RESOLUTIONS OF THE 2ND WORKSHOP OF MICROBIOLOGICAL NRLS, WEYMOUTH, UK, MAY 2003

A workshop of the European National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs was held at CEFAS (The Centre for Environment, Fisheries and Aquaculture Science) on May 7-9 2003. The workshop was hosted by DG Sanco of the European Commission, Brussels, and the attendees comprised representatives from CEFAS Weymouth (the European Community Reference Laboratory), the European National Reference Laboratories and the European Commission Food and Veterinary Office. The workshop produced a number of resolutions to be distributed in the public domain mainly through the CRL website (www.crlcefas.org).

Concerning NRL activities

1. With a few exceptions NRLs reported good progress towards their commitments under Council Decision 1999/313/EC and towards the targets agreed at the 1st workshop of NRLs. Among the NRLs experiencing poor progress lack of funding was stated as the major problem.

2. The NRLs noted that among NRLs accreditation to ISO 17025 by National Accreditation bodies appeared to follow different practices with regard to method specific accreditation or a more 'flexible' accreditation including accreditation of a portfolio of both current methods and procedures for approving future methods. As this did not appear to be a harmonised approach the CRL agreed to seek clarification of the European Accreditation body and report back at the next workshop.

3. Several NRLs reported resourcing problems inhibiting their ability to adequately perform the tasks required in Council Directive 1999/313/EC and their full participation in activities of the NRL network. The Commission stated that it was the responsibility of Member States to adequately fund their designated NRLs in order for them to undertake their required duties and would encourage them to do so.

4. NRLs considered that it was important that the EU and Member States support research activities of the NRLs and other specialist laboratories in order to progress the responsibilities of the laboratory network and to facilitate the development of Community food policy in this area. NRLs noted that EU Framework 6 priority area 8 seemed an appropriate mechanism for such funding.

5. A number of NRLs reported important epidemiological information on shellfish associated outbreaks. However, a systematic approach to the collation and dissemination of this information does not exist among NRLs. It was agreed that it would be important to improve communication of this, and other information, among NRLs in a timely manner. In relation to this it would also be important to consider links to networks of epidemiological data in Europe.

6. Further to the above the CRL agreed to investigate, on a trial basis, extension of the CRL website to include a bulletin board or similar facility for sharing current information among NRLs.

7. NRLs agreed to inform the CRL of any changes in NRL contact details so that the website could be updated.

8. Many NRLs reported useful information related to production practices, characteristics of the trade (for example whether they were gross importers or exporters of shellfish) and classification statistics in their countries. It was considered that it would be beneficial to bring such information together for general information. The CRL agreed to devise and circulate a questionnaire among NRLs and to lodge the responses on the CRL website.

Concerning ring trials

9. NRLs reaffirmed their commitment to the resolutions of the first workshop with regard to achieving competency for FRNA within one year (May 2003) and enteric viruses within 2 years (May 2004).

10. Results from ring trials during 2002/2003 showed that NRLs had generally demonstrated good analytical performance and competency was high among participating laboratories.

11. It was agreed that all Member States should participate in ring trials for the statutory determinants of E.coli and Salmonella. It was preferable if this was the NRL however where the NRL was not performing these tests it was agreed that they could contract a testing laboratory to perform the analysis. However, this should be subject to a written agreement and the alternative laboratory should participate with the same responsibilities as the NRL for performance quality. The NRL should have a system in place to ensure quality assurance in other testing laboratories in accordance with NRL responsibilities for organising comparative testing.

12. Further to the above it was agreed that NRLs would continue to participate in the CEFAS/PHLS shellfish EQA for E.coli and Salmonella testing with 3 distributions during the next year (until the next workshop). It was agreed that NRLs would cover the costs of their own participation in this scheme.

13. Further to the above it was agreed that the CRL would organise a ring trial of either whole animals or homogenates to challenge further aspects of the E.coli method.

14. It was agreed to continue the FRNA bacteriophage ring trials on the existing basis with a further 3 distributions to be conducted during October/November 2003 at a frequency of monthly. The need for continuing ring trials for FRNA bacteriophage would be reviewed at the next workshop.

15. It was agreed to continue the ring trials for hepatitis A virus with a trial of method sensitivity on shellfish matrix (the CRL would send instructions to NRLs prior to the trial) and 2 further trials this year (until the next workshop) using naturally contaminated shellfish. NRL Italy agreed to assist the CRL in obtaining naturally contaminated material for this ring trial.

16. NRLs asked the CRL to give advance notice of ring trials at least 1 month before the planned analysis date.

17. In accordance with the resolutions of the first workshop it was agreed that the CRL would develop a ring trial for Norovirus during the next year. This would follow the approach for

HAV with distributions of faecal material followed by contaminated shellfish. The target was for 3 distributions during the year (until the next workshop).

18. It was agreed that anonymous result summaries for NRL ring trials would be placed in the public domain on the CRL website. This would include additional information on primers used etc.

19. It was noted that (with some exceptions) activity among NRLs in organising or ensuring participation in ring trials among national testing laboratories was at a low level and should be improved in line with NRLs responsibilities under 1999/313/EC. It was considered important that this should address, in particular, ring trials for the statutory determinants of E.coli and Salmonella. This could be done in collaboration with a third institute if appropriate.

Concerning the proposal for a regulation on official controls for foodstuffs (COM(2002)377 final : hygiene 3)

20. The US FDA requirements for sanitary surveys were considered in relation to the new official control (hygiene 3) requirements for identification of pollution inputs to shellfish harvesting areas. It was agreed that the CRL should follow up the invitation from the US FDA to attend the Inter State Shellfish Sanitation Conference and feed back information to future NRL workshops.

21. The NRLs noted that the official control requirements did not include an E.coli criterion for class C shellfish and that this was inhibiting wider uptake of the more scientifically acceptable E.coli specific standard methods.

22. Further to the above all NRLs agreed that official control analysis should be based on E.coli and not faecal coliforms and advise the Commission and Member States that it would be scientifically preferable to remove the faecal coliform criteria from the proposed regulation for official controls.

23. Further to the above the NRLs advise the Commission and Member States that an appropriate E.coli standard for class C shellfish, that would maintain a consistent approach, would be 46,000 E.coli per 100g shellfish.

24. Discussion on the official controls proposal (hygiene 3) highlighted several areas which would benefit from more detailed technical discussion. This included the numerical standards for faecal indicator analysis and the analytical tolerance applied, sampling methods and plans, and the requirements for detailing polluting influences in production areas. NRLs considered that a working group should consider these aspects and make recommendations/guidance as appropriate.

25. Further to the above the CRL agreed to propose an agenda and make recommendations to the Commission. NRLs agreed to subsequently propose possible working group members with details of their expertise.

26. Further to the above the NRLs agreed to supply relevant documents on practices for classification of production areas to the CRL to inform members of the working group.

Concerning the proposals for a regulation on microbiological criteria for foodstuffs (SANCO/4198/2001, rev 5) : detection of pathogenic viruses

27. Good progress has been made on the detection of Norovirus and hepatitis A virus among NRLs and it is clear from NRLs experiences that these PCR based methods are capable of the detection of Norovirus and hepatitis A virus in molluscan shellfish.

28. Further to the above it was clear that although NRLs employ some common approaches, harmonised methodologies had yet to emerge. NRLs agreed that it was important to begin to work towards more standardisation of virus detection methods among European laboratories.

29. Further to the above, and as a first step, NRLs agreed to supply current virus methods and protocols to the CRL who would compile a dossier for information on the website (restricted access). NRLs would supply protocols to the CRL by September 2003.

30. NRLs agreed that because of the problems associated with non-specific amplification, particularly when assaying environmental samples, virus methods should always employ a confirmation stage to ensure result validity. NRLs are currently using probe hybridisation, or sequencing, of PCR products for this purpose and these approaches can be endorsed.

31. Further to the above NRLs agreed that real-time PCR, incorporating a probe stage, would allow the development of more standardised, robust, quality assured, and potentially quantitative assays for detection of viruses in shellfish and that this was a desirable platform for future developments.

32. The DG Sanco research project on Detection of Pathogens in Shellfish was presented to the NRLs who considered it would contribute important information to the developments of methods in its respective areas and to a better understanding of the potential impact to producers of the proposed Microbiological Criteria for FRNA bacteriophage. The CRL would keep NRLs informed of progress in the project.

33. Further to the above it was agreed that it was important to ensure that the real-time PCR primers being designed for Norovirus were capable of covering all relevant circulating strains i.e. those identified both in Europe and elsewhere.

34. NRLs reported different findings with regard to the correlation of enteric viruses and FRNA bacteriophage in shellfish in production areas. Some studies found a good correlation whereas others did not. Correlations appeared to be stronger in more polluted areas and less strong in less polluted areas (e.g. class A areas).

Concerning the proposal for a regulation on microbiological criteria for foodstuffs (SANCO/4198/2001, rev 5) : purification and FRNA bacteriophage

35. On the basis of research data presented the NRLs endorsed the conclusion of the SCVPH report of Norovirus in food that the use of E.coli for determining the efficiency of shellfish purification for removing enteric viruses was not a safe practice and that current commercial depuration practices did not adequately protect the shellfish consumer from viral infections.

36. Further to the above the NRLs agreed that seawater temperature had a major impact on viral elimination during the purification process and that ambient water temperatures during winter months were insufficient to effectively remove viruses.

37. Reported studies from NRLs found very similar findings with regard to FRNA bacteriophage removal kinetics for C.gigas during depuration. Essentially the major influence was temperature with removal to the 90% or more level generally being achieved within about 3 – 5 days at elevated temperatures (above 20°C). Information for other species was very limited.

38. Data presented by the CRL using quantitative real-time PCR showed a good correlation between Norovirus removal during depuration and that of FRNA bacteriophage (for C.gigas). Other data presented using presence/absence analysis for virus suggested that viral contamination may persist for longer than FRNA bacteriophage during depuration.

39. Following discussion of research findings, opinions differed among NRLs as to the proposed FRNA bacteriophage criteria for purification plants. Some laboratories considered the proposals could reduce consumer exposure to viruses in depurated shellfish. Other laboratories considered that the health gain was unlikely to be significant and was outweighed by the potential adverse economic impact. A major concern of some NRLs was the impact the proposal would have on current producer practices and whether they would remain economically viable. It was agreed that ultimately this was a risk management issue and therefore outside the remit of the CRL and NRLs.

40. Further to the above several NRLs identified data gaps with respect to depuration of certain shellfish species (clams, cockles, rope mussels) and that it would be important to have further information before the impact (in relation to product quality and cost) and effectiveness (in relation to virus removal) of the proposals could be established.

41. It was identified by NRLs that it was necessary to ensure that increasing temperature during depuration would not cause the proliferation of potentially pathogenic marine vibrio's or of other bacteria of possible public health concern.

42. NRLs agreed that the DG Sanco research project (see Resolution 32) should proceed as this project would address some of the data gaps with regard to vibrio's, species, impact and effectiveness. It was important for NRLs to make the CRL aware of particular shellfish species of concern.

43. Further to the above, it was also identified as important for NRLs to carry out further research on the proposed FRNA bacteriophage criterion in order to ascertain the levels of bacteriophage in their countries and the impact and effectiveness of the proposals on virus removal during depuration according to commercial practices in their countries. It was important for Member State Competent Authorities to adequately fund such research by NRLs in order to inform the scientific debate and the appropriate development of Community food policy.

44. Following extensive discussion it was agreed that although there were concerns expressed with the FRNA bacteriophage criterion, particularly in regard to a full understanding of the effectiveness and implications of the proposal, NRLs were not able to identify an alternative approach for improving depuration effectiveness with regard to virus removal.

45. NRLs agreed that if depuration cannot be improved in regard to viral elimination then the only alternative for improved consumer protection appears to be restriction of harvesting in contaminated areas. NRLs agreed that it was important to ensure the development and application of viral detection methods to facilitate more effective controls in the future.

46. NRLs agreed that an alternative, longer term approach would be to fundamentally improve water quality in shellfisheries and to adopt a more proactive pollution management system in shellfish harvesting areas. All NRLs agreed that this should be the longer term priority.

Concerning marine vibrio's and E.coli methods

47. NRLs regretted that there was insufficient time during this workshop to discuss activities on marine vibrio's and E.coli methods and asked the CRL to ensure sufficient time was set aside for these discussions during the 2004 workshop. Further to this the following resolutions (numbers 48 and 49) were agreed by correspondence among NRLs there being insufficient opportunity at the workshop.

48. NRLs agreed that additional data should be generated in support of the CRL-recommended reference method for E.coli being progressed as an ISO standard method (and in support of Resolutions 3 and 4 of the meeting held in May 2002).

49. NRLs agreed that the acquisition of data on the effectiveness of the proposed revised ISO methods for vibrios would contribute to the consideration of future use of such methods by NRLs and National Laboratories.

Concerning the next NRLs workshop

50. It was proposed and agreed that the third workshop of NRLs would take place at the ISS, Rome, Italy on Tuesday March 30th – Thursday 1st April 2004.

CRL Weymouth, 19th May 2003