Resolutions of the 3rd Workshop of Microbiological NRLs, ISS Rome, April 2004

A workshop of the European National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs was held at the Istituto Superiore di Sanità, Rome, Italy, on 30th March – 1st April 2004. The workshop was hosted by ISS and the attendees comprised representatives from the European Commission (DG Sanco and the Food and Veterinary Office), the European Community Reference Laboratory (CRL - CEFAS, Weymouth, UK) and National Reference Laboratories (NRLs) from Austria, Belgium & Luxembourg, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, and United Kingdom. The workshop produced a number of resolutions to be disseminated in the public domain by meeting representatives and through the CRL website (www.crlcefas.org).

1. The workshop welcomed EU Accession Countries to the work of the laboratory network.

2. The CRL demonstrated the web forum on the CRL website (www.crlcefa.org) and its purpose to improve rapid dissemination of information, particularly concerning outbreaks of illness associated with bivalve shellfish. The NRLs agreed that it would be important to add e-mail contact addresses to NRL information on the website to facilitate the above and agreed to send e-mail addresses to the CRL by 1st June 2004

3. NRLs supported the development of the Donovan method as an ISO standard for shellfish but expressed concern that the data needed for other food matrices for progression of a horizontal method should not delay progress. The CRL agreed to write to ISO supporting the progression of the Donovan method as a vertical method for shellfish if such delays became apparent.

4. Further to the opportunity to comment on the current proposals for revision of Community food legislation the NRLs recommended to the Commission and Member States that Hygiene 3 (Official Controls Regulation – 2004/C48E/03) should include a reference method for *E.coli* testing in shellfish to mirror that found in the Microbiological Criteria for Foodstuffs (SANCO/4198/2001 rev 9).

5. Further to the above the NRLs recommended that the Donovan method (Donovan et al., 1998, Communicable Disease and Public Health, 1, 188-196) should be the stipulated reference method for *E.coli* analysis in bivalve molluscs.

6. Further to the above the NRLs recommended that the Hygiene 3 (Official Controls Regulations) should also include the possibility to use an alternative method for *E.coli* analysis that was validated according to accepted scientific principles.

7. Further to the above the NRLs agreed that it was necessary to define the appropriate validation criteria for acceptance of alternative methods. NRLs consider that the available ISO protocol ISO 16140 maybe too demanding for this purpose. The workshop asked CRL and NRL attendees of the April 2004 Palma meeting of ISO/CEN to raise this issue for discussion.

8. NRLs agreed to continue the CRL/HPA EQA programme, with 3 distributions per year, as the primary means of *E.coli* and Salmonella comparative testing among NRLs. All NRLs agreed to participate. Accession countries agreed to register with the scheme and to notify the CRL when this registration was complete. The CRL advised participants that the scheme operated on the basis of cost recovery and there was a charge for participation. NRLs were advised that reports are made on an anonymous basis.

9. The workshop consider the need for further ring trials on *E.coli*/Salmonella in naturally contaminated samples but recognised the logistic and sample difficulties. It was agreed to consider further ring trials on naturally contaminated samples at the next meeting following feedback on experiences from a ring trial in France to be conducted during 2004.

10. The workshop agreed that reports for the EQA, shellfish homogenate ring trial for *E.coli*, FRNA bacteriophage ring trials should be placed in the public domain on the CRL website. It was agreed that NRLs should advise the CRL of any factual inaccuracies in the reports by 1st June 2004 following which the reports would be placed on the CRL website. NRLs were reminded that ring trial reports are anonymous.

11. It was agreed that the NV ring trial report was in draft and NRLs needed to advise the CRL of methods used for analysis of ring trial part 3 (shellfish) and also of any factual inaccuracies in the report by 1st June 2004. The CRL would then place in the public domain on the CRL website.

12. Regarding FRNA bacteriophage analysis the NRLs considered that the ring trials had been a useful exercise and demonstrated that for consistent quality of analysis it was important to run the assay on a regular basis. The NRLs therefore agreed to continue the ring trials with 2 further distributions during 2004/5 during November and January. The CRL would invite participation in due course.

13. Further to the above the CRL agreed that it might be possible to include national laboratories in the bacteriophage ring trials if NRLs considered this important and depending on the numbers of laboratories interested. NRLs agreed to provide the CRL with further information for consideration.

14. NRLs agreed that it was essential that laboratories registering to participate in ring trials should ensure that they returned results or, if this was not possible, to inform the CRL of the problem and the reasons. Laboratories are reminded that ring trials are conducted and reported on an anonymous basis.

15. It was agreed that the questionnaire on Proficiency Testing (PT) by NRLs had been a useful exercise and that the results should be placed on the CRL website subject to comments on factual accuracy to be supplied to the CRL by 1st June 2004. With regard to part C the CRL advised, for clarification, that the questionnaire responses should also apply to processed bivalve mollusc but that a 'don't know' response could be lodged if appropriate. The CRL agreed to incorporate any refinement of responses in light of discussion at the workshop providing these were provided by 1st June 2004.

16. Discussion on the *E.coli* standards relevant to imported processed but not cooked (e.g. raw frozen) bivalve molluscs highlighted a lack of clarity on the current legislative requirements. The Commission agreed to reflect on this issue and provide clarification for NRLs.

17. The Commission and NRLs agreed that the current review of food hygiene legislation provided an opportunity to improve Community legislation and that NRL experts should work with their competent authority officials.

18. The questionnaire on PT by NRLs in national laboratories highlighted the current low level of activity by NRLs - for example only 4 countries were carrying out PT for laboratories carrying out official control monitoring of bivalve mollusc production areas.

19. Further to the above it was agreed that all national laboratories carrying out official control monitoring of bivalve mollusc production areas should participate in a proficiency testing programme. It was agreed that this applied to all EU Member States having bivalve mollusc production areas.

20. Further to the above it was noted that 8 NRLs reported plans to initiate PT within 2 years. It was agreed that NRLs should report on the performance of testing laboratories at the annual NRLs workshop. It was agreed to consider and agree the confidentiality status of these reports at the next workshop.

21. Further to above the CRL advised NRLs of the proposed JRC/CRL workshop on PT in October 2004 and invited expressions of interest in attending by 1st June 2004

22. The Commission FVO presented information to NRLs of the role of the FVO and problems found during missions. The FVO stressed the important role of experts on missions and invited NRL experts to advise the FVO if they had an interest to participate in missions. The FVO advised that the aim of the FVO was to have a balanced mission report reporting both positive aspects and deficiencies.

23. NRLs recognized the importance of the Microbiological Monitoring Working Group in developing a best practice guideline and were invited to provide information on classification practices and data handling in their Member States to the WG to inform its discussion. The WG particularly requested scientific data supporting such practices. The WG would feedback to NRLs at the next annual workshop.

24. The workshop considered current industry developments on the transport and retail holding of live bivalve molluscs in tanks of refrigerated seawater. The workshop considered that there were concerns about water quality and recontamination of molluscs using such systems. The Commission advised that re-emersion following leaving a dispatch center was not permitted in legislation and that it could be difficult to comply with requirements for dispatch centers in the retail setting (e.g. in a supermarket). Use of such systems for transport to the dispatch center is not forbidden but is foreseen that recontamination must be avoided –Directive 91/492/EEC states that ' live bivalve molluscs must not be re-immersed in water which could cause additional contamination between harvesting and landing'.

25. Regarding the results of research findings from the CRL and NRLs, the workshop endorsed the scientific opinion that there are problems with the application of current methods for detection, enumeration and identification of pathogenic vibrios. It is considered that the current situation is not satisfactory regarding official controls on imports.

26. Further to the above, the workshop agreed that it was important to make further progress on refinement of methods for vibrios to better reflect the public health significance of isolates. Consequently the CRL agreed to distribute to NRLs the protocols for the probe hybridization method by 1st May for labs to consider comparison with other methods in use.

27. Further to the above, and following consideration of the protocol, the NRLs agreed to indicate their interest in attending a workshop for vibrio methods (focusing on probe hybridization but also other methods). NRLs were to indicate their interest by 1st June with an aim of holding a workshop by the end of September 2004.

28. Following the workshop the CRL agreed to circulate proposals for a ring trial on vibrio's.

29. The workshop identified the lack of epidemiological information on vibrio outbreaks associated with seafood. To assist better dissemination of information, the CRL agreed to set up a 'vibrio outbreak' topic on the CRL forum website. NRLs and other interested parties were encouraged to register an interest on the website for receiving notifications of posts. The usefulness of this approach would be reviewed at the next workshop.

30. The workshop agreed that further to the application of improved methods, and also the acquisition of better epidemiological data, the "Opinion of the Scientific

Committee on Veterinary Measures relating to Public Health on *Vibrio vulnificus* and *Vibrio parahaemolyticus* (in raw and undercooked seafood)" should be reviewed.

31. The workshop discussed the criteria for vibrios in cooked crustaceans and bivalve molluscs in the proposed Regulations on Microbiological criteria for foodstuffs. The workshop recommended to the Commission that the data from the 'Coordinated programme for the Official Control of Foodstuffs for 2003' should be scrutinised before this aspect of the Regulation is finalised.

32. The pilot Norovirus ring trial was found to be a useful exercise, particularly for laboratories new to the analysis, and the NRLs agreed that the CRL should conduct further similar ring trials but incorporating also HAV analysis. Sample would be coded and could contain both, either or neither viruses. The CRL agreed to develop proposals and invite participation with the aim of 3 distributions during 2004/5 in July, October, and January. Participation continued to be voluntary however the workshop agreed that it was important that laboratories committing to participate completed the series of distributions and reported their results.

33. Further to the above the NRLs agreed that methods used for analysis continued to be those in use at the various laboratories. If laboratories wished to use more than one method (e.g. real-time assays) they were invited to notify the CRL who would ensure sufficient material was distributed. The workshop agreed that it would be very valuable to obtain comparative quantitative data using real-time assays.

34. The NRL Italy had identified some naturally contaminated bivalve molluscs which could be used for a ring trial for naturally contaminated HAV. The CRL agreed to evaluate the material and, if appropriate to propose a ring trial using this material during 2004/5. The numbers of participants might need to be restricted depending on the quantity of material available. If necessary the ring trial would be targeted at those laboratories already performing well in the laboratory virus ring trials.

35. The workshop considered the developments on standardisation of methods for viruses in food under CEN and were invited to send shellfish methods to the leader of that aspect of the project (David Lees) for consideration. NRLs were also advised that countries not currently represented could be included in future meetings through designation of experts by their national standardisation bodies.

36. The workshop consider the need for a practical workshop on virus methods. NRLs agreed to consider the issue and provide feedback to the CRL on the possibility and particularly on their suggestions for content by the 1st June. The CRL considered it important to have a clear view of the objectives of the workshop (for example should the focus be training or comparative method evaluation). The CRL agreed to consider the NRL feedback and then make proposals for a practical workshop to be held during 2004/5.

37. The CRL reported the results of the research project funded by DG Sanco on Vibrio's, real-time PCR for Norovirus, and the impact of the FRNA bacteriophage criteria. The NRLs found this to be useful data. The workshop agreed that it was very useful for such data obtained from research projects to be disseminated to NRLs and others via presentations at the workshop and via the circulation of reports.

38. The NRLs discussed the Framework 6 Seafood plus research projects and highlighted the continuing need for research funding to underpin the development of laboratory network activities and the development of Community food safety policy on a sound scientific basis.

39. The workshop reviewed the results of the DG Sanco funded research on depuration criteria. The data showed failure to meet current *E.coli* standards (230 per 100g shellfish) in an average of 6% of samples from current existing commercial depuration systems across Europe. This was surprising and indicated the need to review existing practices to ensure that the current *E.coli* standard of 230 per 100g was meet.

40. Further to the above from data presented by both the CRL and NRL France the meeting confirmed the poor removal of both bacteriophage and viruses from shellfish during depuration using current industry practices. These studies confirmed the correlation between temperature and time on both virus and bacteriophage removal. NRL France data suggested that virus removal was even more protracted than FRNA bacteriophage removal.

41. The DG Sanco funded project also showed that elevated temperatures (20°C) tolerated by oysters (*C.gigas*) could cause mortalities (e.g. up to 15%) in other animals (mussels and clams). The workshop agreed that the impact of elevated temperature depuration on quality and mortality would need to be evaluated for each species/system combination.

42. Further to the above the workshop reviewed the data on the impact of the proposed bacteriophage criteria on current commercial practices and agreed that the criteria would have a significant impact on current commercial depuration practices in Europe. The full report would be circulated to NRLs shortly.

43. The workshop agreed that the research data showed that FRNA bacteriophage levels in bivalve shellfish were highly seasonal in all countries that had investigated this. FRNA bacteriophage levels being much higher during the winter (colder water temperatures) and low or absent in the summer (higher water temperatures). Consequently if the FRNA bacteriophage criteria was adopted it would be necessary to perform the depuration criteria checks when the bacteriophage was present i.e. in the winter months.

44. The meeting discussed alternatives to the bacteriophage criteria and reaffirmed its resolution of the 2nd workshop that improving and protecting water quality in

bivalve mollusc production areas was of fundamental importance in protecting the European shellfish consumer

45. Further to the above the Commission noted the need to improve consumer protection in this area and asked NRLs to consider whether, for example, strengthening current standards based on *E.coli*, or specifying minimum depuration times, could be effective. NRLs were asked to feedback their comments to the Commission (considering the timetable for the finalisation of the Commission proposals). The Commission advised that it was important for NRLs to help the Commission develop proposals for improvement of Community legislation in this area.

46. Further to the above the workshop noted the importance of European Regulations on Water Quality (such as the Water Framework Directive, 2000/60/EC) and encouraged the Member States to adopt a more integrated approach to protection of coastal waters in relation to bivalve mollusc production areas.

47. The Commission considered the need for risk assessment data to underpin legislative decisions. The NRLs advised of several projects in this area and agreed to circulate reports to the Commission and other participants to be determined.

48. The CRL agreed to host a practical training event for Accession Countries relating to analysis of *E.coli*, FRNA bacteriophage and viruses and invited those Accession Countries to register an interest in attending to the CRL. The Commission TAIEX office would be approached to fund the training event.

49. The workshop delegates thanked Dr Croci of the ISS for hosting the meeting in Rome and agreed that the next meeting would be hosted by IFREMER and held in Nantes, France on 15th – 17th March 2005.

CRL Weymouth, UK
6th April 2004