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

# Report of the 13th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs.

# Weymouth, UK, 13th – 15th May, 2014.

## Final report version 2

## 16/12/2014

Contract Reference: C6095B

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|---|---|---|---|
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| **Document approved by:** | Rachel Hartnell | **Classification:** | Unclassified |

## Contents

**Forward**

This document summarises relevant information from the 13th workshop of National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs held at the EURL, Cefas, Weymouth 13th - 15th May 2014. It includes the workshop agenda, delegate contact information, workshop minutes, lists of associated papers, and the resolutions agreed by the meeting. Supplementary supporting information identified in this report can be accessed on the website of the EURL www.eurlcefas.org or may be supplied on request by the EURL. All requests should be made to the EURL co-ordinator.

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| FVO | | Telmo Valinhas | Invited speaker | Telmo.VALINHAS@ec.europa.eu |
| Exeter University | UK | Selina Church | Invited speaker | src211@exeter.ac.uk |

**AGENDA**

**13TH WORKSHOP OF MICROBIOLOGICAL NRLS, 13-14 MAY 2014, UK**

Venue: EURL,
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**Day 1 - Tuesday 13 May 9:30 - 18:00**

**1 Introductory meeting**

1.1 Welcome, introductions and apologies (paper WS13/01).
1.2 Domestic arrangements including reclaim of expenses (papers WS13/02, WS13/03).
1.3 Actions arising from the 12th workshop 2013 (paper WS13/04).
1.4 Agreement of the agenda (paper WS13/05).
1.5 EURL work programme 2014 (EURL) (paper WS13/06).
1.6 The new EURL website (EURL).

Coffee/tea break (10:30 -11:00)

**2 Official controls - Microbiological monitoring and classification**

2.1 Feedback from the latest round of MS audits on LBM (Telmo Valinhas, DG Sanco FVO).
Discussion and questions for NRLs
2.2 Feedback from the Commission Restricted Working Group - EU proposals for amendments to Regulation 854/2004 affecting E. coli and introducing virus standards (EURL).
2.3 Good Practice Guidance
2.4.1 Revisions to Technical Good Practice Guide issue 5 (paper WS13/08) (EURL)
2.4.2 Publication of Community Guide issue 2 (paper WS13/09) (EURL)
2.4 Community Guide practically - Considerations on practical application of some key points of the Community Guide needed for a further discussion on possible critical points and for a better specification. (NRL Italy - Mario Latini)
Discussion and questions for NRLs

Lunch break (13:00 – 14:00)

2.5 Shell Fish Sanitation Programmes - #AGDF-FOOD-SAFETY-QUALITY- FAO proposals for international guidance on best practice for application of shellfish sanitation programmes (paper WS13/10) (EURL)

2.6 Investigations of Arcobacter spp. in bivalve molluscs and Arcobacter butzleri bioaccumulation in mussels (NRL Italy - Francesca Leoni)

**3 Official Controls – Methods and Proficiency Testing (EURL)**

3.1 Published and forthcoming amendments/revisions to ISO (ISO TS) methods with relevance to Official Controls of bivalve shellfish (ISO TS 16649-3, ISO 6579, ISO 7218, ISO 6887-3).
3.2 Proposed amendments to the EURL generic protocol for enumeration of E. coli in bivalve shellfish (paper WS13/11).
3.3 EURL proficiency testing for E. coli and Salmonella (paper WS13/13, paper WS13/14)

Coffee/tea break (15:30 -16:00)

3.4 Investigations into the effects of different homogenisation methods for Common cockles (Cerastoderma edule) on the levels of E. coli – data from UK PT programmes (Ron Lee - UK NRL)

**4 Marine vibrios**

4.1 Identification of vibrio species using whole-cell MALDI-TOF mass spectrometry (NRL Germany)
4.2 Identification of type VI secretion systems in V. vulnificus (Selina Church, Exeter University)
4.3 Outputs from the 2nd International practical vibrio methods workshop - January 2014 (EURL)
4.4 Vibriosis in the USA and progress on trade discussions between the EU and USA (EURL)
4.5 Contributions from NRLs on marine vibrios

Dinner –7.30pm

**<u>Day 2 - Wednesday 14 May 9:00- 19:00</u>**

**5 Method validations**

5.1 Validation of ISO TS 21872 under the CEN mandate (M/381) and revision of ISO TS 21872 (EURL)
5.2 Update on the progress of the validation of ISO TS 15216-1 under the CEN validation (M/381) (Nicole Gustar - EURL)

Coffee/tea break (11:00 -11:30)

**6. Virus proficiency testing and methods**

6.1 EURL proficiency test norovirus and HAV – PT 50 (EURL) (paper WS13/16)
6.2 Quantitative determination of viruses in bivalve shellfish (EURL)
6.3 Quantification and dose response relation of noroviruses in oysters related to disease outbreak (NRL Denmark – Anna-Charlotte Schultz)
6.4 Discussion on future needs and questions for NRLs

Lunch break (1:00 – 2:00 pm)

**7. Virus monitoring and surveillance**

~~7.1 The fate of human noroviruses in shellfish water catchments in England and Wales (NRL UK – Carlos Campos)~~ CANCELLED

7.2 Follow up monitoring in a production area implicated in shellfish related outbreaks from French bivalve shellfish (NRL France – Soizick le Guyader)

~~7.3 Comparison of FRNA bacteriophage concentrations in wastewater and oysters determined by RT-qPCR and plaque assay (NRL Ireland –Sinead Keavney)~~ CANCELLED

7.4 Contributions from NRLs on virus monitoring and surveillance

Coffee/tea break (3:30 pm)

8. Topics of concern for the EURL/NRL network

9. Agreement of Workshop resolutions.

10 Any other business.

11 Date and venue for next meeting.

*Meeting close*

**Minutes of the 13th Workshop of Microbiological NRLs for Bivalve Molluscs, 13th - 14th May, 2014**

## Attendees

| | | |
|---|---|---|
| David Lees (DNL) (chair) | EURL Director | Cefas, UK. |
| Rachel Hartnell (RH) | EURL Coordinator | Cefas, UK. |
| Samantha Arkell (SA) | EURL | Cefas, UK. |
| James Lowther (JL) | EURL | Cefas, UK. |
| Johann Ladstatter (JLa) | NRL Austria | Ages Ilmu Wien, Spargelfeldstr |
| Sarah Denayer (SD) | NRL Belgium and Luxembourg | Scientific Service of Food-borne Pathogens, Brussels |
| Vanya Chikiova (VC) | NRL Bulgaria | National Diagnostic and Research Veterinary Institute, Sofia. |
| Anna Charlotte Schultz (ACS) | NRL Denmark | Institute of Food Safety and Nutrition, Soborg. |
| Soizick Le Guyader (SG) | NRL France | Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER) , Nantes. |
| Pascal Garry (PG) | NRL France | Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER), Nantes. |
| Eckhard Strauch (ES) | NRL Germany | Federal Institute for Risk Assessment, Berlin. |
| Ntina Vasileiadi (NV) | NRL Greece | Institute of Food Hygiene of Athens, Athens. |
| Erzsebet Adrian (EA) | NRL Hungary | Central Agricultural Office, Food & Feed Directorate, Budapest. |
| Elisabetta Suffredini (ELS) | NRL Italy | Istituto Superiore di Sanità (ISS) Rome. |
| Francesca Leoni (FL) | NRL Italy | Centro di Referenza Nazionale per il controllo microbiologico e chimico dei molluschi bivalve vivi, Ancona |
| Mario Latini (ML) | 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. |
| Audinga Verbickiene (AV) | NRL Lithuania | National Food and Veterinary Risk Assessment Institute, Lithuania |
| Irene Pol-Hofstad (IPH) | NRL Netherlands | National Institute of Public Health and the Environment (RIVM), Bilthoven. |
| Iwona Kozyra (IK) | NRL Poland | National Veterinary Research Institute, Pulawy. |
| Remiguusz Pomykala (RP) | NRL Poland | National Veterinary Research Institute, Pulawy. |
| Sonia Pedro (SP) | NRL Portugal | Portuguese Institute of Sea and Atmosphere, Lisbon. |
| Alina Popescu (AP) | NRL Romania | Institute of Diagnosis and Animal Health, Buharest. |
| Urska Henigman (UH) | NRL Slovenia | Institute for Food Hygiene and Bromatology, Ljubljana. |
| Zuzana Kubicova (ZK) | NRL Slovakia | State Veterinary and Food Institute, Janoskova |
| Cristina Acebal (CA) | NRL Spain | Agencia Espanola de Seguridad Alimentaria, Majadahonda, Madrid. |
| Cristina Alvarez Alvarez( CAA) | Invited expert | 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. |
| Craig Baker-Austin (CBA) | NRL UK | Cefas, Weymouth. |
| Ines Skoko (IS) | NRL Croatia | Croatian Veterinary Institute, Split. |
| Franklin Georgsson (FG) | NRL Iceland | Matis, Reykjavik. |
| Liv Marrit Rorvik (LMR) | NRL Norway | The Norwegian School of Veterinary Science, Oslo. |
| Mette Myrmel (MM) | NRL Norway | The Norwegian School of Veterinary Science, Oslo. |
| Telmo Valinhas (TV) | Invited expert | Representing FVO, European Commission |
| Selina Church (SC) | Invited expert | Exeter University, Exeter |

Apologies
Paolo Caricato DG SANCO, European Commission
Sinead Keaveney, Bill Dore, NRL Ireland

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

**Acronyms**

| | | | |
|---|---|---|---|
| CA | Competent Authority | NFRDI | National Fisheries Research and Development Institute |
| CG | Community Guidance | NoV | Norovirus |
| CEN | Comité Européen de Normalisation | NRL | National Reference Laboratory |
| DG SANCO | Directorate General for Food and Consumers | NSSP | National Shellfish Sanitation Program |
| DNA | Deoxyribonucleic acid | PHE | Public Health England |
| EQA | External Quality Assessment | PT | Proficiency Testing |
| EU | European Union | SC | Scientific Committee |
| EURL | European Union Reference Laboratory | SD | Standard Deviation |
| FAO | Food and Agriculture Organisation | STW | Sewage Treatment Works |
| FDA | Food and Drug Administration | SS | Sanitary Survey |
| FVO | Food and Veterinary Office | TAG | Technical Advisory Group |
| GPG | Good Practice Guide | TS | Technical Specification |
| GI, GII | Genogroup I and Genogroup II | UK | United Kingdom |
| HAV | Hepatitis A Virus | US | United States |
| ISO | International Standard Organisation | WP | Work Programme |
| KSSP | Korean Studies Summer Programme | WG | Working Group |
| LBM | Live Bivalve Molluscs | WHO | World Health Organisation |
| MOF | Ministry Of Finance | WWTP | Waste Water treatment plant |
| MS | Member State | | |

**1 Welcome meeting**

**1.1 Welcome and introduction**

DNL opened the meeting, followed by round table introductions.

**1.2 Domestic arrangements including reclaim form (WS13/02, WS13/03)**

Delegates were given instructions on electronic submission of forms and supporting information to enable payment of expenses.

**1.3 Actions arising from the 12th workshop (paper WS13/04A)**

Actions captured in paper WS13/04A are included as Annex I of this report. All actions identified were either complete or covered separately as agenda items. Delegates were asked to review the accuracy of papers WS13/04B1 (waiving monitoring results), WS13/04C1 (prohibition zones) and WS13/04D1 (management of outbreaks) within two weeks of the workshop and report any changes in practices. The documents would then be updated and posted on the EURL website.

**Action 1 – NRLs to forward any comments regarding papers WS13/04B1, WS13/04C1 and WS13/04D1 to DNL** — **NRLs**

**1.4 Agreement of the agenda (WS13/05)**

The workshop agenda (WS13/05) was agreed.

**1.5 EURL Work Programme 2014 (WS13/06)**

RH presented the EURL work programme agreed with DG Sanco for the calendar year 2014.

**1.6 EURL website**

The new EURL website was launched with NRL delegates. The website had undergone substantial changes in 2013/14. The philosophy of the new website was to enable public access to all documentation excepting unpublished data or presentations given at the annual workshop. A restricted papers section including workshop information required registration and password access. RH would register all NRL attendees and distribute a password. **RH**

**Action 2 – RH to send information to all NRLs regarding registration for the website.** **RH**

**Action 3 – RH to register and send all NRLs a password to access new website.**

NRL delegates were asked to check the network page of the website and notify the EURL (SA and RH) if the information given regarding addresses and contact details of the NRL was incorrect. **NRLs**

**Action 4 – NRLs to forward any updated contact details to SA for inclusion on website.**

**2 Official Control - Microbiological monitoring and classification**

**2.1 Feedback from the round of MS audits on LBM (TV)**

TV presented the summary audit findings with emphasis on official control methods. It was noted that the audits which were completed between 2011 and 2013 were the first undertaken since the implementation of the new hygiene package.

TV informed the workshop that the majority of deficiencies identified in the latest round of audits were resolved or in progress. MS noted that in some MS additional classifications existed e.g. 'D' prohibited. ES asked for an opinion regarding timeframes for completing sanitary surveys (EU Community guidance states by 2015, *cf.* Regulation 854/2004 does not specify). It was acknowledged that in most MS there is now an acceptance that the Community guidance should be followed.

**2.2 Feedback from the Commission Restricted Working Group – Codex *E. coli* standards**

DNL gave an overview of developments from the MS restricted WGs since the last workshop. In summary, there was support for the removal of the *Salmonella* criterion for LBM, in accordance with Codex and EURL opinions. Inclusion of provision for detection of *Salmonella* Typhi and Paratyphi were expected for at risk products. Further progress would be reported in due course.

The Codex *E. coli* criterion for end-product testing and standards for Class A production areas was discussed. In brief, there was support for application of a three class plan (3CP) for class A areas where n=5, c=1, m=230 and M=700 *E. coli* MPN/100g applied over time within a specified review period. Application of the 3CP as a microbiological criterion for end products was less popular amongst member of the WG and an outcome was likely to take longer to reach. This was in particular acknowledgment of the increased additional burden on Food Business Operators (FBO). Optional retention of retaining one sample of ≤ 230 *E. coli* MPN/100g was under discussion. Further progress would be reported in due course.

TV questioned the current linkage between Reg 854/2004, 853/2004 and 2073/2005 with respect to LBM placed on the market. DNL confirmed that within the restricted WG MS supported the separation of classification requirements and those for end product.

With respect to viruses, DNL summarised the progress and discussion around the

introduction of virus controls (Annex II – virus discussion paper presented in 2013 – FOR INFORMATION ONLY). In brief, all MS represented on the restricted WG agreed that the issue of virus contamination needed to be tackled to protect public health. However, a minority do not support the introduction of a virus criterion or criteria. Specifically, the UK has expressed the view that virus contamination should be addressed through HACCP and the introduction of exclusion zones and was reported to be developing an alternative proposal for submission to the Commission. The UK NRL confirmed that it was not familiar with the technical details of this proposal. SG noted that the use of prohibition zones was a good approach; this view was generally supported by the workshop.

All members of the restricted WG supported the proposed baseline survey *cf* Commission Decision 2010/75/EU on *Listeria* to supplement existing datasets examined in the EFSA report on noroviruses in oysters. This view is not backed by the Commission who consider existing EFSA opinion as sufficient evidence for the introduction of criteria for viruses. DNL reported that it was expected that the EURL would be asked to coordinate a call for existing datasets, to potentially increase the knowledge base. The EURL would develop quality assurance criteria for acceptance of data.

SG reported that following discussion at the CA level, it was likely that France would take the Commission position on the lack of need for a baseline survey. Furthermore SG informed the workshop that it was the view of NRL France that a qualitative (presence-absence) standard was preferable to a quantitative criterion. A qualitative criterion would, in their view, obviate the requirement for a baseline survey. DNL noted that this view had not been stated by France at the restricted WG.

ACS questioned the scientific benefits of a baseline survey given the demonstrable seasonality and large range of environmental conditions that can impact viral contamination. DNL confirmed that for a baseline survey to generate usable data it would need to be extensive, it was acknowledged that this would require substantial commitment of resources. An alternative approach might be to establish the requirement for monitoring but allow a substantial lead in period for implementation of controls. This would enable generation of data over an extended period and assist in establishing both technical capabilities across the network and provide valuable information on virus levels across a broader range of MS than considered in the EFSA report (France, Ireland and UK).

MS questioned the likelihood of the retention of existing classifications (A, B, C) if virus criteria were introduced. DNL confirmed that discussions had centred on the application of virus criteria in the production area with maintenance of existing classifications. Virus criteria may therefore be more analogous to biotoxin monitoring programmes operated in MS. With closure and reopening requirements based on viruses. MS concluded that quantitative criteria may be problematic because of observed differences in performance amongst laboratories testing for viruses in LBM. It was acknowledged that an implementing period would be required for laboratories to adopt harmonised methods.

The option of cooking LBM where viruses were either present or products were not tested was discussed (see Annex II for further details). Following discussion, it was noted that across the EU there were different societal preferences with respect to cooking bivalve shellfish. In parts of Europe (e.g. Sardinia and Denmark) cooking labels were already used for some products. DNL confirmed that the EURL interpretation of the proposed legislation was that norovirus positive product could be placed on the market if a cooking label was used. SG considered that an upper limit for cooked product should be considered as levels of $>10^5$ genome copies per gram may not be destroyed through cooking.

RL identified that there may be an inconsistency in the proposed option for cooking with respect to viruses as cooking was not by definition an approved heat treatment (Reg 853/2004). Non-depurated or relayed class B, or class C product required approved heat treatment prior to placing on the market; a cooking label lacked this level of stringency.

SP asked about the potential for the use of processes other than cooking. DNL confirmed that neither this, nor the precise wording on a cooking label had been discussed at the restricted WG level.

**2.2.1 Community Principles Guidance - issue 2 (WS13/09)**

DNL presented the amendments to the guidance with specific respect to the annexes setting out requirements for export of LBM to the US. DNL outlined the main issues identified by US FDA with respect to the EU LBM sanitation programme:

- The lack of prescriptive detail in EU regulations,
- Unacceptably high levels of faecal contamination permitted in harvesting areas, other than Class A,
- The lack of sufficient controls across the EU.

The US FDA had confirmed that for export to the US, the FDA would audit MS against the Community Principles Guidance and the Good Practice Guide. DNL asked that NRLs should communicate the requirements for audit to their CA (Resolution 1)

**NRLs**

RL noted that prohibition zones as applied in the US are based on bacteriological criteria not viruses. The presence of a recent publication on norovirus levels and prohibition zones in the US was noted (Goblick et al 2011, https://eurlcefas.org/media/13633/ws12_09b.pdf) . It was confirmed that for US zones size was generally calculated on the theoretical probability of meeting the 14 FC MPN/100ml standard assuming an untreated discharge. In addition, a 1:1000 dilution was expected for treated discharges.

**2.2.2 Revisions to Technical Good Practice Guide (GPG) issue 5 (WS13/08)**

RL presented paper WS13/08 outlining the proposed changes to the GPG. The final draft was due for completion by the end of May 2014.

The EURL agreed to provide assistance to NRLs on the calculation of the required sizes for prohibition zones on request.

RL requested views from the workshop on a number of outstanding issues:

- What is the minimum sampling frequency,

It was confirmed that baseline sampling frequency should be monthly. Following presentation of evaluation of datasets from NRLs (RL) it was agreed that descriptive statistics could not be used to establish criteria for reduced bimonthly

- Sample transport criteria
- Expression of measurement uncertainty
- Assessment of classification
- Responses to alert states

RL presented results of evaluation of multiple datasets supplied by NRLs. These demonstrated that it was not possible to identify a robust procedure using descriptive statistics to inform stability assessments to provide justification for decreased monitoring frequency. Therefore following discussion it was agreed that and that this would be noted in the Good Practice Guide[2]. 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 Community Guide and considerations on the practical application of some key points leading to further discussion on possible critical points for a better specification**

NRL Italy (ML) presented on areas that in the view of NRL Italy required further scientific

consideration.

**2.5 FAO proposals for international guidance on best practice for application of shellfish sanitation programmes (WS13/10)**

RH presented paper WS13/10 and explained that 2 approaches exist globally, the United States 'National Shellfish Sanitation Program' (NSSP) and the EU Food Hygiene Regulations. The International workshop on Molluscan Shellfish Safety (IWMSS) has held workshops in both 2009 and 2012 with the recommendations to develop a technical and scientific best practice. Providing a flexible framework for the technical application of current shellfish sanitation programs and the development of new programs by countries that do not currently have such health controls. Also to enable a broader application of shellfish sanitation programs in reference to protecting a larger proportion of the world's population from shellfish-associated infections and to assist a wider range of countries to trade in safe shellfish. The proposal put forward to the WHO and the FAO by IWMSS, has been supported by the FAO under their Food safety and quality programme. Moving forward the FAO will produce a more detailed scoping document, with a call for experts and the formation of an expert working group, 2 meetings will be organized with subgroups covering topic headings by means of electronic working groups. The FAO will then publish a technical guide. RH suggested that MS NRLs could possibly contribute; the EURL will keep the group informed.

**2.6 Investigation of *Arcobacter spp.* in bivalve molluscs and *A. butzleri* bioaccumulation in mussels. FL outlined the 15 different species and where they are commonly found**

NRL Italy (FL) gave a presentation on *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* and human infections, outlining the source, cause and symptoms. The potential for association with shellfish was discussed, it was noted that to the best of their knowledge no shellfish associated illness had been reported.

**3 Official Controls – Methods and Proficiency Testing (EURL)**

**3.1 Published and forthcoming amendments/revisions to ISO (ISO TS) methods with relevance to Official Controls of bivalve shellfish (ISO TS 16649-3, ISO 6579, ISO 7218 and ISO 6887-3)**

The EURL (RH) informed the group about the progress of ISO standards currently under revision. In brief ISO TS 16649-3 would be published as a full standard following the FDIS vote, publication was expected in early 2015. ISO 6579 was within the mandate (381) and therefore publication would follow the timelines set by AFNOR and project leaders. ISO 7218 was under full revision, following the publication of the amendment in 2013. ISO 6887-3 was nearing the end of the process and would be submitted for FDIS within the next few months in parallel with the other parts.

**3.2 Proposed amendments to the EURL generic protocol for enumeration of *E. coli* in bivalve shellfish (WS13/11)**

The EURL (RH) presented paper WS13/11, and discussed those points which were highlighted in the paper and asked for any comments to be forwarded to the EURL after the close of the workshop. RH also demonstrated the MPN table calculator which is particularly useful if more than 3 dilutions are being undertaken. The group suggested that a footnote be included to explain why the difference (Resolution 10).

**NRLs/EURL**

**3.3 EURL proficiency testing for *E. coli* and *Salmonella* (WS13/13 and WS13/14)**

The EURL (RH) presented results for PT 49 which consisted of 2 samples, sample 1 Pacific Oyster (*C. gigas*) and sample 2 Common mussels (*Mytilus edulis*). Fifty laboratories
(26 NRLs) participated in the distribution (WS13/13).

*E.coli*: Thirty three (33) laboratories returned results within +3 SD of participants' median for sample 1 (Oyster) with 40 laboratories returning results within +3 SD of participants'

median for sample 2 (Mussels). Eleven (11) laboratories for sample 1 and 4 laboratories for sample 2 reported results which fell outside ±3 SD of participants' median. Nineteen (19) laboratories had score deductions for MPN value(s) not consistent with guidance in ISO 7218 for interpretation of 5 x 3 MPN tables

*Salmonella* spp.: Forty-six (46) laboratories returned results and reported the presence of *salmonella* in sample 1 as expected. *Salmonella* spp. reference results were considered void for sample 2 due to laboratory quality control failures, although the results that were reported did indicate that the sample contained a low level of naturally acquired contamination which was close to the limit of detection (LOD) or the levels throughout the batch were not consistent, so for this reason participants results were not allocated a score for this distribution.

The EURL (RH) presented the results reported by NRLs for the EURL/PHE EQA scheme for *E. coli* and *Salmonella* spp. detection PT 52 (WS13/14). Samples consisted of LENTICULE™ discs containing fully characterised bacterial isolates. Twenty laboratories (19 Member state NRLs and 1 EFTA country) participated in one, two or all of the distributions. All laboratories achieved a cumulative total of >70% for the EURL whole animal and 1 or more EURL/PHE distributions

**3.4 Investigations into the effects of different homogenisation methods for common cockles (*Cerastoderma edule*) on the levels of *E. coli* – data from UK PT programmes**

The UK NRL (RL) presented the results of a comparison in homogenisation methods 2011 and 2012. RL detailed why this comparison had taken place, stating that it arose after it became apparent that 2 types of homogenisation are used, stomaching or blending. UK OCLs were asked to complete a questionnaire to determine their preference with 10 laboratories using a stomacher while 5 laboratories use a blender. Mean $\log_{10}$ results from the UK NRL proficiency test distributions in 2008, 2009 and 2010 were compared using general linear modelling. The bivalve species used in those PT distributions were *Crassostrea gigas* (Pacific oysters) and *Mytilus* sp. (mussels). In 2011 and 2012 *Cerastoderma edule* (cockles) were used in the PT distributions along with Pacific oysters, where it was found that the variability of cockle results appeared to be greater than that of the Pacific oyster results, an investigation followed in conjunction with the UK NRL laboratory network. The conclusions being:

- Homogenisation method significantly affects the *E. coli* results for cockles
- But not for Pacific oysters or mussels
- Tendency to both more variable and lower results when a stomacher is used
- UK NRL advice to UK OCLSs
- Use blending for samples of cockles

A discussion followed regarding the use of blenders or stomachers in reference to the homogenisation of clams and cockles. Five of those present (Spain, Croatia, France, Portugal and The Netherlands) reported they used a stomacher. It was suggested that the next PT distribution should include clams. (Resolution 11)

**EURL**

**4 Marine vibrios**

**4.1 Identification of vibrio species using whole-cell MALDI-TOF mass spectrometry.** NRL Germany presented some interesting data on the application of MALDI-TOF to identification of *Vibrio* spp.

**4.2 Investigating pathogenesis and virulence of the human pathogen, *Vibrio vulnificus***

SC gave an overview of the work being carried out by Exeter University in regards to the pathogenesis and virulence of the human pathogen *Vibrio vulnificus*. The aims being to identify novel virulence factor in *V. vulnificus* and to carry out WGS and genome annotation of 10 *V. Vulnificus* isolates, (5 clinical and 5 environmental).

**4.3 Outputs from the 2nd International practical vibrio methods workshop January 2014**

CB-A outlined the aims of the workshop stating that the focus was to bring together researchers from across Europe that were interested in *Vibrio* pathogens, in particular vibrios of human health relevance, with the objective to provide a means of sharing data, ideas and experience in the area of pathogenic vibrios from the perspective of shellfish safety. Presentations were given that encompassed may relevant aspects in regard to vibrios in a European context. Several important outputs from the workshop were agreed upon, including;

1. Greater collaborative research effort across the EU and also internationally, in particular greater efforts to share strains between and within countries and to help provide a central resource for *Vibrio* isolates,
2. Provision of *Vibrio* data (e.g. abundance, distribution, spp) on a range of shellfish matrices other than pacific oysters. Sharing of methods both between and within laboratories,
3. Efforts to establish an electronic 'working group' on vibriosis in Europe,
4. Dissemination of 'lessons learned' from vibriosis issues in the USA.

CB-A intimated that possibly a 3rd workshop could take place in summer of 2015.

**4.4 Vibriosis in the USA and progress on trade discussions between the EU and USA .** CBA provided an update on the situation with respect to trade between the EU and US and potential issues of vibrio related illness.

**5 Method Validations**

**5.1 Validation of ISO TS21872 under the CEN mandate (M/381) and revision of ISO TS 21872**

RH gave an overview of the revision of ISO TS 21872 parts 1 – 2, these being;

1. Two parts have been merged into a single standard,
2. PCR included as an optional method for identification of the target pathogens,
3. Removal of the saline triple sugar iron agar test,
4. Removal of the ornithine decarboxylase test (lysine decarboxylase and arginine dihhydrolase considered sufficient),
5. Modify the growth in peptone water by adding:
   +/- at 6% NaCl for *V. Cholerae*
   +/- at 10% NaCl for *V. parahaemolyticus*

RH updated the group on the progress so far;

- Revised draft submitted to ISO October 2013 following comments from this group and the expert working group
- Consultation at ISO level agreed to register on the ISO work programme – vote 100% positive, with 33 comments (US, ES and NL) 20 technical, 5 general and 8 editorial)
- Comments addressed via the working group, new draft in preparation (waiting for the validation)
- Now at the CD stage, DIS to be registered after this meeting and before the ISO plenary in June.

There was a selection of PCR targets carried out in PT38, these were;

- *ToxR* for *V. parahaemolyticus* (Kim *et al*, 1991, Powell *et al*, 2012)
- *trh* and *tdh* for the detection of pathogenicity markers of *V. parahaemolyticus* (Bej *et al*, 1999, Nordstrom *et al*, 2007)

- *VVH* for *V. vulnificus* (Hill *et al*, 1991, Campbell and Wright 2003)
- *prVC* or HLYA for *V. cholerae* (Chun *et al*, 1999, Lyon 2001)

July 2013 - distribution 1 – *V. parahaemolyticus* was sent out, the matrix being, seafood, food type fresh, raw bivalve molluscs. The sample was made up of depurated oysters spiked with log phase bacterial cultures.

November 2013 - distribution 2 – *V. vulnificus* and *V. cholerae* was sent out, the matrix being seafood; food type cooked crustacean. The sample was made up of cooked, frozen, peeled prawns from a commercial retail establishment.

Data was received by participating laboratories for *V. parahaemolyticus*, and the ILS generated a very large dataset comprising hundreds of separate results for;

1. Biochemical and molecular species identification
2. Identification of pathogenicity markers (*tdh* and *trh*)
3. Primary and secondary enrichments at 37°C and 41.5°C

RH summarised the results;

- For *V. parahaemolyticus* in raw seafood (oysters) results (provisional)
  - Sensitivity -between 72 and 97% dependent upon the level of contamination
  - Specificity - 90 - >100% dependent upon method of calculation (to be discussed)
  - $LOD_{50}$ = 0.67 (0.49-0.91) cfu/g (result expressed in 25g)
- For *V. vulnificus* in raw seafood (oysters) results (provisional)
  - Sensitivity -between 34 and 62 % at low contamination level dependent upon the method of calculation (to be discussed)
  - Specificity – 97%
  - $LOD_{50}$ = 40 (22.4-60.7) cfu/g (result expressed in 25g)

The EURL proposed a vibrio PT distribution in 2015.

**5.2 Update on the progress of the validation of ISO TS 15216-1 under the CEN validation (M/381)**

The EURL (NG) gave an overview of the validation of ISO TS 15216-1 stating that TAG4 is carrying out the validation TS 15216-1 (quantification) under CEN mandate M/381. The method describes the detection and quantification of HAV and NoV genogroups I and II from foodstuffs, with the ISO being split into 2 parts;

- Part 1 describes the quantification of norovirus GI and GII and HAV,
- Part 2 describes the qualitative detection of these 3 targets.

The technical specification was published in 2013 and the final (validated) method is due to be re-published as a full standard in 2017. The work was started in 2012 and there were 7 matrices and 3 targets. The experimental work was completed by April 2014, and the final report being due by year end, 2014.

- The validation study comprised of 2 main elements;
  - The single laboratory performance characterization – Part 1,
  - The interlaboratory study (ILS) – Part 2.
- Performance characterisation tested samples contaminated with 0.5 log dilution series of target viruses.
- The performance characterisation study has provided data to determine
  - Linearity
  - Limit of detection
  - Limit of quantification
  - Repeatability

The preliminary work for oysters comprised of;

- Establishing a method to generate a serial dilution of oyster test samples,
- Several alternative methods included;
  - bioaccumulating oysters with a dilution series of a faecal suspension,
  - spiking shellfish homogenates with a serial dilution of faecal suspension,
  - using a blender to homogenise positive digestive gland to produce serial diluted samples using negative glands as diluents,
- Blending approach a fairer test of the method cf. bioaccumulation and tests more of the method than spiking; but needed to establish proof-of-principle,
- Bioaccumulated positive material was used to spike negative glands and blended to make a homogenous sample (down to $10^{-5}$).

The interlaboratory study;

- The ILS data will be used to show the repeatability and the reproducibility of the method,
- Expert laboratories were required to generate matrix specific test material for distribution to those participating laboratories,
- The expert laboratories carried out testing on multiple sub-samples of the test material to generate reference results at different time points,
- Duplicate samples at four levels of contamination for each target virus (negative, low, medium and high) were supplied,
- Analysis was carried out within a specific time-frame for norovirus GI, GII and HAV.
- 6 laboratories out of the 10 involved in testing the oyster matrix for the ILS were NRLs.

NG summarised the progress to date;

- All the data for each of the 7 matrices has been received,
- Analysis of the data to determine the method characteristics is underway,
- The report is due to be submitted at the end of 2014 with the conversion of ISO TS 15216 to full standard by 2017.

The discussion that followed established that Part 2 of ISO TS 15216 was not included in the mandate and will not be published in 2017 according to the mandated item timeframes..

## 6 Virus proficiency testing and methods

### 6.1 EURL proficiency test norovirus and HAV – PT 50 (WS13/16)

The EURL (JL) gave an overview of PT50 (WS13/06), detailing the samples used, participation, laboratory performance, harmonisation of methods, quantification and the new proficiency testing scoring method.

Six samples were distributed, these being;

- Shellfish Sample 1 : Shucked Pacific oysters (C. gigas) bioaccumulated with GI and GII norovirus from human faeces,
- Shellfish Sample 2 : Shucked purified Pacific oysters from a UK commercial harvesting area. Tested negative for GI, GII and HAV,
- Shellfish Sample 3 : Shucked purified Pacific oysters bioaccumulated with HAV cell culture supernatant,
- Shellfish Sample 4 : Common mussels (M. edulis) bioaccumulated with GI and GII norovirus from human faeces and HAV cell culture supernatant,
- LENTICULE™ 1 – Prepared using mix with GI from human faeces,
- LENTICULE™ 2 – Prepared using mix with GI and GII norovirus from human faeces and HAV cell culture supernatant.

Of the 44 laboratories which received test material, 17 were NRLs 15 of which were within MS's while 12 were third country laboratories. Two NRLs did not return results. One laboratory tested for HAV only, 3 tested for norovirus only of which 1 tested only shellfish samples

Overall accuracy has improved with 97%; 33/42 laboratories scoring 100% for all sample/determinand combinations. Overall specificity has reached 99%, with only 2 false positive results. 96% sensitivity in lenticule samples has been achieved with sensitivity in shellfish samples reaching 93%.

There has been a continued increase in CEN TAG4/ ISO 15216 type methods for shellfish samples;

- 39/42 laboratories (93%) use CEN-type ProK virus extraction methods
- 31/42 laboratories (74%) use Nuclisens magnetic RNA extraction reagents
- 40/42 laboratories (95%) use one-step real-time RT-PCR
- 17/42 laboratories (40%) use Ultrasense PCR reagents

- NOTE 16/33 (48%) of laboratories scoring 100% accuracy use Ultrasense, cf. 1/9 (11%) of laboratories scoring <100%

JL informed the group that large spreads of both quantities and Ct values have been reported with 39 of 42 laboratories provided Ct values for at least one sample/determinand and 28 of 42 laboratories provided quantities for at least one sample/determinand.

A new proficiency testing scoring method was introduced, this being a 3 tier system,

- A = 100% accuracy; "satisfactory"
- B = maximum of one mistake; "questionable"
- C = two or more mistakes; "unsatisfactory"

with Norovirus (GI and GII) and HAV being scored separately.

JL summarised the conclusions as, a continued improvement in the overall performance (qualitative), with continued harmonisation of the methodology, with quantification being closer for laboratories using similar methodologies although there is still a wide variation in reported quantities.

**6.2 Quantitative determination of viruses in bivalve shellfish**

The EURL (JL) gave a presentation on the problems with dsDNA Quantification Standards, with JL outlining;

The control materials,

- TS 15216-1 which was developed by TAG4,
- CEN bench protocols (and EURL generic protocol v2),
- Numerous protocols and publications,
- Cefas has used circular DNA diluted in water ~2006-2012 which have produced generally good results.

The problems with dsDNA,

- From 2012-2013 increasing number of unusual results with dsDNA control materials:-
  - Linearity of standard curves,
  - Between-aliquot reproducibility,
  - Stability of stored solutions.
- Investigations pinpoint use of water as diluent root cause of problems
- Possibly due to pH changes due to temperature or atmospheric contamination with e.g. dust,
- Cefas switched to use of TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA) for dilution with much improved and very consistent results,
- No evidence of PCR inhibition by 1mM EDTA,
- 2ng/ul sheared salmon sperm DNA added as carrier for long term storage.

Circular vs. linear DNA,

- Over course of investigations it has emerged that circular DNA may be amplified at lower efficiency cf. linear,
- Investigations at Cefas have shown that circular DNA preparations produce higher Ct values than the equivalent concentration of linear DNA (e.g. linearised plasmid or PCR product),
- That there is some variability between different preparations of plasmid DNA (different conformations?),
- Linear DNA molecules less variable and with higher PCR efficiency,
- Cefas now uses linear PCR product amplified from plasmid using pTAG primer sets as dsDNA quantification control,
- New version of EURL generic protocol released to incorporate change to use of linear DNA diluted with TE buffer as quantification control,
- TAG4 discussed changes to TS 15216-1 in June.

Sequence issues,

- It has emerged that there are sequence errors in norovirus GI and GII inserts in control plasmids used at Cefas,
- For GI errors are all in non primer-binding regions; minimal effect on qPCR efficiency,
- For GII, preps contain mixture of two different inserts,
  - Y sequence contains major mismatches in probe binding region,
  - Z sequence contains minor mismatches at extreme 3' end of amplicon (REV primer binding).
- Y sequence, Z sequence and mixtures of both sequences provide different qPCR efficiencies and curve shapes,
- Labs receiving GII plasmid from Cefas may have received either a mixture of Y and Z, or Y inserts only,
- Currently generating new constructs (GI and GII) with no sequence errors for distribution to all labs requiring replacements.

Effects of issues with dsDNA standards,

- Labs using water as diluent may experience problems with linearity, reproducibility and stability of standards,
- Labs using circular plasmids may overestimate concentrations in samples cf. use of linear DNA,
- Labs using GII Y sequence may overestimate concentrations in samples cf. Z sequence or other sequence with no errors,
- PT50 distributed standard materials were linear DNA molecules diluted in buffer, GII Z sequence used,
- For 135 samples (at 17 labs) quantified using both distributed and lab's own standards; in 110 cases (81%) levels lower using distributed standards (Ct values for distributed standard materials lower).

Conclusions

- Cefas recommends use of linear DNA molecules for quantification,
- Dilution in TE buffer (with salmon sperm DNA for storage),
- Generating new constructs with no sequence errors – will provide replacement aliquots to NRLs,
- New version of EURL protocol issued; changes to TS 15216-1 to be discussed,
- Commercial/ready-to-use dsDNA (lenticules) in development.

There followed a discussion in which JL recommended those attending download version 3 of the Generic Protocol, and also stating that the EURL would be willing to supply NRLs

with free lenticels.

**6.3 Quantification and dose response relation of noroviruses in oysters related to disease outbreak**

ACS gave a presentation on quantification and dose response in relation to norovirus in oysters related to disease outbreaks. There followed a discussion regarding outbreaks, in which ACS stated that it has been noted that active surveillance shows an exceptional level of under-reporting. ACS also stated that French producers are testing for norovirus in various labs with variable laboratory performance.

**7 Virus monitoring and surveillance**

**7.1 Cancelled**

**7.2 Follow up monitoring in a production area implicated in shellfish related outbreaks from French bivalve shellfish**

SLG gave a presentation outlining the follow up monitoring of a production area which was implicated in a shellfish related outbreak from French bivalve shellfish, which covered the winter norovirus protocol, this being an official document which has been distributed to all regions. This protocol gives an explanation of how shellfish may be contaminated, and gives instructions on how and why an area is closed and also the re-opening procedure. There followed a discussion on the number of samples received over a 12 month period, 2012 – 2013, and the number of outbreaks recorded from various production areas.

**7.3 Cancelled**

**8 Topics of concern for the EURL/NRL network**

RH gave an overview of the topics of concern for the EURL/NRL network, these being,

Commission initiative

- To improve harmonization across the network of EURLs in the Food and Feed and Animal Health sectors
  - Introduction of numerical performance indicators for EURL, based on Baldridge Criteria,
    - Proficiency testing
    - Method development
    - Training
    - Qualifications of staff
    - Accreditation
    - Publication/presentations
  - Change to Financing procedure to include a Commission work programme

Performance of NRLs

- Specifically relates to the Commission Protocol which covers *inter alia*:
  - underperformance (i.e. failure in proficiency test)
  - lack of collaboration by the NRLs with the EURL
- Two step **two step protocol**
- **Phase 1 (confidential)**
- Contact the NRL and provide assistance
- **Phase 2**
- Inform the Commission

Feedback from NRLs

- Equally important that we receive feedback to help us to improve the provision to NRLs
- Your opportunity to raise issues
- Please contact us if you have suggestions for improvements

| | |
|---|---|
| **9 Agreement of the Workshop resolutions**<br>The workshop resolutions were agreed.<br><br>**10 Any other business**<br>No other business was raised.<br><br>**11 Next meeting**<br>The workshop accepted that the next meeting would be held at IFREMER, Nantes, 19th - 21st May 2015 **(Resolution 18)**, **POST MEETING NOTE** the dates have now been revised to 20th – 22nd May 2015 by mutual consent. | |

**Actions of the 13th Workshop of Microbiological NRLs for Bivalve Molluscs, Cefas, Weymouth, 13th - 15th May, 2014.**

| Action | Owner | Completed | Notes |
|---|---|---|---|
| **General Administration** | | | |
| 1. NRLs to forward any comments regarding papers WS13/04B1, WS13/04C1 and WS13/04D1 to DNL | NRLs | | |
| 2. RH to send information to all NRLs regarding registration for the website. | RH | | |
| 3. RH to register and send all NRLs a password to access new website. | RH | | |
| 4. NRLs to forward any updated contact details to SA for inclusion on website. | NRLs | | |
| **Official Control - Microbiological monitoring and data interpretation** | | | |
| 1. Regarding export of LBM to US - NRLs in MS with an interest in export to US to inform CA that US FDA would audit against compliance with the Community Guidance (issue 2) and Good Practice Guide (issue 5) | Priority NRLs | | |
| 2. Regarding stability assessment – EURL to include in the Good Practice Guide (issue 5) text stating that it was not possible to provide justification for decreased monitoring frequency using descriptive statistics (Note. reduced sampling frequency only justified in areas meeting the definition of remote in the Community Guide and Good Practice Guide) | EURL | | |
| 3. Regarding control of sample transport – Good Practice Guide (issue 5) to include reference to ISO 6887-3 (as revised) for sample transport criteria | EURL | | |
| 4. Practices on waiving results of monitoring programmes - A summary of all NRLs responses would be collated by the EURL | EURL | | |
| 5. EURL generic protocol for enumeration of *E. coli* in LBM – NRLs advised to use EURL generic protocol for enumeration of *E. coli* in LBM which have been corrected with respect to errors in 5x5 MPN tables in ISO7218:2007/FDAM1:2013 | NRLs | | |
| 6. EURL generic protocol for enumeration of *E. coli* in LBM – EURL to include note explaining the technical justification for deviations from ISO7218:2007/FDAM1:2013 for accreditation bodies | EURL | | |
| **Proficiency Testing** | | | |
| 7. The EURL would include a small species of BM (e.g. *Cerastoderma edule*) in the next matrix proficiency testing distribution | EURL | | |
| 8. The EURL would offer proficiency testing for *Vibrio parahaemolyticus* and *V. vulnificus* in 2014/15 | EURL | | |
| 9. The EURL would offer two PT distributions in 2014/15 in June 2014 (Lenticules™ only) and autumn/winter 2014/15 (BM matrix), matrix distribution to contain at least one sample with anticipated levels of $<10^3$ genome copies per gram | EURL | | |

**Resolutions of the 13th workshop of NRLs for bacteriological and viral contamination of bivalve molluscs, 14th 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.

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.

[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

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.

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

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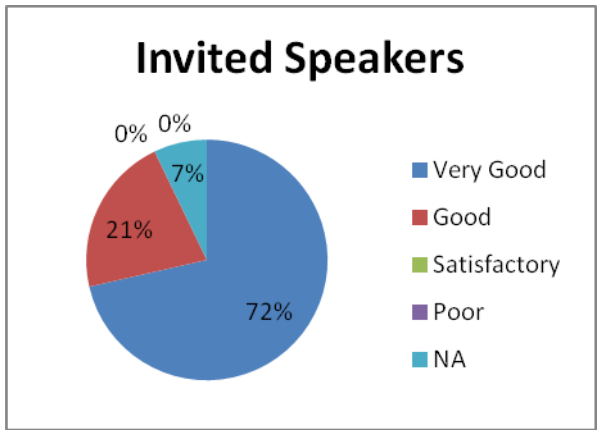
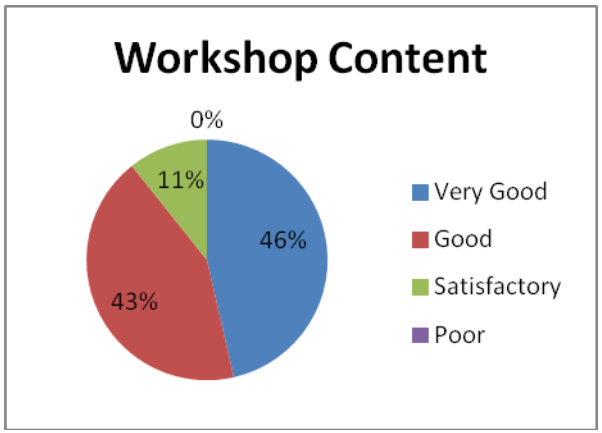
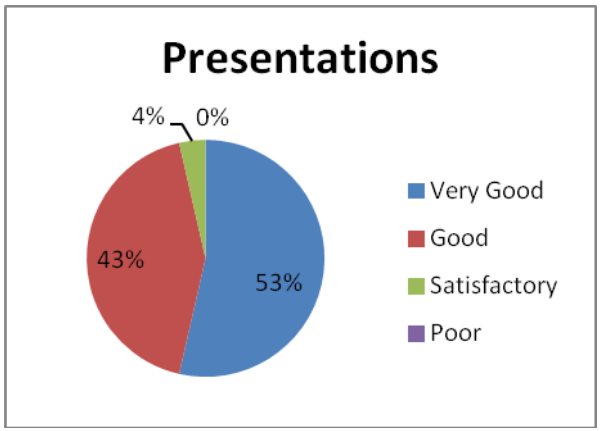
## List of papers for 13th Workshop of Microbiological NRL's

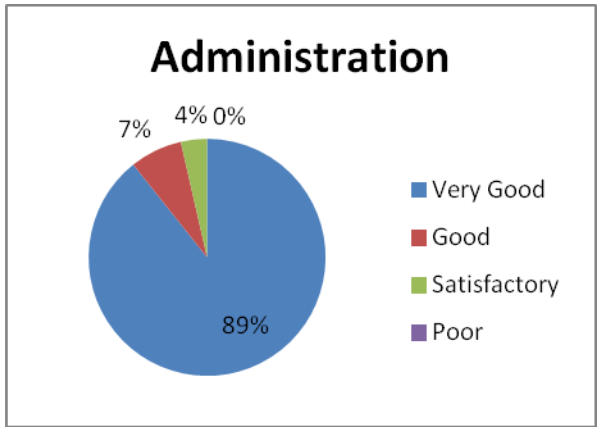
| | |
|---|---|
| **WS13/00** | List of papers |
| **WS13/01** | Delegates List |
| **WS13/02** | Instructions regarding expenses claim form |
| **WS13/03** | Expenses Claim Form |
| **WS13/04** | Workshop Report and Minutes - Final |
| **WS13/04A** | Actions from 12th workshop |
| **WS13/04B** | Details of Criteria for Waiving Results of Monitoring Programmes - Final |
| **WS13/04C** | Prohibition Zones Workshop of NRLs for Bacteriological and Viral Contamination of Bivalve Molluscs - Final |
| **WS13/04D** | Management of Outbreak Workshop of NRLs for Bacteriological and Viral Contamination of Bivalve Molluscs - Final |
| **WS13/05** | Agenda |
| **WS13/06** | EURL Microbiological Contamination of Bivalve Molluscs Final Work Programme 2014 |
| **WS13/07** | WITHDRAWN |
| **WS13/08** | Microbiological Monitoring of Bivalve Mollusc Harvesting Areas – Guide to Good Practice : Technical Application |
| **WS13/09** | Community Guide Microbiological Monitoring Bivalve Mollusc Harvesting Areas |
| **WS13/10** | ICMSS Project Scoping Document |
| **WS13/11** | EURL Generic Protocol *E.coli* Enumeration in Bivalve Shellfish draft issue 10 |
| **WS13/12** | WITHDRAWN |
| **WS13/13** | PT49 Final Report |
| **WS13/14** | PT52 EQA Report Samples 44, 45 and 46 Final |
| **WS13/15** | WITHDRAWN |
| **WS13/16** | T50 Final Report version 3 |

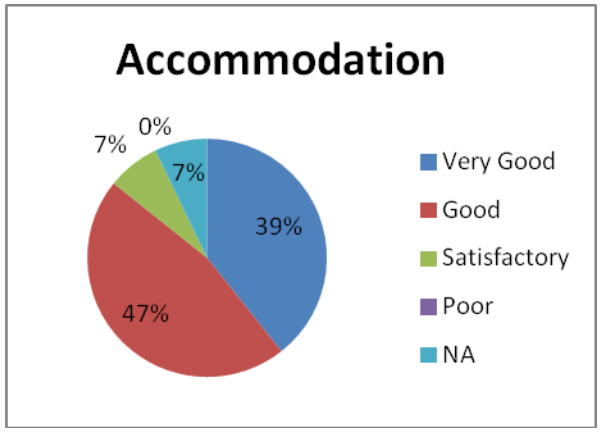
# EURL Workshop 13th to 14th May 2014, Weymouth
## Confidential Participant Feedback Results

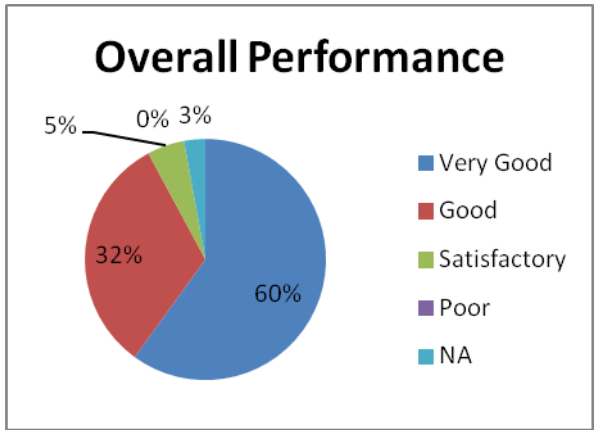


Comments :-

1. a. Please don't put me on the inside of the seating. I suffer from a bad back!
   b. Perhaps less talks, and try to ensure that delegates stick to time – 1 speaker over-ran by nearly 1 ½ hours!

2. a. Presenters should be given a time limit so it will be easier to keep the schedule.

3. a. I propose avoid presentation for PT E.coli and Sal unless there is something exceptional to mention or someone's requirement, because people has the report at the lab. This time could be use for discussion.

4. a. Running behind schedule.
   b. Discussions too elaborate on certain subject. Keep tight to time schedule.

5. a. Separate day of workshop for countries with harvesting areas.

6. a. Too much program? Or maybe too little discipline in the audience?
   b. Ok meeting though.

7. a. Select one topic to discuss more?
   b. Less ISO/CEN discussion.
   c. More research topic.

8. a. Stick to the schedule, efficiency.
   b. Allow discussions on viruses as they are also important.

**Workshop declaration**

This technical report is submitted in accordance with the requirements of Commission Implementing Regulation (EC) No 926/2011 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 Weymouth 13th & 14th May 2014.

Dr David Lees — December 2014
EURL Director

Dr Rachel Hartnell — December 2014
EURL Co-ordinator