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

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

Technical Report for Calendar Year 2005

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Legal functions and duties

The functions and duties of the CRL are specified in Article 32 of Council Regulation (EC) 882/2004 (Official Journal of the European Communities No L165).

Introduction

The work programme for the CRL for 2005 was approved by the European Commission in December 2004. This report details consequential activities of the CRL according to the work programme 2005 (Annex I), additional tasks described under the resolutions of the 4th workshop of microbiological NRLs held in Nantes 2005 (Annex II) and other responsibilities outlined in Council Regulation (EC) 882/2004 for the calendar year 2005.

1. Scientific advice and support

To the European Commission.

The CRL has chaired the expert working group on microbiological monitoring of harvesting areas, the primary output of which was the draft Good Practice Guide for the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas. In addition the CRL provided presentations on microbiological monitoring and depuration criteria to the expert working group of Member States in June 2005. Scientific assistance has been provided to the Food and Veterinary Office through missions to Chile, South Korea and South Africa. The CRL has supplied specific advice to a number of MS Competent Authorities, NRLs and third country institutes, including the Canadian

Shellfish Sanitation Programme and New Zealand government. Notably on validation of alternative rapid methods, relaying of class B bivalves to class A areas, equivalence of the EU and USFDA approach to sampling and verification of results, and analytical tolerances in microbiological analyses. A number of method specific queries were received from NRLs and in country testing laboratories relating to initial sample preparation, uncertainty of measurement in the 3x5MPN for *E. coli* enumeration, and various aspects of virus detection in bivalve molluscs. A full register of advice provided by the CRL is available from the CRL co-ordinator on request.

Other Scientific activities

The CRL has been actively involved with ISO/CEN. Specifically, in chairing the CEN/TC 275/WG6/TAG4 expert working group on viruses in foods, including bivalve molluscs, representation at the annual ISO meeting in Warsaw, June and in participation in the ISO SC9 working group on method validation. The CRL participated for the first time in the biennial Interstate State Sanitation Conference (ISSC) and presented CRL research and proficiency testing activities at the preceding ICMSS intregrated science and policy workshop in Alabama, US. In 2005 CRL staff presented research a number of high profile international scientific conferences including the Coastal ERA-NET, Water Quality in Shellfish Growing Areas conference in Tarragona, Spain, the Camden and Chorleywood International Food Microbiology Conference, U.K, 2nd Seafood plus conference in Granville, France and Vibrio 2005, Ghent, Belgium. CRL research and progress on viral method standardization was also presented at the UK Water Virology Research Meeting at the UK Health Protection Agency.

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 as the primary means of dissemination of information (www.crlcefas.org). The web-site provides technical information and guidance for interested parties. The activities page documents events taking place at the CRL and invites relevant laboratory participation. The website is very well used by NRLs and others and is a successful and efficient means of disseminating information. Users must register and log in to access data held in the information centre (downloadable files and other data). In

2005 the CRL website was expanded to incorporate a 'welcome page' to alert users to newly posted information in this section. User statistics for the CRL website for 2005 are given in the graph at Annex III. Application of website statisitics software (T-Stats) demonstrate that the reports section within the "Information Centre" was most frequently viewed, representing an average of 16% of the total session usage over the year. Currently there are 208 registered users representing a 32% increase from 2004.

CRL workshop March 2005, IFREMER, Nantes, France

The IFREMER Institute, Nantes, France hosted the fourth workshop of European NRLs on 15-17 March, 2005. NRL experts from 18 Member States, 2 accession countries and 2 members of ETFA attended. The meeting was very productive, with knowledgeable, vigorous scientific discussion on a number of important and varied diverse issues relating to bivalve shellfish sanitation. Eleven formal resolutions were passed by the meeting. The resolutions of the workshop, associated discussion documents and presentations given by delegates were disseminated via the CRL website. The resolutions of the meeting are attached as Annex II of this document.

Training and technical assistance

Formal training workshops

A three-day formal training workshop in detection of norovirus and hepatitis A was organised at the CRL in February 2005. Thirteen delegates attended from 11 NRLs. The workshop was predominantly aimed at newer Member State and accession country NRLs without extensive pre-existing experience of viral methods. Attendees were provided with hands-on experience of methods to detect norovirus and hepatitis A from environmentally contaminated bivalve shellfish using real-time and nested PCR. The training programme is included as Annex IV of this report.

Ad hoc training

Additional *ad hoc* training was provided on a number of occasions in the form of specific requests for standard operating procedures, method review or other supporting information. The CRL provided dedicated technical training to staff from NRL Ireland, NRL Netherlands and, through TAIEX to visiting scientists from the Cyprus on *E. coli* and FRNA bacteriophage enumeration, *Salmonella* spp. detection and methods to enumerate human pathogenic *Vibrio* spp. and norovirus/hepatitis A detection.

3. Proficiency testing and quality assurance

Participation of Member State NRLs, accession, and third countries in CRL organised ring trials in 2005 is summarised in Annex V. Proficiency testing (ring trial reports) summarised below are presented in Annexes VI, VII, VIII and IX. All reports are available as public domain documents on the CRL website.

E.coli / *Salmonella* External Quality Assurance Scheme (EQA)

The CRL collaborates with the UK Health Protection Agency (HPA) on the application of a Shellfish External Quality Assurance (EQA) Scheme for proficiency testing among laboratories analysing bivalve molluscan shellfish. The Shellfish EQA scheme is targeted at analysis of the statutory determinants *E. coli* and *Salmonella* spp. in bivalve molluscan shellfish and is the primary means of ensuring comparative performance by NRLs. Three EQA distributions between the March 2005 workshop and the end of February 2006 are covered in the report presented in Annex VI. Under the rules of the CRL/HPA EQA scheme this enables a full performance assessment of participating NRLs over three consecutive distributions.

The uptake of NRLs in the CRL/HPA Shellfish scheme is very good with 20 NRL participants registered as of January 1 2005. Taken as a whole participation and performance of NRLs in the CRL/HPA Shellfish scheme is good with all designated MS NRLs, excepting Latvia and Poland, now registered. Eighteen NRLs returned results for three distributions and were thus subject to a full performance assessment.

Five laboratories, subject to full performance assessment over the three consecutive distributions in 2005/6, failed to achieve cumulative scores of >70%. Failures were generally attributable to non-return of results. All other laboratories scored >78% with eight NRLs returning scores of 100%. Trouble-shooting information was added to the individual laboratory reports in 2005 comprising a checklist of recommended preventive measures and corrective actions. It was strongly recommended that laboratories introduced mechanisms into their quality systems to prevent EQA failure due to non-return of results.

Vibrio parahaemolyticus ring trial

At the 4th annual workshop of NRLs Nantes 2005 it was resolved that the CRL should organise a pilot ring trial for detection of enterotoxigenic *V. parahaemolyticus.* It was the intent, that the ring trial, would generate information of comparative efficacy of in-

house largely molecular based laboratory developed procedures, and in the absence of other formal external quality assessment schemes provide laboratories with limited data in support of internal quality procedures. The full report of laboratory performance is included as Annex VII of this document.

In summary, most laboratories correctly identified *V. parahaemolyticus* to the species level and identified *Vibrio* spp. as non- *V. parahaemolyticus* correctly. Laboratories were less successful with respect to the overall designation of strains containing the putative pathogenicity markers (thermostable direct (*tdh*) and direct related haemolysins (*trh*)), with just 40% returning the intended result for both markers. Several laboratories produced highly variable results for this phase of the ring trial and it is recommended that method standardisation for detection of potentially pathogenic strains of *V. parahaemolyticus* is progressed prior to further consideration of incorporation of this determinant in the context of official controls.

FRNA bacteriophage ring trials

FRNA bacteriophage proficiency testing was scaled down in 2005 to reflect the reduced emphasis on this determinant following the removal of the proposed FRNA bacteriophage process criterion from the Community hygiene legislation (Commission Regulation (EC) 2073/2005). FRNA bacteriophage is however still used throughout the EU by a number of NRLs as complementary assay in the assessment of BMS sanitary quality for research purposes. In some of those laboratories the ISO 10705-1, as modified for use with bivalve mollusc tissue, has been incorporated into the suite of ISO 17025 accredited methods. Outside of the CRL FRNA ring trial scheme, no external quality assessment (EQA) scheme exists for enumeration of FRNA bacteriophage.

Further to the minutes of the 4th workshop of NRLs, the CRL organized a single FRNA ring trial distribution in 2005. The summary report of NRL performance in this ring trial is included as Annex VIII of this document

Uptake of the fourth FRNA ring trial was not as high as that in 2004, with just five MS NRLs returning results. The distribution was extended to MS in country testing laboratories although again the uptake was limited with just one NRLs requesting additional material. The results of this demonstrate that participating NRLs can run the FRNA bacteriophage assay reproducibly and consistently. All NRL laboratories

obtained results within the expected range. The existence of standard methods (ISO 10705-1) and the training exercises previously performed have helped to achieve this good performance.

Norovirus and Hepatitis A ring trial

At the 4th annual workshop of NRLs Nantes 2005 it was resolved that the CRL should organise further ring trials for both NoV and Hepatitis A virus (HAV) and that these should include analyses of live bivalve molluscs. In the continued absence of internationally or nationally accepted standard or procedures of equivalent standing analyses should be undertaken using laboratory in house procedures. Twenty seven participants registered for the ring trial (17 MS NRLs, 1 accession country, and 9 third countries including laboratories in the U.S., New Zealand, Canada, South Korea, Hong Kong and Chile). This represented a greater than 70% increase in uptake of the scheme from 2004. The full report of laboratory performance is included as Annex IX of this document.

The data generated from the 2005 ring trial identified a number of key issues and recommendations with respect to the application of Norovirus and hepatitis A detection methods. In brief, it was clear that the methods of analysis utilised by laboratories were numerous and diverse. For example at least 29 different viral nucleic acid extraction methods were in use. However, the increase in numbers of laboratories generating real-time quantitative data was judged as encouraging and greatly enhanced the ability to assess both method and laboratory performance. Laboratory constructed samples irrespective of titre were effectively analysed using a number of approaches and most methods successfully detected high titre virus (GII norovirus) in bivalve shellfish matrix. However, low titre virus (GI and GII) in bivalve shellfish (representative of natural levels of contamination, typically found in harvesting areas) presented a significant challenge to laboratories. It was concluded that standardisation of reference material and methods are is required to enable accurate method assessment and between laboratory performance.

Reference materials

On request the CRL has provided reference materials in the form of Nov (GI and GII) (faecal material), HAV (tissue culture), *V. parahaemolyticus* TDH, TRH –ve and +ve strains, *V. vulnificus*, *V. cholerae* non 01/0139, *Salmonella* Typhimurium (WG49) to most NRLs in the network.

4. Confirmatory testing

During 2005 the CRL has maintained it's accreditation to ISO 17025 (EN 45000) for the following methods: 'Examination of shellfish for *Salmonella* spp. other than *Salmonella* Typhi', 'Enumeration of *Escherichia coli* in molluscan bivalve shellfish'. The scope of accreditation was extended to include 'Detection of *Vibrio parahaemolyticus* in bivalve molluscan shellfish' in November 2005. Accreditation of the FRNA bacteriophage enumeration assay was suspended in xx 2005.

The CRL has maintained its laboratory testing capabilities for norovirus and hepatitis A virus contamination of bivalve molluscs. SOPs for all aspects of viral analyses including preparation and use of control materials have been elaborated. Accreditation to ISO 17025 for the detection of norovirus using real-time PCR is pending following submission of SOPs and initial encouraging discussion with the United Kingdom Accreditation Service (UKAS) in November 2005.

5. Development of analytical methods

The CRL has also continued to undertake practical work in support of the ongoing revision of the ISO standard (ISO/TS 21872-2) for the detection of vibrios, other than *V. cholerae* and *V. parahaemolyticus*. Specifically, with investigations into the use of selective and semi-selective solid media for the enhanced recovery of *V. vulnificus.* Further standardisation of the Taqman assay for quantitation of NoV and HAV have been made throughout 2005. Principally these have comprised establishing control limits for assessment of extraction efficiencies, evaluation of one-step and two-PCR procedures and preliminary use of ssRNA constructs for use as internal RT-PCR/PCR controls.

Sustained progress has been made in the production of stable, non-friable, easily transportable reference materials for use as controls within assays and as proficiency testing materials. Previous work utilising carboxymethylcellulose/trehalose disks (LENTICULES) as surrogate virus carriers has been continued. Norovirus LENTICULES have been produced and initial experiments indicate good viral recovery and between LENTICULE repeatability (using quantitative RT-PCR) can be achieved using this approach. Work on LENTICULES is ongoing with focus on stability, production of high and low concentration material and hepatitis A.

Annex I- WORK PROGRAMME FOR THE CRL FOR BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS, 2005

LEGAL FUNCTIONS AND DUTIES

The functions and duties of the CRL are specified in Articles 4 and 5 of Council Decision 1999/313/EC (Official Journal of the European Communities No L 120 of 8.5.1999).

In the 2005 work programme year 25 Member States and 3 candidate countries are considered eligible for CRL assistance and invited to participate in CRL organised training programmes, ring trials/external quality assessments schemes etc. The full integration into the European Union of new Member States continues to be a priority area, and is facilitated via the provision of additional training and assistance.

WORK PROGRAMME, 2005

	Duration
1. Scientific advice and support	
1.1. Provide scientific assistance to DG Sanco towards further development of EU Community food legislation as it impacts bivalve molluscs (advice, participation in working groups, etc). For 2005 this will include completion of the work of the Commission WG on microbiological monitoring of shellfish harvesting areas.	• 30 days
1.2. Assist DG Sanco with U.S. equivalence negotiations relating to bivalve molluscs and with specialist assistance in relation to veterinary inspections of MS, Candidate Countries and Third Countries (on request). Assist DG TAIEX with targeted assistance towards training of new EU MS NRLs.	• 10 days
1.3. Prepare for and participate in EU and International scientific fora/committees relating to contaminants and contamination of bivalve molluscs (EU SVC, WHO/FAO, ISO/CEN, etc). During 2005 the CRL will continue to actively participate in ISO/CEN working groups to progress methods for *E.coli*, Salmonella and marine vibrios. The CRL will also be chairing a CEN working group on methods for detection of viruses in foodstuffs (including bivalve molluscs). The CRL also anticipates participation in follow up activities to the WHO workshop on Quality Management and Shellfish Safety.	• 35 days
1.4. Undertake CRL missions in support of above. During 2005 missions are foreseen in relation to the annual meetings of ISO and CEN (up to 2 missions, venues to be confirmed); the CEN, TAG4 working group on	• 20 days • €5000 (travel costs)

Viruses in Food (2 missions); participation in the 2005 Interstate Sanitation Conference (ISSC) Biennial Meeting in Alabama U.S.; the IWA Health-Related Water Microbiology Conference, September 2005, UK. In addition up to 3 European missions are foreseen in support of NRLs and DG Sanco activities.	
2. Co-ordination of activities of NRL network and provision of technical assistance and training	
2.1. Participate in annual CRL Directors co-ordination meeting and other CRL co-ordination meetings/workshops as appropriate	• 5 days
2.2 Organise, host, and participate in, fourth annual NRL workshop and produce resolutions and other workshop outputs (March 2005, Nantes). Including CRL administrative assistance.	• 40 days
2.3 Undertake CRL activities and commitments agreed in resolutions of 2004 and 2005 annual workshops (as posted on www.crlcefas.org).	• As agreed in resolutions (up to 100 days)
2.4 Maintenance of CRL laboratory competence and expertise on analytical methods for monitoring bacteriological contaminants of bivalve molluscs (*E.coli*, Salmonella, FRNA bacteriophage, marine vibrios). Supply information and specialist advice to NRLs (particularly new MS NRLs and CCs), testing laboratories, and third county laboratories. Including assistance on implementation and accreditation to ISO17025 of methods by development and provision of CRL SOPs and transfer of other technical information.	• 70 days
2.5 Maintenance of CRL laboratory competence and expertise on analytical methods for monitoring virological contaminants of bivalve molluscs (Norovirus and hepatitis A virus). Supply information and specialist advice to NRLs, testing laboratories, and third county laboratories. Including assistance on implementation and accreditation of methods by development and provision of CRL SOPs and transfer of other technical information.	• 70 days
2.6 Provide specialist training and/or training courses to NRLs and CC NRLs in relation to analysis of *E.coli*, *Salmonella*, *Vibrio* spp., FRNA bacteriophage, Norovirus and hepatitis A virus.	• 40 days • €1000
2.7 Develop with NRLs and other interested parties improved means for exchange of epidemiological information on outbreaks of illness associated with molluscan shellfish.	• 20 days

2.8	Maintenance and further development of CRL website (www.crlcefas.org) as primary means of dissemination of information to NRLs and others. Incorporate "welcome page" to highlight latest new documents and important information, for more efficient notification.	• 30 days • €6720 (sub contracting costs)
3	**Ring trials, comparative testing and quality assurance**	
3.1	Continue use of the CRL/HPA shellfish EQA scheme as the primary means of comparative testing among NRLs for *E.coli* and *Salmonella* in bivalve molluscs. To establish widespread uptake of the scheme amongst MS (see resolution 8 of 3rd workshop) analyse results and produce report and recommendations.	• 20 days
3.2	Undertake Norovirus and hepatitis A ring trials (see resolution 32 of 3rd workshop) with 3 planned distributions during 2004/05. Analyse results and produce report and recommendations (by March 05).	• 40 days • €2000
3.3	Update dossier of virus detection methods used in ring trials (including quantitative aspects) to assist in standardisation of virus methods under CEN WG (see resolutions 33 and 35 of 3rd workshop)	• 5 days
3.4	Further to the above undertake virus method development workshop with invited participation from targeted key laboratories to progress method standardisation and to feed into the CEN procedure.	• 15 days • €1500
3.5	Undertake FRNA bacteriophage ring trials (see resolutions 12 and 13 of 3rd workshop) with 2 planned distributions during 2004/05. Extend provision of ring trial material, methods of analysis etc to national testing laboratories if required by NRLs. Analyse results and produce report and recommendations (by March 05).	• 25 days • €1000
3.6	Delivery of ring trial programme for March– Dec 2005 as agreed in Resolutions of the 4th workshop in March 2005 (may include among further ring trials for *E.coli* in shellfish homogenate/whole animals, FRNA bacteriophage, *Vibrio* spp. HAV, and Norovirus).	• 42 days • €3000 • €11625 (sample transport)
4	**Confirmatory testing**	
4.1	Contribution to costs for maintenance of CRL ISO 17025 (EN 45000) accreditation for tests for *E.coli*, *Salmonella* spp, and FRNA bacteriophage in bivalve molluscs.	• 20 days • €3000
4.2	Contribution to costs for maintenance of CRL capability to perform analysis of Norovirus and hepatitis	• 25 days • €4000

A virus in bivalve molluscs.

4.3	Contribution to costs for maintenance of CRL capability to perform analysis for marine vibrios in bivalve molluscs.	• 10 days • €2000
4.3	Performance of above tests on outbreak material or on occasion of disputed test results (on request of DG Sanco).	• Costs included in above

5 Development of analytical methods (undertaken at CRL)

5.1	Co-ordination and participation (as appropriate) in laboratory studies to support progression of the CRL-recommended reference method for *E. coli* as an ISO standard method	• 20 days • €2000
5.2	In the absence of appropriate International standards, development of agreed CRL/NRL criteria and procedures for validation and acceptance of alternative methods of analysis (see resolution 7 of 3rd workshop). During 05 this focuses in particular on *E.coli* analysis.	• 25 days
5.3	Continue to co-ordinate and participate in laboratory studies in support of the proposed horizontal ISO methods for detection of *Vibrio* spp. of human significance (ISO 21872 parts 1 and 2).	• 20 days • €1000
5.4	Further development of stable hepatitis A virus, Norovirus and FRNA bacteriophage, standard reference materials to facilitate future quality assurance and ring trials, with particular focus on the production and evaluation of biological lenticules.	• 80 days • €4500
5.5	Participation in CEN co-ordinated laboratory validation studies in support of the development of a standard CEN/ISO method for the detection of viruses in food.	• 65 days • €2000

Annex II- Resolutions of the 4th workshop of Microbiological NRLs for Bivalve Molluscs, Nantes, France, 15-17th March, 2005

Proficiency testing

1. NRLs agreed to maintain commitment to the CRL/HPA EQA as the primary means of proficiency testing for *E.coli* and *Salmonella* spp. amongst the NRL network.

2. NRLs reaffirmed commitment (by 5th Workshop) to establishing proficiency testing for statutory determinants (*E.coli* and *Salmonella* spp.) amongst national laboratories conducting testing for classification of production areas.

3. The workshop agreed that the CRL would conduct a *V. parahaemolyticus* ring trial for detection, enumeration and determination of pathogenicity principles using the methods of the laboratories own choice. The CRL will invite expressions of interest for one distribution planned for autumn 2005.

4. The workshop agreed that the CRL should organise further ring trials for detection of NoV/HAV and that this should include shellfish analyses. The CRL will invite expressions of interest for two distributions the first focusing on analysis of NoV/HAV in faecal samples/tissue culture material in September 2005 and the second comprising whole shellfish in winter 2005/6.

Risk assessment

5. The workshop identified the difficulty of interpretation of PCR positive results in shellfish. The CRL agreed to make proposals at the next workshop on the design of studies to progress a risk assessment for viruses in shellfish in consultation with relevant risk assessment experts.

Commercial practices

6. The workshop identified that trade practices relating to trans-shipment of B and C shellfish for final processing in other Member States lacked clarity with regard to the controls actually applied and acknowledged that this could have consumer health implications. NRLs agreed to investigate this issue and the CRL would circulate a protocol for an audit with the results to be reported at the next workshop.

7. The workshop discussed the remit of NRLs in relation to industry “own-checks” and identified that the EU legislation was not precise on this point. The CRL agreed to seek clarification from the Commission and feedback to the network with a view to further discussion at the next workshop.

8. The workshop discussed the need for establishment of a minimum specified duration for the commercial depuration process in order to ensure compliance with statutory bacteriological standards. The workshop resolved to inform the Commission via the CRL of their agreement on this need and for a working group to determine the minimum period and the optimum physiological conditions for each species in order to define best practice with regard to commercial depuration as it effects bacterial contamination.

Monitoring of harvesting areas

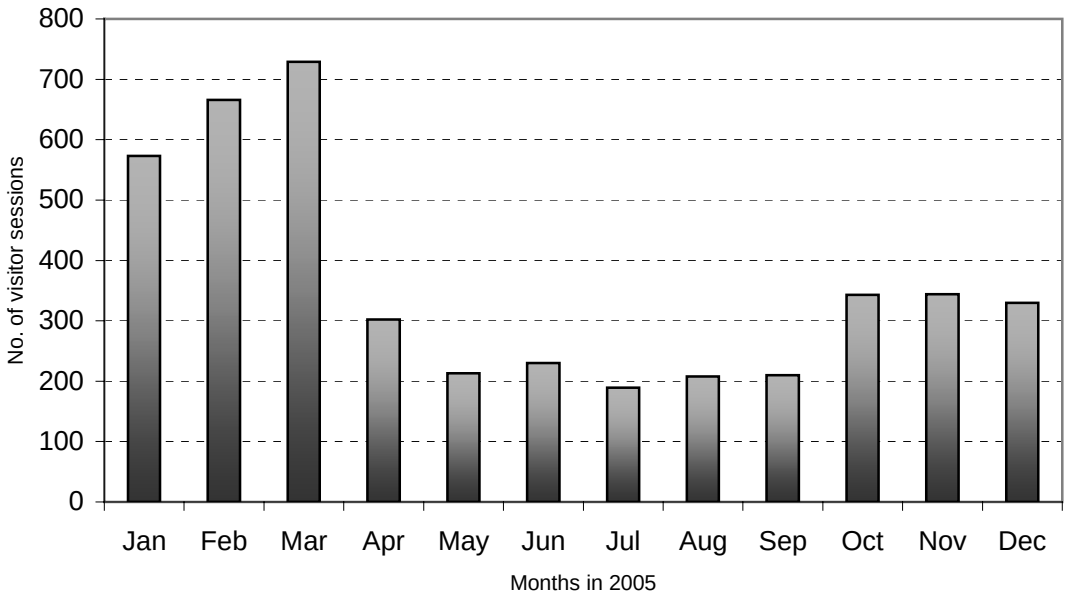
9. NRLs endorsed the draft output of the microbiological monitoring of bivalve mollusc harvesting areas working group and noted the timetable for comment on the review document of 25th March 2005. The draft good practice guide is scheduled for distribution to NRLs for comment in June 2005.

10. In order to finalise the Microbiological Monitoring Good Practice Guide the WG requested clarification of the need to monitor *Salmonella* and *Listeria* in class A production areas as part of official control surveillance programmes. The CRL agreed to seek clarification from the Commission and to report back to NRLs.

Human health

11. The workshop acknowledged the importance of sharing information relating to bivalve mollusc related human health incidents and resolved to share information on this, and related epidemiological information, in a dedicated session at the annual workshop.

Annex III-CRL website visitor statistics for 2005 (www.crlcefas.org)



The graph shows the number of individual visitor sessions recorded on the website per month during 2005. 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 included. A hit is defined as any request for data such as a web page, bitmap, CGI gateway or file.

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PRACTICAL TRAINING WORKSHOP IN METHODS TO DETECT NOROVIRUS AND HEPATITIS A, FROM ENVIRONMENTALLY CONTAMINATED SHELLFISH- NESTED PCR AND REAL-TIME APPROACHES.

DAY 1 - Tuesday 15 February

09:30 Conference room (Room 107A)

- Introduction and course content – Dr Kathy Henshilwood
- Development of viral standards- Dr David Lees
- Development of molecular methods for the detection of Norovirus - Dr James Lowther

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10:45 Coffee break

11:15 Microbiology laboratory (Room 210)

- Practical session on virus extraction/concentration from the digestive gland of the oysters (*Crassostrea gigas*).
  - *Gland excision*
  - *Proteinase K treatment*

12:00 CATIII Laboratory (Room 212)

- Practical session. Modified boom procedure.
  - *Nucleic acid extraction*
  - *Precipitation of RNA*

13:30 Buffet style lunch (Foyer)

14:15 Conference Room (107A)

- Degenerate primers and sequencing – Dr Kathy Henshilwood

15:45 Tea break

16:00 Conference Room (107A)

- Tour of the laboratory

17:30 Close

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PRACTICAL TRAINING WORKSHOP IN METHODS TO DETECT NOROVIRUS AND HEPATITIS A, FROM ENVIRONMENTALLY CONTAMINATED SHELLFISH- NESTED RT-PCR AND REAL-TIME APPROACHES.

DAY 2 - Wednesday 16 February

09:00 CATIII (Room 212)

- Practical session on reverse transcription.
  - *RT1*
  - *RT2*

11:15 CATIII (Room 212)

- Practical session on cDNA preparation for Real-Time PCR and nested protocols
  - *cDNA pooling and aliquoting*
  - *First round PCR*

12:30 Buffet style lunch (Foyer)

13:30 CATIII (Room 212)

- Practical session on Real Time preparation.
  - *Master Mix preparation*
  - *Sample Loading*.

15:00 PCR Laboratory (Room 213)

- *Practical session on set up and analysis of samples on the ABI7000 Real-Time PCR machine*

16:00 Tea break

16:30 PCR Laboratory (Room 213)

- Demonstration on sequencing, outputs and analysis.
- Strain identification
- Demonstration on 'Blast' searching undefined sequences.

17:30 PCR Laboratory (Room 213)

- Practical session. Nested PCR

18:00 Close

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PRACTICAL TRAINING WORKSHOP IN METHODS TO DETECT NOROVIRUS AND HEPATITIS A, FROM ENVIRONMENTALLY CONTAMINATED SHELLFISH- NESTED PCR AND REAL-TIME APPROACHES.

DAY 3 - Thursday 17 February

09:00 PCR Laboratory (Room 213)

- Practical session. Gel electrophoresis.
  - *Gel preparation*
  - *Nested PCR products loading and running*

10:30 Coffee break

11:00 PCR Laboratory (Room 213)

- Practical session. Gel electrophoresis continued.
  - *Image analyses of electrophoresis gels*
  - *Interpretation and discussion of results*

11:30 PCR Laboratory (Room 213)

- Real Time PCR results.
  - *Setting analysis preferences*
  - *Analysis of data*
  - *Curve interpretation and discussion*

12:30 Buffet style lunch (Foyer)

13:30 Conference room (Room 107A)

- Round table discussion

15:00 Close

Annex V -Summary of participation amongst NRLs and others in CRL organised proficiency testing

CRL ring trial reference number	Ring trial description	Austria	Belgium and Luxembourg	Czech Republic	Denmark	Estonia	Finland	France	Germany	Greece	Ireland	Italy	Latvia	Lithuania	Netherlands	Poland	Portugal	Slovakia	Slovenia	Spain	Sweden	United Kingdom	Hungary	Bulgaria	Romania	Turkey	Iceland	Norway	United States	New Zealand	South Korea	Chile	Hong Kong	Canada
RT11a	FRNA bacteriophage, 2005	x	x	x	x	x	✓	✓	x	x	✓	x	x	x	✓	x	x	x	x	r	x	✓	x	x	x	x	x	x	x	x	x	x	x
RT13	*E. coli/Salmonella* EQA 2005-6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	r	✓	x	✓	✓	✓	✓	✓	✓	x	x	✓	x	x	✓	nk	nk	nk	nk	nk	nk
RT14	*V. parahaemolyticus* 2005	x	✓	✓	x	✓	✓	✓	✓	✓	x	x	✓	x	✓	✓	✓	✓	x	✓	x	✓	x	x	x	✓	x	x	✓	x	x	x	x	✓
RT15	Norovirus/Hepatitis A 2005	x	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	x	✓	x	x	r	x	x	✓	✓	✓	✓	✓	✓	✓

r- registered but did not return results by stated deadline
nk-no known