European Union Reference laboratory for monitoring bacteriological and viral contamination of bivalve molluscs

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Resolutions of the 13th workshop of microbiological NRLs for bivalve molluscs, 13-15th May 2014

Official Controls – Microbiological monitoring and data interpretation

1. Regarding export of live bivalve molluscs to the United States; the EURL informed NRLs that for Member States wishing to export to the US, that the US FDA would audit the Competent Authority against compliance with the Community Guidance[1] and the Good Practice Guide[2] including the annexes concerning exports to the USA. For priority MS, NRLs interested were advised to communicate this to their Competent Authority.

2. Following extensive evaluation undertaken by the EURL using multiple datasets supplied by NRLs, NRLs agreed that based upon these evaluations it was not possible to determine a procedure using descriptive statistics to inform stability assessments, to provide justification for decreased monitoring frequency and that this would be noted in the Good Practice Guide[2].

3. Further to the above, NRLs agreed that based upon the technical and statistical evaluation reduced sampling frequency could not be justified on the basis of the data, it was agreed however that reduced sampling frequency could be justified for areas meeting the definition of remote in the Community Guide[1] and the Good Practice Guide[2] .

4. NRLs discussed the proposed amendments to the Good Practice Guide[2] with respect to acceptable sample transport temperatures. It was agreed that for official controls sample transport criterion described in ISO 6887-3[3] should be referenced. A note should be included in all relevant guidance and protocols to permit sample transport temperature criterion other than stated in ISO 6887-3[3], provided that Competent Authorities had undertaken appropriate verification studies, and that the results of those verification studies demonstrate that there was no effect on the quality of test results.

Official Controls – Microbiological methods

5. The importance of the EURL and the NRLs network in the procedure for approving validation of alternative methods for official controls, validated against EU stipulated reference methods, was agreed. It was acknowledged that although there was no legal basis for formal approval of validation studies by the EURL that this approach could assist laboratories and was good practice.

[1]Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Molluscs Production and Relaying Areas with regard to Regulation 854/2004 (Issue 2).
[2]Microbiological Monitoring of Bivalve Mollusc Harvesting Areas: Guide to Good Practice: Technical Application (Issue 5: May 2014).
[3] Currently ISO/DIS 6887-3Microbiology of food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 3: Specific rules for the preparation of fish and fishery products

6. Further to the above, the EURL agreed to review datasets and information relating to validation of alternative methods for official controls on request, and to communicate this to the network of NRLs at the annual workshop as appropriate.

7. With respect to analysis of live bivalve molluscs, the inclusion of measurement uncertainty (addition or subtraction) in microbiological test results for official control purposes is not foreseen in EU legislation (Regulation (EC) No. 854/2004 and Regulation (EC) No. 2073/2005). NRLs agreed that measurement uncertainty (MU) or confidence limits should not be included in reported results, but MU could be determined by laboratories as part of their quality procedures and may be provided for information alongside reported results or on request.

8. NRLs noted that there was a discrepancy between the 5 x 3 MPN tables in ISO7218:2007/FDAM1:2013 and the EURL generic standard operating procedure for enumeration of *E. coli* in bivalve molluscs (Issue 10). The 5 x 3 MPN tables in ISO7218:2007/FDAM1:2013 contain a small number of errors when compared to the ISO MPN calculator (referenced in ISO7218:2007/FDAM1:2013). Errors have been corrected in the EURL generic standard operating procedure (Issue 10) which NRLs are advised to use.

9. In addition to the above, NRLs noted that there was a discrepancy between the 5 x 3 MPN tables in ISO7218:2007/FDAM1:2013 and the EURL generic standard operating procedure for enumeration of *E. coli* in bivalve molluscs (Issue 10) with respect to the estimated MPN given for tube combinations of 0, 0, 0 and 5, 5, 5. NRLs agreed that it was technically and scientifically justified in microbiology to report combinations of 0, 0, 0 and 5, 5, 5 as per Appendix 2 of the EURL generic standard operating procedure (Issue 10).

10. Further to resolution 9, the EURL would include an explanatory note in the EURL generic standard operating procedure (Issue 10).

Proficiency testing and validation

Statutory determinands

11. NRLs noted the work carried out by the UK NRL on proficiency testing for *E. coli* amongst official control laboratories in the UK on Common cockles (*Cerastoderma edule*). The observed increased variability in reported results compared to larger species (e.g. oysters) was attributed to initial sample preparation (blending *vs* stomaching). NRLs agreed that this issue may have wider relevance across the NRLs network and would be examined in the next EURL matrix proficiency testing distribution.

Marine Vibrios

12. The EURL expressed its thanks to the NRLs network in their participation in the completed validation of the *Vibrio* standard (ISO TS 21872) under the CEN mandate (M/381). The EURL would recommence proficiency testing for *V. parahaemolyticus* and *V. vulnificus* for NRLs in 2014/15.

Viruses

13. NRLs supported continuing virus proficiency testing offered by the EURL. For 2014/15 the EURL would offer two proficiency testing distributions for norovirus and hepatitis A virus. One non matrix distribution (June 2014) and a matrix distribution in late 2014. The matrix distribution would comprise at least one sample with anticipated virus levels $<10^3$ genome copies per gram.

14. The EURL informed NRLs that the EURL generic protocol for quantitative detection of norovirus and hepatitis A virus in bivalve molluscan shellfish had been amended in line with recent technical improvements in the preparation and storage of dsDNA quantification standards. The EURL generic protocol (Issue 3) was now available to download from the EURL website (www.eurlcefas.org)

15. The EURL also informed NRLs that new norovirus (GI and GII) dsDNA quantification standards were being prepared to correct some identified deficiencies and would shortly be available for distribution on request.

16. NRLs noted the presentation of data on norovirus levels in shellfish samples associated with outbreaks showed that low genome copy levels/g digestive tissue, including levels below the quantification limit, were found in some samples.

17. NRLs noted the development of the 'winter norovirus protocol' in France which requires the closure of areas associated with outbreaks for 28 days and norovirus analysis in oysters from the production areas. Experience from NRL France showed extended persistence of norovirus in oysters in the production area in some cases.

18. Date of the next meeting – 19th – 21st May at IFREMER, Nantes, France.