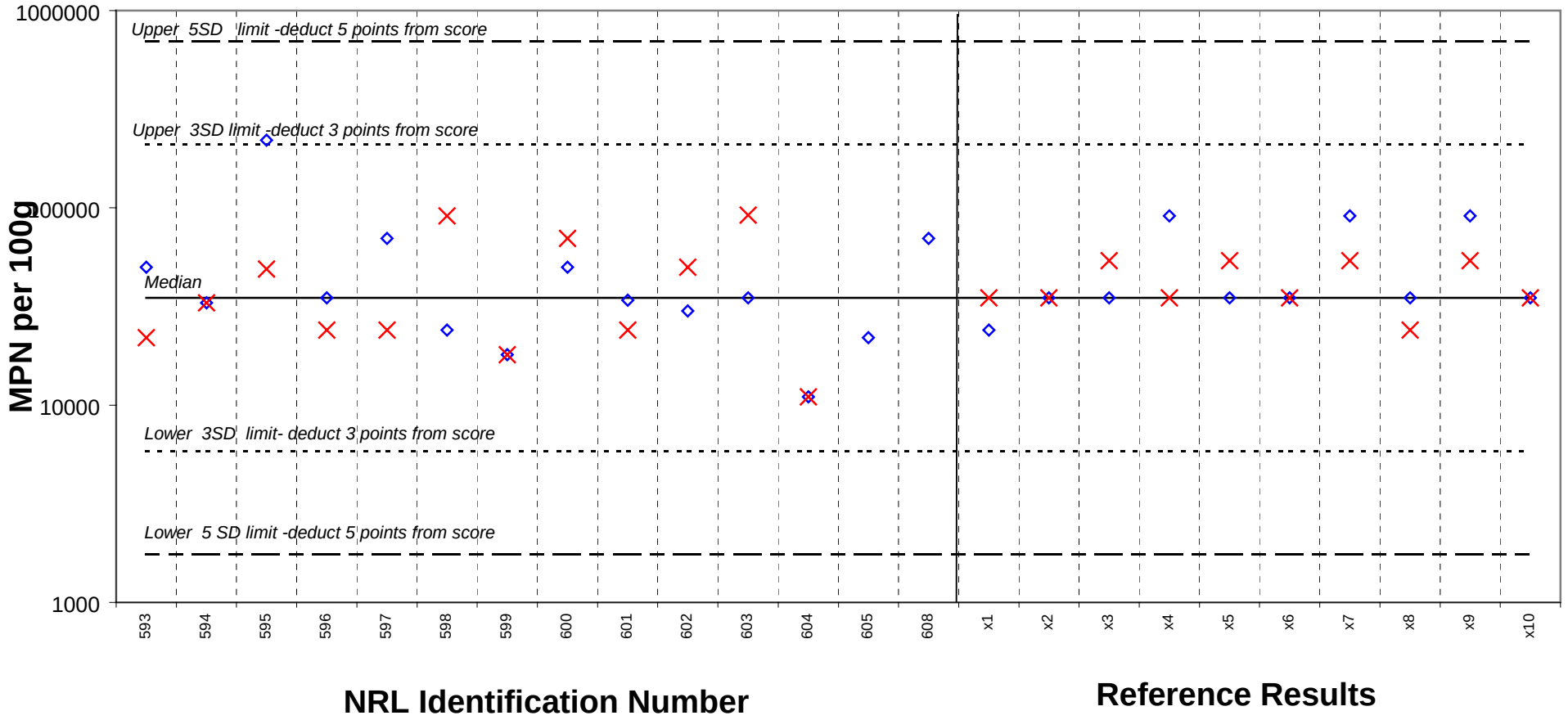
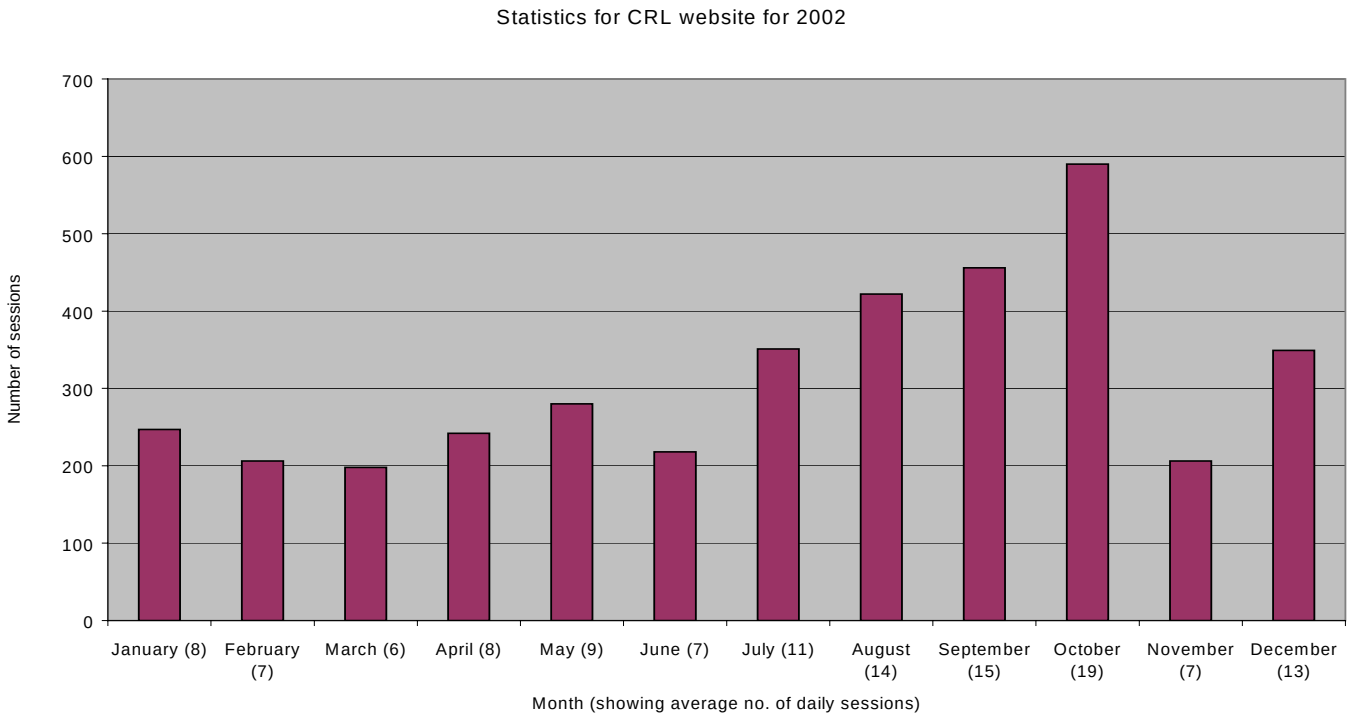


Fig 2: SF0035 MPN Results



Appendix 5

CRL Website : User Statistics for 2002



The graph shows the number of sessions recorded on the website per month with the average number of daily sessions shown in brackets after each month. A session reports individual users for any given time interval. Sessions are tracked per IP address and must register at least one hit to be counted. (A hit is considered to be any request for data such as a web page, bitmap, CGI gateway or file).