Cefas

European Union Reference Laboratory for monitoring bacteriological and contamination of bivalve molluscs

Report of the 9th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs.

Ancona, Italy, 18th – 19th May, 2010.

Contents

Forward

This document comprises relevant information arising from the 9th workshop of National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs held in Ancona, Italy on 18-19th May 2010. It includes the workshop agenda, delegate contact information, workshop minutes, lists of associated papers and presentations, and the resolutions agreed by the meeting. All supplementary supporting information identified in this report can be accessed in full via the information centre of the website of the European Union Reference Laboratory www.crlcefas.org or may be supplied on request by the EURL. All requests should be made to the EURL co-ordinator.

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Agenda

9th Workshop of Microbiological NRLs, 18 - 19 May 2010

Venue: **Yatch Club,**
Ancona,
Italy.

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Day 1 - Tuesday 18 May 9:00 – 18:00

1 Welcome meeting
- **1.1** Welcome and introductions.
- **1.2** Domestic arrangements including reclaim of expenses (papers WS09/01, WS09/02).
- **1.3** Actions arising from the 8th workshop 2009 (paper WS09/03).
- **1.4** Agreement of the agenda (paper WS09/04).

2 Microbiological monitoring – statutory determinands
- **2.1** Update of the good practice guide (version 3, April 2010) (WS09/06) – EURL.
- **2.2** Codex vs. EU *E. coli* standards (WS09/07; WS09/08) - EURL.
- **2.3** Validation of ISO 16649-2 (TBX method) for enumeration of *E. coli* - (NRL Netherlands).
- **2.4** Classification status across the EU (WS09/09) - EURL.
- **2.5** EU / US equivalence – EURL.

3 Microbiological monitoring- sanitary surveys
- **3.1** Current position with respect to application of sanitary surveys in EU Member States (WS09/10) - EURL.
- **3.2** Experiences in application of requirements for sanitary surveys of Regulation EC 854/2004 in Italy: an update - NRL Italy.
- **3.3** National study for inventory of sources of contamination of human and animal origin and atlas of cartographic information - NRL France.

4 Viruses
- **4.1** Activities at EFSA and Codex (noroviruses and hepatitis A).
- **4.2** Virus standard and validation - EURL.
- **4.3** Experiences with norovirus outbreaks in Denmark associated with live bivalve molluscs from multiple Member States - NRL Denmark.
- **4.4** Monitoring of Norovirus contamination in three shellfish species from the delta area of the Po river - NRL Italy.
- **4.5** Norovirus outbreaks linked to oyster consumption in the UK, Norway, France, Sweden and Denmark 2010 (WS09/11).
- **4.6** Discussion – current situation and future direction of virus controls in EU Member States – All.

Day 2 - Wednesday 13 May 9:00 - 18:00

5 Vibrio session

5.1 Progress of the NRL methods expert working group for *Vibrio* spp, activities at CEN TAG3 (WS09/12) – EURL.

5.2 Colony hybridization method for the enumeration of total and pathogenic *Vibrio parahaemolyticus* – NRL Italy.

5.3 Development of extraction and RT-PCR methods for the *in-situ* detection and enumeration of pathogenic bacteria from shellfish matrices – EURL.

5.4 Selection of molecular targets for the detection of *V. parahaemolyticus* in naturally contaminated bivalve molluscs - NRL France.

5.5 Discussion- outstanding activities and timetable for Vibrio controls in the EU - All.

6 Proficiency Testing Session

6.1 EURL/HPA shellfish scheme for *E. coli* enumeration and detection of *Salmonella* spp. -HPA.

6.2 NRLs participation and performance assessment Shellfish EQA scheme for *E. coli* and *Salmonella* (EURL) (paper WS09/13) – EURL.

6.3 Participation of Italian OCLs to Food EQA Shellfish Scheme - NRL Italy.

6.4 *E. coli* and *Salmonella* spp. proficiency testing in live oysters and mussels, performance assessment and follow-up - NRL France.

6.5 NRLs participation and performance assessment in the whole animal distribution for *E. coli* and *Salmonella* (paper WS09/14) - EURL.

6.6 NRLs participation and performance assessment in the *Vibrio* spp. PT (paper WS09/15) - EURL.

6.7 NRLs participation and performance assessment in the norovirus and hepatitis A PT (paper WS09/16) – EURL.

6.8 PT performance assessment and follow-up activities – development of best practice across the network (WS09/17) - All.

7 Agreement of Workshop resolutions 16:30-18:00

8 Date and venue for next meeting

Meeting close

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Report of the 9th workshop of Microbiological NRLs for Bivalve Molluscs, Ancona, Italy, 18th – 19th May, 2010.

Attendees

David Lees (DL) (chair)	EURL Director	Cefas, UK.
Rachel Rangdale (RR)	EURL Coordinator	Cefas, UK.
Louise Stockley (LS)	EURL	Cefas, UK.
Johann Ladstatter (JL)	NRL Austria	Austrian Agency for Health and Food Safety, Wien.
Sarah Denayer (SD)	NRL Belgium & Luxembourg	Scientific Institute of Public Health, Brussels.
Vanya Chikiova (VC)	NRL Bulgaria	Pencho Slaveikov, Sofia.
Anna Charlotte Schultz (ACS)	NRL Denmark	Institute of Food Safety and Nutrition, Soborg.
Leila Rantala (LR)	NRL Finland	Finnish Food Safety Authority Evira, Helsinki.
Martial Catherine (MC)	NRL France	Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER), Nantes.
Soizick Le Guyader (SG)	NRL France	Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER), Nantes.
Dominique Hervio-Heath (DH)	NRL France	Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER), Nantes.
Eckhard Strauch (EDS)	NRL Germany	Federal Institute for Risk Assessment, Berlin.
Anna Ntala (AN)	NRL Greece	Institute of Food Hygiene of Athens, Athens.
Erzsebet Adrian (EA)	NRL Hungary	Central Agricultural Office, Food & Feed Directorate, Budapest.
Luciana Croci (LC)	NRL Italy	Istituto Superiore di Sanità (ISS) Rome.
Mario Latini (ML)	NRL Italy	Centro di Referenza Nazionale per il controllo microbiologico e chimico dei molluschi bivalve vivi, Ancona.
Elisabetta Suffredini (EAS)	NRL Italy	Centro Nazionale per la Qualita degli Alimenti e per i Rischi Alimentari, Elena, Roma.
Elena Rocchegiani (ER)	NRL Italy	Centro di Referenza Nazionale per il controllo microbiologico e chimico dei molluschi bivalve vivi, Ancona.
Francescia Leoni (FL)	NRL Italy	Centro di Referenza Nazionale per il controllo microbiologico e chimico dei molluschi bivalve vivi, Ancona.
Gita Tupe (GT)	NRL Latvia	National Diagnostic Centre of Food & Veterinary Service (FVS), Riga.
Ewelina Bigoraj (EB)	NRL Poland	National Veterinary Research Institute, Pulawy.
Remigiusz Pomykala (RP)	NRL Poland	National Veterinary Research Institute, Pulawy.
Sonia Pedro (SP)	NRL Portugal	Instituto de Investigacao das Pescas e do Mar (IPIMAR), Lisbon.
Alina Popescu (AP)	NRL Romania	Institute of Diagnosis and Animal Health, Buharest.
Julia Habovstiakova (JH)	NRL Slovakia	State Veterinary and Food Institute, Dolny Kubin.
Andrej Kirbis (AK)	NRL Slovenia	National Veterinary Laboratory, Ljubljana.
Urska Henigman (UK)	NRL Slovenia	National Veterinary Laboratory, Ljubljana.
Cristina Acebal (CA)	NRL Spain	Agencia Espanola de Seguridad Alimentaria, Majadahonda, Madrid.
Cristina Alvarez (CAZ)	NRL Spain	Centro de Control da Calidade do Medio Marino, Pontevedra.
Magnus Simonsson (MS)	NRL Sweden	National Food Administration, Uppsala.
Ron Lee (RL)	NRL UK	Cefas, Weymouth.
James Lowther (JL)	NRL UK	Cefas, Weymouth.
Liv Marrit Rorvik (LMR)	EFTA Norway	The Norwegian School of Veterinary Science, Oslo.
Mette Myrmel (MM)	EFTA Norway	The Norwegian School of Veterinary Science, Oslo.
Huseyin Dede (HD)	Accession Country Turkey	Ministry of Agriculture & Rural Affairs, - 42080, Konya.

Attendees (cont.)

Skype		
Bill Dore (BD)	NRL Ireland	Marine Institute, Galway.
Apologies		
Franklin Georgsson (FG)	EFTA Iceland	Matis, Reykjavik.
Ciska Schets (CS)	NRL Netherlands	National Institute of Public Health and the Environment (RIVM), Bilthoven.
Julie Russell (JR)		HPA Colindale, London, UK.
Paolo Caricato (PC)		European Commission, Brussels.

Representatives from NRLs in The Czech Republic and Lithuania did not attend the workshop.

Note

All presentations can be viewed in the Information Centre of the EURL website under the Workshops section www.crlcefas.org

1 Welcome meeting ***Action***

1.1 Welcome and introduction

DNL opened the meeting, welcoming NRL representatives to Ancona, Italy. Delegates were reminded that all eligible receipts must be submitted to the EURL for authorisation to enable repayment of expenses. The format of the workshop was outlined (WS09/04), it was also noted that the meeting would take place over 2 days (Tuesday 18th – Wednesday 19th). It was identified that the afternoon of Wednesday 19th would be dedicated to Proficiency Testing (PT).

1.2 Actions arising from the 8th workshop

The resolutions and report from the 8th workshop were reviewed (WS09/03). All actions had been progressed, outcomes are either summarised below or covered in subsequent sections.

A further EURL practical training course had been held on the application of sanitary surveys in October 2009. No further training courses in this area were anticipated in 2010. The EURL reported that following discussion with DG SANCO it had been proposed that formal acceptance of Bactrac 4300 as an alternative method for *E. coli* enumeration should be through publication in the Official Journal of the European Union. The EURL would propose text for consideration by the Commission. EURL.

The lack of explicit microbiological parameters in the Water Framework Directive (WFD, 2000/60/EC) was discussed. The EURL noted that these concerns had been raised with DG SANCO. The workshop expressed the continuing concerns at the potential health impact of the absence of microbiological standards. Following discussion NRLs agreed to advise their Competent Authority, and to recommend that these concerns were highlighted at a European level (**Resolution 1**). All NRLs.

2 Microbiological monitoring – Statuary determinands

2.1 Good Practice Guide - EURL

RL presented the revised Good Practice Guide (GPG) revision 2009/10 (Version 3, April 2010). Previous comments received from both NRLs and the GPG working group had been collated and included in the revision. The amendments comprised largely the addition of references to legislation and ISO standards, identification of minimal requirements for performing and reporting sanitary surveys, and recommendations for transport of bivalves. The EURL requested that NRLs forward the revised GPG document (WS09/06) to their Member States (MS) authorities responsible for Official Control testing and return comments back to RL by the 18th June 2010 (**Resolution 2**). NRLs were informed of the perceived importance of the GPG with respect to assessment of EU LBM production by third parties (including EU FVO). DL noted that the United States (U.S.) FDA had requested the GPG from the Commission; it was felt that this indicated that for the U.S this document would be used as the technical basis informing application of the European programme *cf* the N.S.S.P. All NRLs with LBM production.

2.2 Codex vs EU *E.coli* standard – EURL

The EURL introduced the differences between the CODEX STAN 292-2008 3 class plan (CP) approach to *E. coli* MPN criteria in LBM (n=5, c=1, m=230, M=700 *E. coli* MPN/100g) and the Commission Regulation (EC) No. 2073/2004 based on a 2 CP with all samples≤ 230 MPN *E. coli*/100g (n=1, c=0, m=0, M=230). To harmonise the approach of CODEX and the EU, NRLs were requested to consider two scenarios:

- Analysis of historic class A datasets against the CODEX 3 class but applied over time.
- To look at the statistical equivalent 2 CP to the Codex 3 CP end-product standard which is calculated as all samples to be less than or equal to 330 *E. coli* MPN /100g.

NRLs agreed that the adoption of CODEX for end-products was in line with approaches used in other food stuff **(Resolution 3)** and agreed to look at their existing class A datasets with respect to the above scenarios. Based on the outcome of this the EURL agreed to produce recommendations for circulation amongst NRLs and the Commission **(Resolution 4).**

All NRLs with LBM production. EURL.

2.3 Validation of ISO 16649-2 (TBX method) – NRL Netherlands

NRL Netherlands were due to report on the validation of ISO 16649-2 (TBX method) against ISO TS 16649-3 but due to travel difficulties the representative was not present at the meeting. The EURL had been consulted throughout the validation and gave a brief overview of progress. To date an expert laboratory study and inter-laboratory study had been performed on bio-accumulated samples. The organising laboratory for this validation was RIKILT, The Netherlands; the validation was co-ordinated by MicroVal. It was agreed by NRLs that the validation should include sufficient naturally contaminated material (**Resolution 5**). It was confirmed that ISO 16649-2 included a 2 hour recovery step at 37°C before transfer to 44°C.

2.4 Classification status across the EU - EURL

The percentages of harvesting areas across Europe classified as A, B, C and prohibited (actual classifications) had been requested from NRLs earlier in 2008-9. Information was collated in workshop paper (WS09/09). This was presented to the group for information. All NRLs were requested to check the accuracy of information recorded in the paper and to inform the EURL of any amendments within 2 weeks of the NRL meeting.

All NRLs with LBM production.

2.5 EU/US equivalence – EURL

The EURL reported on the ongoing EU US trade discussions on equivalence of the EU and US shellfish sanitation programmes. A temporary agreement on export of LBM from the US to the EU was due to expire on July 1st, no exports from the EU to the US had been allowed for over 20 years. No further extension to the bilateral trade agreement was considered likely and the focus of the way forward was to demonstrate (or not) the equivalence of the two systems. To date equivalence negotiations had started with an initial meeting in Brussels in Jan 2010, and an agreement to set up technical workings groups the following areas:

- Classification and sanitary surveys.
- Biotoxins and contaminants.
- Post harvest controls (with the potential for the use of epidemiological data to assess the efficacy of the two systems).

In addition an exchange of questionnaires requesting details of the two approaches has been circulated. The EU (FVO) audited the US in 2009 although the final report was not yet available on the Commission website. It was considered likely that a reciprocal audit would take place in the EU, focusing on MS who had expressed an interest in export to the US. These MS included Denmark, France, Ireland, Italy, The Netherlands, Spain and the UK.

DL noted that analysis of existing datasets where paired water and flesh samples were available indicated that EU procedures for Class A compliance (according to EU Regs) were more stringent in terms of allowable faecal contamination than US approved areas status. HD noted that in Turkey both shellfish flesh and shellfish waters were monitored. Questions were raised with respect to US control of *V. vulnificus*. DL explained that risk based control plans were implemented in the US requiring preventative action (control plans) if risk of *V. vulnificus* presence was indicated. It was noted that control plans did not extend to oysters removed from the shell.

3 Microbiological monitoring – Sanitary surveys

3.1 Application of sanitary surveys in EU Member States - EURL

The EURL gave a brief presentation on the recent training course on the application of sanitary surveys. In addition information on activities with respect to sanitary surveys in each country was presented in draft (WS09/10). NRLs were asked to update the workshop with any additional information (within two weeks of the meeting) (**Resolution 6**). The paper would then be finalised and placed on the EURL website for information.

All NRLs with LBM production.

3.2 Experiences in the application of sanitary survey requirements – NRL Italy

ML presented on the approach to classification, monitoring and application of sanitary surveys in Italy. Responsibility for sanitary surveys was devolved to local sanitary authorities (ASL), microbiological testing was undertaken by IZS laboratories and classification was determined regionally. There were 15 regions with sea coasts and approximately 400 classified areas. Approximately 50% of areas were classified as class A. No information was held centrally, it was noted that this could cause problems for Italy in the provision of co-ordinated national information.

3.3 National study for inventory of sources of contamination of human and animal origin and atlas of cartographic information – NRL France.

MC presented a French initiative to publish a cartographic atlas (CD) identifying of all potential sources of contamination and their impact on production areas. The atlas was not intended for the public domain, the aim was to use it as a common information source for IFREMER in the desk-based element of sanitary surveys.

4 Viruses

4.1 Activities at EFSA and Codex

DL and SG presented on the activities at EFSA and Codex on viruses in foods. At EFSA the working group had been tasked with carrying out a review of biology, epidemiology, diagnosis and public health importance of food-borne viruses covering all aspects of shellfish production, identification of possible production controls and assessments the implications of establishing food safety criteria for viruses. The work was ongoing and would be reviewed at the next meeting in June 2010. The workshop discussed the importance of continued improvements to water quality. It was agreed that this remained of paramount importance. RL noted that under EU Food Hygiene Regulations greater responsibilities were placed upon the producers to identify the high risk processes and to address them through HACCP. It was noted that depuration had been shown to not to be an effective control measure for high risk products. The importance of sanitary surveys was stressed. It was highlighted that whilst the outcome of sanitary surveys did not always result in an altered classification they could redefine the boundaries of a production area which could lessen the impact of pollution sources. DL explained that in the US a mandatory dilution factor of 1:1000 from the source of pollution was implemented with harvest prohibitions in place any closer to the source.

At Codex the aim was to produce a guidance document reducing food borne illness associated with viruses. A specific annex covering LBM was under development. The preliminary draft document was presented in San Diego in July 2009 and was accepted with minor comments. A number of areas required further elaboration such as procedures for closing/reopening areas after incidents, reduction in inputs through improvements in sewage treatment works and labeling and traceability. The need for validated, accredited methods was also considered. The target date for completion of the Codex guide was December 2011.

4.2 Virus standard and validation

The EURL presented an update on the progress of CEN/TC275/WG6/TAG4 responsible for the development of a standard method for the determination of norovirus and Hepatitis A virus in bivalve shellfish and other food stuff. Part 1 of the standard (quantitative) was launched at CEN WG6 and ISO SC9 for preliminary consultation in April 2009. Comments arising were addressed at the meeting of TAG4 Jan 2010. In addition, method controls were rationalised and a draft of part 2 (qualitative) of the standard was agreed. Parts 1 and 2 were re-launched in April 2010 formal technical enquiry. The importance of the inclusion of adequate controls was stressed (SG). SG further noted that formal validation of the method should be for all at risk foodstuffs (not solely shellfish). Further technical comments were expected at the end of the year. All NRLs agreed that it was important to fully validate the CEN method if it was to be used for Official Control purposes. Concerns were expressed on the continued delay in a decision on the outcome of the EU mandate to CEN on methods in microbiology (M/381). It was agreed that if funding (or a decision on a roadmap to validation) was not available through the EU mandate during 2010 the EURL should progress a mechanism with DG SANCO to take this validation forward **(Resolution 7).**

EURL

4.3 Experiences with norovirus outbreaks from multiple MS – Denmark

AC (NRL Denmark) reported 6 clusters (27 cases) of norovirus gastro-enteritis in Denmark associated with the consumption of oysters imported from other European countries. Mixed infections (GII, GI.7, GII.1 and GI.7 and GII.2) were identified in 3 cases. Both GI and GII detected in implicated oysters (GII >3,800 genome copies per gram, GI 1 -2 logs lower).

4.4 Monitoring of norovirus contamination in the Po river – Italy

LC presented on a research project that was carried out on 3 shellfish species in 2 class B areas with different salinity and variability levels in North Italy. The analysis of samples was performed using qualitative real time PCR procedure (based on CEN/TAG4). A total of 36 samples (51.4%) were positive for the presence of norovirus with the majority of samples from both areas to be positive (94.4%) during the winter period (October and April). It was shown that there was no statistically significant difference between the presence of norovirus (GI or GII), and site or species. It was recommended that mussels could be used as an indicator for viral contamination.

4.5 Norovirus outbreaks linked to oyster consumption

BD presented risk management actions following an outbreak of norovirus associated with oysters harvested from Carlingford Lough. Over 70 cases of gastroenteritis had been reported in a number of clusters. The implicated site was closed and product recalled. Quantitative norovirus testing (CEN method) was used to establish levels at site both before and after the outbreaks. Norovirus GII was present in both the harvesting area, and in oysters collected from 2 restaurants associated with incidents (>1000 genome copies per gram) *cf* prior testing where levels were low (≈200 genome copies per gram). Throughout, *E. coli* monitoring data showed that the site was in compliance with a class A status. Additional controls on oysters from the area were implemented comprising 2 weeks relay in clean water followed by depuration for 3-5 days at 15-17°C. Norovirus testing throughout indicated that levels reduced to "background" using this approach. It was suggested that routine monitoring and action limit of 1000 norovirus genome copies per gram could be used to prevent outbreaks. And that relaying and depuration could reduce significant levels of norovirus contamination to "safe" levels.

4.6 Discussion

The workshop noted the high incidence of norovirus outbreaks in Northern Europe during 2009/10. It was suggested that the winter had been severe and this may have been a contributory factor **(Resolution 8)**. It was noted that in several of the preceding presentations quantitative approaches to norovirus testing had been shown to be useful in risk evaluation **(Resolution 9)**. JL presented quantitative data from LBM (*C. gigas* and *O. edulis*) tested at the EURL since 2007, samples implicated in outbreaks were all positive at >100 genome copies/g. It was suggested that this could form the basis of a potential "safe" limit, as opposed to absence of virus which would have a substantial effect on LBM harvest particularity during the winter months. Following discussion no agreement on absolute risk of low levels (<100 genome copies /g of norovirus) could be established. However, whilst the health significance of low virus levels was uncertain it was agreed that additional data were required to help clarify risk **(Resolution 10)**. Delegates agreed that current EU controls did not adequately address the public health risk from norovirus and needed improvement. Possible control options were discussed including: focussing on high risk products; reducing human faecal inputs based on sanitary surveys; using relaying in clean waters; improving the effectiveness of depuration; and the possibility of virus standards based on RT-PCR analysis **(Resolution 11)**.

5 Vibrio session

5.1 Progress of CEN/TC275/WG6/TAG3 – EURL

At the 2009 NRLs workshop a number of resolutions relating to vibrios had been agreed. Since then an expert working group comprising NRL representatives from Denmark, France, Italy, Norway, Portugal and UK had identified the important issues, these were summarised in a workshop paper (WS09/12). The EURL informed the workshop of the progress at TAG3 on the development of the new CEN standard for the detection of human pathogenic vibrios. Two proposals were currently under consideration (real time PCR and nucleic acid hybridisation) but required further practical evaluation. Delegates agreed that a single method was preferable as a potential the reference method, but that more than one approach could be taken forward as an ISO standard **(Resolution 12)**. NRLs were informed of the Codex request for further data and experts, and asked where possible to contribute to this **(Resolution 13)**.

All NRLs with relevant data.

5.2 Colony hybridisation method of V. parahaemolyticus – NRL Italy

The development of an enumeration method for *V. parahaemolyticus* was presented by EAS, this work was a conntinuation of the EU funded SEAFOOD plus project. The methodology uses hybridisation of a single filter/petri dish for enumeration of total and pathogenic *V. parahaemolyticus*. Separate probes target *toxR*, *tdh* and *trh*. Results obtained from the validation study showed >95% sensitivity and accuracy. RL asked if the issue associated with the overgrowth of *V. alginolyticus* on non-selective media had been resolved. EAS reported that some work had been carried out on altering media salt levels to inhibit growth of *Vibrio* spp. and prevent swarming. EDS raised some concern over the subjective interpretation of positive results and stressed the importance of good controls and training.

5.3 Development of real time qPCR method for V. vulnificus and V. parahaemolyticus – EURL

The EURL presented preliminary data from real time PCR testing directly from hepatopancreas of bioaccumulated oysters. Sample extraction was following the CEN protocol for LBM for norovirus and hepatitis A. The *V. vulnificus* assay targeted both clinical and environmental *V. vulnificus* strains enabling differentiation of clinically significant isolates. Previously published *V. parahemolyticus* primers and probes had also been tested directly from shellfish. The method was rapid process and in limited studies had a sensitivity of approximately 50 cells/g. Extensive further studies were required to demonstrate that it was applicable to naturally contaminated shellfish.

5.4 Molecular targets for the detection of *V. parahaemolyticus*

DH (NRL France) presented work on selection of molecular targets and development of real-time PCR for the detection and enumeration of total and enteropathogenic *V. parahaemolyticus* in naturally contaminated shellfish. Molecular targets were *toxR*, (total) and *tdh* and *trh*. Data presented indicated between 98% and 100% inclusivity/exclusivity. Work to date has focused on detection from 4-6 hour enrichment broths. Additional use of an alternative species marker (*dnaJ* gene) had shown some promise with respect to increasing sensitivity.

6 Proficiency testing session

6.1 NRL performance in the EURL/HPA Shellfish EQA scheme RT 36 - EURL

Twenty-two NRLs participated either fully or partly in the EURL/HPA co-organised shellfish EQA scheme for *E. coli* and *Salmonella* spp (WS09/13). Performance assessment was carried out participants who had analysed all 3 distributions. Of 19 NRLs, 17 achieved a cumulative performance assessments of >70%. Two NRLs scored < 70% which is considered as a measure of poor performance. This was due to failure to return results or reporting of high censored results. Performance over three years was also scrutinised. Of the 22 NRLs who had participated in the last 3 years (2007 – 2009) distributions 3 achieved 100% and 19 NRL obtained >70%. It was noted that NRLs performance in the scheme was very good and all were in agreement that NRL participation should remain mandatory. Several NRLs highlighted the difficulty in participating in the numerous PT schemes and asked whether NRLs could participate in only 2 distribution of the EURL/HPA scheme. The EURL agreed to analyse historical data and produce some recommendations on participation frequency (**Resolution 14**).

6.2 Italian OCLs participation in the EURL/HPA EQA shellfish scheme – NRL Italy

EURL

In 2009 NRL Italy used the EURL/HPA scheme to assess 35 Official Control laboratories. Performance assessments were performed on laboratories. Thirty (88%) participating laboratories obtained >70% with 4 laboratories returning results <70% over 2 or more distributions. The frequency of participation within the scheme was biannual for OCLs. The Italian NRL had agreed to produce operating procedures to assist OCL in follow-up procedures for failure in the scheme.

6.3 PT of live shellfish in France – NRL France

In France the NRL organised another 2 PT distributions using whole shellfish. MC explained how the samples were prepared, distributed and the analysis of the results. Performance amongst OCLs in both distributions was good with respect to *E. coli* and *Salmonella* spp. testing by: The scoring system and the follow up procedure for E-coli and Salmonella, based on the CRL guidance, was presented using the assessment method of the performance of participating laboratories.

6.4 Whole animal PT for *E. coli* and *Salmonella* spp. RT 31 - EURL

Thirty-three laboratories (18 NRLs and 15 other laboratories) participated in the EURL whole animal ring trial (WS09/14). All temperature loggers recorded the internal temperature of less than 10˚C. Results were returned from all participants for both enumeration of *E.coli* and the detection of *Salmonella* spp. All 33 laboratories reported results for *Salmonella* spp. that correspond to EURL's expected results, 18 cited ISO 6579 as their analyses method. Thirty laboratories returned 2 replicate

E. coli results (91%) within the expected range. Two laboratories (Laboratories 10 and 22) reported 1 replicate result between 3 and 5 SD of the participants' median (PM). Laboratory 19 reported both replicates outside 5 SD of the PM. Twenty-two laboratories cited ISO TS 16649-3 (Anon 2005) or a derivative of this method. A number of laboratories reported inconsistencies in reported *E. coli* MPN/100g and the tube combinations from the EURL MPN tables. A further performance assessment was carried out on 23 NRLs who had participated in the last 3 years (2007 – 2009) distributions with 15 NRLs achieved 100%, 7 NRLs obtain >70% and only NRL 19 obtaining <70%.

6.5 *V. parahaemolyticus* PT RT 34 - CRL

Twenty-seven laboratories participated in the *Vibrio* spp. ring trial (WS09/15). From the returned results 73% of laboratories, an increase of 32% from 2009, correctly identified the presence / absence of *V. parahaemolyticus* in the 5 vials distributed. Approximately 50% of laboratories correctly detected the presence of *V. vulnificus* and *V. alginolyticus* in vial 2 and vial 5 respectively. Between 12 and 15 laboratories reported results for the detection of *tdh* and *trh* genes. The majority of laboratories correctly assigned the presence / absence of both *tdh* and *trh* with an accuracy rate of 96% and 100% respectively. Laboratories 7 and 33 reported false *tdh* positives. Laboratory 39 reported false *tdh* negatives. Informal performance assessment was performed on 10 NRLs who had participated in the last 3 years (2008 – 2010) distributions, 1 NRL achieving 100% and 9 achieving >70%. NRLs that agreed for the PT should continue with the focus targeted toward method development **(Resolution 17)**. EURL

6.6 Norovirus/ Hepatitis A lenticule PT RT 33 - CRL

Thirty laboratories participated in the virus distribution consisting of 4 LENTICULES™ (WS09/16). Thirteen (43%) participating laboratories obtained the intended results for all LENTICULES™, as determined by EURL reference designations. For individual viruses, 76%, 72% and 72% of laboratories returned intended presence/absence results for norovirus GI, GII and hepatitis A respectively. Four laboratories did not examine for HAV. The false positive reporting rates for GI, GII and HAV were 3%, 9% and 10% respectively. The false negative reporting rates for GI and GII were 12% and 11% respectively. Nineteen laboratories (66%) returned semi-quantitative data expressed at C_t values. Seven laboratories returned quantitative data expressed as detectable genome copies per LENTICULE™. Reported virus quantities were variable, with 3-4 log differences in reported levels for GI and GII. Performance assessment was performed on 12 NRLs who had participated in distributions over the last 3 years (2007 – 2009), based on qualitative results only 2 NRLs achieved 100% and 10 achieved >70%. All workshop delegates agreed that the virus PT was a valuable, and the EURL were asked to include matrix material for the next distribution (**Resolution 15**). EURL

6.7 Performance assessment and follow-up activities

The EURL highlighted the main issues and problems that can occur when analysing PT material. The EURL presented a paper on suggested on performance assessment and follow-up activities. This will be available to NRLs on the EURL website along with other supplementary papers offering information of proficiency testing. The workshop noted that progress with respect to assessment of OCLs in comparative testing was good. Several NRLs presented data describing assessments based upon the use of scoring systems and had developed follow-up procedures to address unsatisfactory performance **(Resolution 16)**. EURL

Date and venue of next meeting

The 10th workshop will be held in Weymouth, UK, 10th, 11th and 12th of May 2011 (**Resolution 18**).

Resolutions of the 9th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs, Ancona 18 - 19th May 2010.

Micriobiological monitoring

1. The workshop expressed concern at the potential public health impact of the continued absence of microbiological standards in the water framework directive (WFD, 2000/60/EC). NRLs agreed to advise their Competent Authority, and to recommend that these concerns were highlighted at a European level.

2. The CRL presented the redraft of the Good Practice Guide, NRLs resolved to disseminate the document to their Competent Authority (to the authority responsible for Official Control and monitoring) and invite comments. Comments must be received by the 18th June 2010. Comments should be addressed to Ron Lee at the CRL ron.lee@cefas.co.uk

3. NRLs agreed that adoption of CODEX STAN 292/2008 (c=1, n=5, m=230 and M=700) for end product standards was in line with the approach taken with other foodstuffs, was scientifically justified, and should be reflected in EU controls.

4. The CRL agreed to circulate proposals for monitoring for class A areas which would give equivalence to the CODEX end-product standards. NRLs agreed to analyse class A datasets against these proposals and report back to the CRL with their opinion by the end of June 2010. The CRL would inform the Commission of the outcome.

5. With respect to the TBX (ISO 16649-2) method validation against the EU reference method (ISO TS 16649-3) for enumeration of *E. coli* and further to resolution 9 of the 8th workshop, NRLs agreed that it remained important to include comparative testing of an environmentally relevant range of naturally contaminated samples.

6. NRLs agreed to provide updated information on the number of completed full sanitary survey reports and the numbers of production areas covered by those reports within two weeks of this meeting.

Viruses

7. The workshop noted that it was important to validate the CEN virus method if it was to be used for Official Controls. In the absence of implementation of CEN mandate (M/381) NRLs supported the CRL proposal to approach DG SANCO for funding to formally validate the virus standard. It was noted that the validation should address all matrices (bottled water, food surfaces, soft fruit, salad vegetables and bivalve molluscs).

8. The workshop acknowledged the high reported incidence of norovirus outbreaks observed in several EU MS during 2009/10. The winter of 2009/10 was unusually severe in Northern Europe which may have contributed to this.

9. Data presented from several NRLs showed that virus detection methods provide useful data for risk evaluation.

10. The workshop noted from data presented that the use of a presence/absence virus standard for bivalves would be likely to have a significant impact during the winter months. The health significance of low virus levels (<100 copies per gram) was extensively debated and opinions and data varied. It was agreed that it is important to continue to generate data on this issue.

11. The workshop noted that current EU controls did not adequately address the public health risk from norovirus and needed to be improved. The workshop discussed possible control options including: focussing on high risk products; reducing human faecal inputs based on sanitary surveys; using relaying in clean waters; improving the effectiveness of depuration; and the possibility of virus standards based on RT-PCR analysis.

Vibrios

1. The workshop agreed that the target was to develop a single reference method for detection and enumeration of pathogenic *Vibrio* spp.. Currently, two proposals were under development based upon nucleic acid hybridisation for *V. parahaemolyticus* and real-time PCR. Further data on performance and applicability would be presented in due course.

2. NRLs were informed of the call for data and experts on *Vibrio* spp. in Europe from Codex, NRLs were encouraged to send information directly by May 31st. The CRL would provide contact details immediately after the meeting.

Comparative testing

3. NRLs performance in comparative testing for statutory determinands (*E. coli* and *Salmonella* spp.) was good. The workshop agreed that participation in comparative testing for *E. coli* and *Salmonella* spp. was mandatory for all NRLs. The frequency of distribution for CRL/HPA EQA was discussed, the CRL was asked to examine historic data and make recommendations on the minimum frequency of participation.

4. The workshop agreed that virus ring trials were valuable and should be continued to include matrix samples, focus should be on analysis of quantitative data, where available, in addition to presence/absence assessments.

5. The workshop noted that progress with respect to assessment of Official Control laboratories in comparative testing was good. Several NRLs presented data describing assessments based upon the use of scoring systems and had developed follow-up procedures to address unsatisfactory performance.

6. The workshop agreed that further proficiency testing for *Vibrio* spp. should be focused on targeted ring trials to assist in methodology development.

Date and time of next meeting

7. The next meeting would be in Weymouth U.K. on the 10th- 12th of May 2011.

List of papers for 9th Workshop of Microbiological NRL's

WS09/00	List of papers
WS09/01	Instructions on how to complete your expenses claim form
WS09/02	Expenses claim form
WS09/03	Report on the 8th Workshop of National Reference Laboratories for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs
WS09/04	Agenda
WS09/05	Delegates List
WS09/06	Microbiological Monitoring of Bivalve Mollusc Harvesting Areas Guide to Good Practice: Technical Application
WS09/07	Proposals for the application of analytical variability in the interpretation of *e. Coli* monitoring data for the classification of live bivalve mollusc production and relay areas
WS09/08	Comparison of Two and Three Class plans for evaluating E-coli levels in Live Bivalve Molluscs
WS09/09	Comparison of bivalve mollusc harvesting area classifications under EC Reg 854/2004 across EU member states (as of November 2009)
WS09/10	The Application of Sanitary Surveys in EU Member States
WS09/11	Norovirus outbreaks linked to oyster consumption in the UK, Norway, France, Sweden and Denmark 2010
WS09/12	Progress of the NRL methods expert working group for *Vibrio* spp, activities at CEN TAG3
WS09/13	NRLs participation and performance assessment Shellfish EQA scheme for *E. coli* and *Salmonella*
WS09/14	Community Reference Laboratory (CRL) - Proficiency Testing Schemes Enumeration of Escherichia coli and the detection of Salmonella spp. in Pacific oysters
WS09/15	NRLs participation and performance assessment in the *Vibrio* spp. PT
WS09/16	NRLs participation and performance assessment in the norovirus and hepatitis A PT
WS09/17	Community Reference Laboratory (CRL) guidance on performance assessment in proficiency testing and follow-up activities

Workshop declaration

This technical report is submitted in accordance with the requirements of Commission Regulation (EC) No 1754/2006 laying down detailed rules for the granting of Community financial assistance to Community reference laboratories for feed and food and the animal health sector, following the workshop of National Reference Laboratories for bacteriological and viral contamination of bivalve molluscs held in Ancona 18-19th May 2010.

Dr David Lees 20th July 2010

CRL Director

Dr Rachel Rangdale 20th July 2010

CRL Co-ordinator

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