



# Report of the 14<sup>th</sup> workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs.

**Nantes, France, 20<sup>th</sup> – 22<sup>nd</sup> May, 2015.**

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## Foreword

This document summarises relevant information from the 14<sup>th</sup> workshop of National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs held at IFREMER, France 20<sup>th</sup> – 22<sup>nd</sup> May 2015. 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](http://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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## AGENDA

### 14<sup>TH</sup> WORKSHOP OF MICROBIOLOGICAL NRLS, 20-22 MAY 2015, FRANCE

**Venue:** IFREMER  
Rue de l'Île d'Yeu  
44300 Nantes  
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#### **Day 1 - Wednesday 20 May 9:00 - 17:30**

##### **1 Introduction**

- 1.1 Welcome to IFREMER (Murielle Millot, IFREMER, France)
- 1.2 Introductions and apologies (paper WS14/01)
- 1.3 Domestic arrangements including reclaim of expenses (papers WS14/02, WS14/03)
- 1.4 Actions arising from the 13<sup>th</sup> workshop 2014 (paper WS14/04)
- 1.5 Agreement of the agenda (paper WS14/05)
- 1.6 EURL work programme 2015 (EURL) (paper WS14/06)

##### **2 Official controls**

- 2.1 Commission Restricted Working Group and trade negotiations (EURL) (paper WS14/07 )

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

- 2.2 Community Guide and Good Practise Guide (EURL)
- 2.3 Sanitary Survey of Ría de Aldán, special case for export to United States (Cristina Alvarez, INTECMAR)
- 2.4 The new national database for control of shellfish harvesting areas (NRL Italy)
- 2.5 Shellfish production in land-based systems: how to manage shellfish quality (NRL Netherlands)
- 2.6 Microbiological monitoring, classification and sanitary surveys in Galicia (Cristina Alvarez, INTECMAR)
- 2.7 The International guide (EURL)

##### ***Lunch break (13:00 – 14:00)***

##### **3 *E. coli* and *Salmonella***

- 3.1 *E. coli* and *Salmonella* methodology (EURL)
- 3.2 EURL proficiency testing for *E. coli* and *Salmonella* (EURL) (papers WS14/08, WS14/09)
- 3.3 Detection of *E. coli* contamination in urchins (NRL France)
- 3.4 Optimizing the preparative sample for enumeration of *E. coli* in *Venus gallina* by ISO/TS 16649-3 (NRL Italy)
- 3.5 Bivalve-related outbreaks of non-Vibrio bacterial pathogens (EURL)

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

##### **4 Viruses**

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

- 4.2 Amendments to the EURL generic protocol (EURL) (paper WS14/10)
- 4.3 EURL proficiency testing for norovirus and HAV – PT 53 and PT 55 (EURL) (paper WS14/11, WS14/12)
- 4.4 Proficiency Testing for viruses in shellfish in Italy (2012-2015) (NRL Italy)

**Day 2 - Thursday 21 May 9:00 - 17:30**

- 4.5 International linkage of two food borne-HAV clusters in the Netherlands in 2012 through trace back of mussels harvested in a non-endemic area (Ingeborg Boxman, Food and Consumer Product Safety Authority, the Netherlands)
- 4.6 HAV in Naples area: investigation and control measures (NRL Italy)
- 4.7 Norovirus persists in oysters and may be implicated in an outbreak (NRL France)
- 4.8 NoV Concentrations in Individual Oysters – implications for sampling and consumer exposure (NRL Ireland)

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

- 4.9 Detection and phylogenetic analysis of noroviruses from bivalve molluscs at production areas in the republic of Croatia (NRL Croatia)
- 4.10 Norovirus analyses in 2 Dutch production areas with low levels of *E.coli* (NRL, the Netherlands)
- 4.11 EU harmonised baseline survey
  - Opinion of Member States (EURL) (paper WS14/13)
  - Provisional sampling plan (EFSA) (paper WS14/14)
  - Analytical considerations including quantification (round-table discussion)

***Lunch break (13:00 – 14:00)***

**5 Marine vibrios**

- 5.1 Management of bivalve-related Vibriosis in the USA (Anna Newton, CDC, United States)
- 5.2 Validation of ISO TS 21872 under the CEN mandate (M/381) and revision of ISO TS 21872 (EURL)
- 5.3 Update on EURL proficiency testing for vibrios (EURL)

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

- 5.4 A miniaturized MPN real-time PCR method for rapid quantification of total and enteropathogenic *Vibrio parahaemolyticus* in shellfish (NRL France)
- 5.5 Overview of microbiological criteria for vibrios (EURL) (paper WS 14/15)

**Day 3 - Friday 22 May 9:00 - 12:00**

**6 Any other business**

***Coffee/tea break (10:15 - 10:45)***

- 7 Agreement of Workshop resolutions**
- 8 Date and venue for next meeting**

***Meeting close***

After the meeting close lunch will be provided and there will be an opportunity to visit the IFREMER laboratories (optional).



### **List of papers for 14th Workshop of Microbiological NRL's**

<b>WS14/00</b>	List of papers
<b>WS14/01</b>	Delegates List
<b>WS14/02</b>	EURL Workshop Payment Details Form
<b>WS14/03</b>	EURL Workshop Expenses Claim Form
<b>WS14/04</b>	Actions from 13th workshop
<b>WS14/05</b>	Agenda
<b>WS14/06</b>	EURL Work Programme 2015
<b>WS14/07</b>	PT54 Report
<b>WS14/08</b>	PT57 Report
<b>WS14/09</b>	Method characteristics for detection of viruses in bivalve shellfish using ISO 15216
<b>WS14/10</b>	Generic Virus Protocol
<b>WS14/11</b>	PT53 Report
<b>WS14/12</b>	PT55 Report
<b>WS14/13</b>	Blank Questionnaire for EU virus in LBM baseline survey 18-12-14
<b>WS14/14</b>	Conclusions from questionnaire for EU virus in LBM baseline survey EURL Feb 2015
<b>WS14/15</b>	Number of Production Areas and Dispatch Centres for each Member State
<b>WS14/16</b>	Validation of ISO TS 21872 under the CEN mandate
<b>WS14/17</b>	Overview of microbiological criteria for vibrios

## Minutes of the 14<sup>th</sup> Workshop of Microbiological NRLs for Bivalve Molluscs, 20<sup>th</sup> – 22<sup>nd</sup> May, 2015

### Attendees

EURL Director	David Lees (DNL) (chair)	Cefas, Weymouth, UK
EURL Coordinator	James Lowther (JL)	Cefas, Weymouth, UK
EURL	Louise Stockley (LS)	Cefas, Weymouth, UK
EURL	Ron Lee (RL)	Cefas, Weymouth, UK
EURL	Lisa Cross (LC)	Cefas, Weymouth, UK
NRL Austria	Johann Ladstaetter (JLa)	AGES ILMU, Wien
NRL Belgium and Luxembourg	Nadine Botteldoorn (NB)	Scientific Service of Food-borne Pathogens, Brussels
NRL Bulgaria	Vanya Chikiova (VC)	National Diagnostic and Research Veterinary Institute, Sofia
NRL Croatia	Ines Skoko (IS)	Croatian Veterinary Institute, Split
NRL Denmark	Anna Charlotte Schultz (ACS)	Institute of Food Safety and Nutrition, Søborg
NRL Finland	Elina Vatunen (EV)	Finnish Customs Laboratory, Espoo
NRL France	Soizick Le Guyader (SG)	Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER), Nantes
	Pascal Garry (PG)	
	Dominique Hervio-Heath (DHH)	
	Sylvain Parnaudeau (SPa)	
	Jean-Côme Piquet (JCP)	
NRL Germany	Eckhard Strauch (ES)	Federal Institute for Risk Assessment, Berlin
NRL Greece	Ntina Vasileiadi (NV)	Institute of Food Hygiene of Athens, Athens
NRL Hungary	Zsuzsanna Sreterne Lancz (ZL)	Central Agricultural Office, Food & Feed Directorate, Budapest
NRL Iceland	Franklin Georgsson (FG)	Matis, Reykjavik
NRL Ireland	Bill Dore (BD)	Marine institute, Galway
NRL Italy	Elisabetta Suffredini (ELS)	Istituto Superiore di Sanità (ISS) Rome
	Francesca Leoni (FL)	Istituto Zooprofilattico Sperimentale, dell'Umbria e delle Marche, Ancona
	Mario Latini (ML)	
NRL Latvia	Natalja Ivanova (NI)	National Diagnostic Centre of Food & Veterinary Service (FVS), Riga
NRL Lithuania	Audinga Verbickiene (AV)	National Food and Veterinary Risk Assessment Institute, Lithuania
NRL Netherlands	Irene Pol-Hofstad (IPH)	National Institute of Public Health and the Environment (RIVM), Bilthoven
NRL Norway	Mette Myrmel (MM)	Norwegian School of Veterinary Science, Oslo
NRL Poland	Ewelina Bigoraj (EB)	National Veterinary Research Institute, Pulawy
NRL Portugal	Sonia Pedro (SPe)	Portuguese Institute of Sea and Atmosphere (IPMA), Lisbon
NRL Romania	Alina Popescu (APo)	Institute for Diagnosis and Animal Health, Bucharest
NRL Slovakia	Zuzana Kubicova (ZK)	State Veterinary and Food Institute, Dolny Kubin

NRL Slovenia	Urska Henigman (UH)	Institute for Food Hygiene and Bromatology, Ljubljana
NRL Spain	Cristina Acebal (CA)	Agencia Espanola de Seguridad Alimentaria, Majadahonda, Madrid
NRL Sweden	Magnus Simonsson (MS)	National Food Administration, Uppsala
NRL UK	Craig Baker-Austin (CBA)	Cefas, Weymouth
Invited expert	Anna Newton (AN)	Centre for disease control, Atlanta, United States
Invited expert	Ingeborg Boxman (IB)	Food and Consumer Product Safety Authority, The Netherlands
Invited expert	Federica Barrucci (FB)	EFSA, Parma, Italy
Invited expert	Cristina Alvarez Alvarez(CAA)	Centro de Control da Calidade do Medio Marino, Pontevedra
Observer	Ainhoa Pare (APa)	Ministry of Agriculture, Agrifood, and Forestry, Paris, France

### Apologies

Paolo Caricato DG SANTE, European Commission

### **Acronyms**

CA	Competent Authority	NRL	National Reference Laboratory
CG	Community Guidance	OCL	Official Control Laboratory
CEN	Comité Européen de Normalisation	PCR	Polymerase Chain Reaction
DG SANTE	Directorate-General for Health and Food Safety	PHE	Public Health England
EFSA	European Food Safety Authority	PT	Proficiency Testing
EU	European Union	RASFF	Rapid Alert System for Food and Feed
EURL	European Union Reference Laboratory	RNA	Ribose Nucleic Acid
FBO	Food Business Operator	SS	Sanitary Survey
GPG	Good Practice Guide	TAG	Technical Advisory Group
HAV	Hepatitis A Virus	TS	Technical Specification
ISO	International Standard Organisation	UK	United Kingdom
LBM	Live Bivalve Molluscs	US/USA	United States of America
MPN	Most Probable Number	WG	Working Group
MS	Member State	WS	Workshop
NoV	Norovirus		

<p><b>1. Introduction</b></p> <p><b>1.1. Welcome to IFREMER</b> Murielle Millot from IFREMER welcomed the delegates to the institute.</p> <p><b>1.2. Introduction and apologies</b> DNL opened the meeting, followed by round table introductions.</p> <p><b>1.3. Domestic arrangements including reclaim form</b> Delegates were given instructions on electronic submission of forms and supporting information to enable payment of expenses.</p> <p><b>1.4. Actions arising from the 13th workshop</b> All actions identified were either complete or covered separately as agenda items.</p> <p><b>1.5. Agreement of the agenda</b> The workshop agenda (WS14/05) was agreed.</p> <p><b>1.6. EURL Work Programme 2015</b> JL presented the EURL work programme (WS14/06) agreed with DG SANTE for the calendar year 2015.</p> <p><b>2. Official Controls</b></p> <p><b>2.1. Commission Restricted Working Group and trade negotiations</b> DNL gave an overview on the developments within the Restricted working group. In summary, it was agreed to adopt the Codex criterion for <i>E. coli</i> in end-product, to make corresponding revisions to the criteria for class A, and to retain the <i>Salmonella</i> criterion for end-product testing. Regarding control options for norovirus the Restricted WG had decided to follow the previous recommendation given by EFSA and to undertake a baseline survey for viruses in shellfish in MS, to be coordinated by EFSA with EURL support.</p> <p>DNL also provided an overview of the proposed amendment to Commission Regulation 2073 on microbiological criteria for foodstuffs.</p> <p>The use of heat treatment on imported products and NoV was discussed with reference to RASFF alerts against Vietnamese products. It was noted the interpretation of PCR results on heat treated produce was difficult. DNL stated EFSA have been given a mandate to investigate the application of community heat treatment requirements.</p> <p>DNL finished by reporting that the EU/US trade equivalence negotiations had been finalised and highlighted the latest amendments.</p> <p><b>2.2. Community Guide and Good Practice Guide</b> RL presented the latest changes to the CG and GPG including guidance on prohibited zones (based on sewage levels). Some issues in applying the GPG were raised amongst NRLs and some discussion of application of method uncertainty to microbiological results was held.</p>	<p><b>Resolutions 1-2 Actions 1-4</b></p> <p><b>Resolution 3 Actions 5-8</b></p>
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<p><b>2.3. Sanitary Survey of Ría de Aldán, special case for export to United States (Cristina Álvarez Álvarez, INTECMAR, Spain)</b> CAA presented on the SS work undertaken in the Ria de Aldan estuary in preparation for possible exportation to the US of mussels and oysters from 2 specified harvesting areas within the estuary. It was evident from the data presented that a lot of work was involved to complete this SS and a very thorough job had been done. DNL requested an update on the progress of the export negotiations at the next WS.</p> <p><b>2.4. The new national database for control of shellfish harvesting areas (NRL Italy)</b> A database for controlling production areas in Italy was presented by ML. OCLs can easily upload their data with each production area having a unique code, map and specified sampling points. At present the information retained is not available in the public domain.</p>	<p><b>Action 9</b></p>
<p><b>2.5. Shellfish production in land-based systems: how to manage shellfish quality (NRL Netherlands)</b> IPH presented to the group the work NRL Netherlands had undertaken regarding the development of land-based shellfish production areas. It was noted that there is no guidance written in legislation identifying how classification of a land-based production areas should be performed. It was agreed that clarify was needed from the CA.</p>	<p><b>Resolution 4 Actions 10-11</b></p>
<p><b>2.6. Microbiological monitoring, classification and sanitary Surveys in Galicia (Cristina Álvarez Álvarez, INTECMAR, Spain)</b> CAA presented information on management of shellfish monitoring in Galicia. Some discussion of the approach to results associated with anomalous rainfall or sewage events, both in Galicia and across the EU was undertaken.</p>	<p><b>Resolution 5 Actions 12-13</b></p>
<p><b>2.7. The International guide (EURL)</b> RL gave a presentation on the developing International Guide (Technical Guidelines for the Development of Shellfish Sanitation Programmes), and explained that this guide was designed to help third countries with implementation of regulations to enable safe shellfish sanitation systems.</p>	
<p><b>3. E.coli and Salmonella</b></p>	
<p><b>3.1. E.coli and Salmonella methodology (EURL)</b> RL provided updates on the progress with publication of new versions of the ISO standards relevant to the <i>E.coli</i> and <i>Salmonella</i> methods and confirmed that the EURL generic protocols would need to be updated as a result.</p>	<p><b>Actions 14-16</b></p>
<p><b>3.2. EURL proficiency testing for E. coli and Salmonella (EURL)</b> LS presented a summary of the performance of the NRLs network in the last two <i>E.coli</i> and <i>Salmonella</i> proficiency testing schemes (PT54 and PT57). Problems arising from incorrect application of the MPN calculator spreadsheet referenced in ISO 7218 were discussed, and a number of actions to improve the situation identified.</p>	<p><b>Resolution 6 Actions 17-18</b></p>
<p><b>3.3. Detection of E.coli contamination in urchins (NRL France)</b> JCP gave a presentation on research into <i>E.coli</i> in sea urchins from an area in southern France. The WS discussed the surprisingly high levels detected and noted the potential implications for classification and human health.</p>	<p><b>Resolution 7</b></p>

<p><b>3.4. Optimizing the preparative sample for enumeration of <i>E. coli</i> in <i>Venus gallina</i> by ISO/TS 16649-3 (NRL Italy)</b></p> <p>FL presented an investigation into the use of reduced sample weights (25g cf. 75-100g) for the clam <i>Venus gallina</i> in the standard <i>E.coli</i> method. Although no statistically significant differences between the sample weights were noted the WS noted some possible non-significant effects, possibly requiring extra investigation. The WS then moved onto a discussion of certain technical difficulties surrounding the testing of sea cucumbers for <i>E.coli</i>.</p>	<p><b>Action 19</b></p>
<p><b>4. Viruses</b></p> <p><b>4.1. Update on the progress of the validation of ISO TS 15216-1 under the CEN validation (M/381) (EURL)</b></p> <p>JL presented the group with the progress with the virus validation, with particular focus on the bivalve mollusc matrices. The method characteristics determined through the validation exercise, and particularly the approach used to generate LOD and LOQ characteristics were discussed, and it was agreed that guidance for laboratories for determination of in-house LOD and LOQ values would be important. The issue of viruses in non-bivalve foods was also briefly discussed, and the WS agreed that while this issue fell outside of the scope of the NRLs network, they would be interested in developments in this area.</p>	<p><b>Resolutions 8-9 Action 20</b></p>
<p><b>4.2. Amendments to the EURL generic protocol (EURL)</b></p> <p>JL detailed the changes made to the virus generic protocol to harmonise with the new draft of ISO 15216.</p>	
<p><b>4.3. EURL proficiency testing for norovirus and HAV – PT 53 and PT 55 (EURL)</b></p> <p>JL gave a presentation of the performance of the NRLs in the last two virus proficiency testing round. Encouraging progress was noted in terms of participation, method harmonisation and qualitative detection. Some variation in the quantitative results was noted, with evidence that some laboratories were making incorrect calculations presented. The WS agreed that provision by the EURL of a calculation spreadsheet would be helpful. In addition it was recognised that in-house generation of quantification controls was a potential source of error that could be improved through e.g. development of commercially available standards.</p>	<p><b>Resolutions 10-14 Actions 21-23</b></p>
<p><b>4.4. Proficiency Testing for viruses in shellfish in Italy (2012-2015) (NRL Italy)</b></p> <p>ELS provided details of the virus proficiency testing schemes in operation in Italy in the last years. Through this initiative improvements in performance across the OCL network in Italy had been effected.</p>	
<p><b>4.5. International linkage of two food borne-HAV clusters in the Netherlands in 2012 through trace back of mussels harvested in a non-endemic area (Ingeborg Boxman, Food and Consumer Product Safety Authority, the Netherlands)</b></p> <p>IB provided an in-depth analysis of an investigation into an outbreak of HAV in the Netherlands linked to consumption of mussels harvested in another MS. Sequences from the Dutch clusters matched those from a non-foodborne cluster in the catchment of the mussel production area, and the timings of the cluster were consistent with a causal relationship mediated</p>	

via contamination of the mussels in the production area. The index case in the production area cluster had a history of travel to an HAV endemic region.

**4.6. HAV in Naples area: investigation and control measures (NRL Italy)**

ELS presented data on an outbreak of HAV in the Naples area with consumption of locally-produced shellfish as a risk factor. HAV presence was detected in shellfish samples from local production areas. Sequencing of clinical and shellfish positives indicated a linkage, and ruled out a linkage with a large outbreak of HAV related to consumption of frozen berries. The sequences clustered with those found in an HAV endemic region outside Europe. Following on from this and the previous presentation the WS considered the risks posed by HAV in shellfish in the EU, and discussed possible improvements to risk mitigation practices.

**Resolutions 15-17**

**4.7. Norovirus persists in oysters and may be implicated in an outbreak (NRL France)**

SPa gave a presentation on a case study of outbreaks of norovirus linked to a single oyster production area in France in the years 2011-2015. In one case the area was re-opened following an outbreak, despite the persistence of norovirus signal in the oysters, and some weeks later a further illness outbreak was linked to the area. The delegates considered the implications of this for risk management.

**Resolution 20**

**4.8. NoV Concentrations in Individual Oysters –implications for sampling and consumer exposure (NRL Ireland)**

BD presented a study where norovirus concentration in 30 individual oysters within a single growing bag was assessed. Although variation from oyster-to-oyster was noted, statistical assessment suggested that pooling 10 oysters as in ISO 15216-1 should give a result that was representative of the levels in the population.

**Resolution 19**

**4.9. Detection and phylogenetic analysis of noroviruses from bivalve molluscs at production areas in the republic of Croatia (NRL Croatia)**

IS presented the results of an initial survey into norovirus in production areas in Croatia. Although no NoV illness has yet been linked to consumption of Croatian shellfish, viral RNA was found in ~10% of samples.

**4.10. Norovirus analyses in 2 Dutch production areas with low levels of E.coli (NRL, the Netherlands)**

IPH detailed a small survey of 2 Dutch stable class A sites for norovirus. Although one site was negative for norovirus, high levels were found in the other, highlighting the limitations of E.coli as an indicator of viral risk. The WS discussed this issue and also noted the encouraging use of ISO 15216 methods in this and previous presentations.

**Resolution 21**

**4.11. EU harmonised baseline survey**

The WS considered emerging plans for an EU-wide harmonised survey for viruses in oysters.

**Resolution 18  
Action 24**

**• Opinion of Member States (EURL)**

DNL prepared a summary of responses from the MS CAs to a questionnaire designed to define the scope of the survey.

- **Provisional sampling plan (EFSA)**

APa provided an overview of the responsibilities and activities of EFSA, particularly with regards to their previous organisation of EU baseline surveys, and presented the basics of the draft sampling plan. The WS discussed the survey and agreed that additional considerations e.g. logistics and cost were important and should be taken into account.

- **Analytical considerations including quantification (round-table discussion)**

The WS discussed analytical considerations regarding the baseline survey.

## **5. Marine vibrios**

### **5.1. Management of bivalve-related Vibriosis in the USA (Anna Newton, CDC, United States)**

AN provided a comprehensive overview of the US system for management of shellfish-related Vibriosis including the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system, and presented the data generated therein. The WS discussed how the lessons of the US system could be used to improve the understanding of shellfish-related Vibriosis in the EU.

### **5.2. Validation of ISO TS 21872 under the CEN mandate (M/381) and revision of ISO TS 21872 (EURL)**

CBA presented a summary of the progress of the validation of the vibrio ISO including the results. NRLs that had taken part in the validation noted that they had not seen the full data set and were not able to put their results in context. The EURL agreed to collate and distribute the full data set. The WS discussed the findings of the validation and all agreed that the methods as currently included in the ISO were not sufficiently fit-for-purpose and needed improvement. DNL suggested that the NRLs network should take a lead on developing improved methodology since the network had the relevant expertise. Some delegates expressed confusion at the current state of vibrio standardisation within the CEN/ISO organisation due to the emergence of a new vibrio group TAG15 with CEN TC275/WG6, while up to that point vibrio standardisation had fallen within the remit of TAG3. The EURL agreed to investigate and clarify the situation.

### **5.3. Update on EURL proficiency testing for vibrios (EURL)**

LS provided a brief update on the ongoing vibrio proficiency testing scheme PT 58. The WS agreed that this and future vibrio PT schemes should be used to try to improve methodology.

### **5.4. A miniaturized MPN real-time PCR method for rapid quantification of total and enteropathogenic *Vibrio parahaemolyticus* in shellfish (NRL France)**

DHH gave a presentation on the development of a PCR-based method for enumeration of total and pathogenic *Vibrio parahaemolyticus*, which has been identified by ISO/CEN as a priority for future methods development. The WS noted that this was a welcome development and that it would be helpful to share the protocol within the network to further method development and harmonisation, DHH agreed subject to final revisions of the method.

**Resolution 22  
Action 25**

**Resolution 23  
Actions 26-29**

**Action 30**



<p><b>5.5. Overview of microbiological criteria for vibrios (EURL)</b>  In response to a request from NRL Germany, CBA gave a presentation on international microbiological criteria for vibrios in shellfish and other foods. A wide diversity of approaches was noted and the WS agreed that there was currently no international consensus, and it was therefore difficult to make recommendations for criteria within the EU.</p> <p><b>6. Any other business</b>  No other business was raised.</p> <p><b>7. Agreement of Workshop resolutions</b>  The workshop resolutions were agreed by the delegates.</p>	<p><b>Resolution 24</b></p>
<p><b>8. Date and venue for next meeting</b>  The workshop agreed with the proposal that the next meeting would be held at the Federal Institute for Risk Assessment, Berlin, Germany, 25<sup>th</sup>-27<sup>th</sup> May 2016.</p>	<p><b>Resolution 23</b></p>

**Actions of the 14<sup>th</sup> Workshop of Microbiological NRLs for Bivalve Molluscs, Ifremer, Nantes 20<sup>th</sup> – 22<sup>nd</sup> May, 2015**

Action	Owner	Completed	Notes
<b>Official controls</b>			
1. EURL to revise Note 16 (regarding pooled samples) to the proposed amendment to Commission Regulation 2073 on microbiological criteria for foodstuffs	EURL		
2. EURL to check text of 2073 to see whether stipulation of recording unit as MPN/100g in Table 1.25 of the proposed amendment is appropriate	EURL		
3. Gastropods to be added to note 5 of the proposed amendment for consistency	EURL		
4. EURL to update guidance documents following publication of the amended regulations	EURL		
5. EURL to distribute Commission discussion document on measurement uncertainty (MU) to the NRLs network	EURL		
6. EURL to include advice on how to determine MU in revised guidance documents	EURL		
7. NRLs to advise the EURL on any issues they have had when applying the guidance documents so that these issues can be included in the revision	NRLs		
8. EURL to check that all community guidance has been changed regarding alternative methods for <i>E.coli</i> .	EURL		
9. Cristina Alvarez Alvarez (CAA) to provide feedback to the next workshop on negotiations/audits relating to export of LBMs from Spain to the USA	CAA		
10. NRL Netherlands to ask Netherlands Competent Authority to seek clarification on the legislative requirements for classification of land-based LBM production systems	NRL Netherlands		
11. EURL to consider how to address monitoring of land-based and multi-trophic systems in the Good Practise Guide	EURL		
12. CAA to send the Galician classification protocol to the EURL	CAA		
13. EURL to include further clarification in the Good Practice Guide on waiving results due to high rainfall events	EURL		
<b><u>E.coli and Salmonella</u></b>			
14. EURL to review and, where necessary, update E.coli generic protocol following publication of new version of ISO 16649-3	EURL		
15. EURL to review and, where necessary, update Salmonella generic protocol following publication of new	EURL		

versions of ISO 6579 part 1			
16. EURL to review and, where necessary, update E.coli and Salmonella generic protocols following publication of new version of ISO 6887-3	EURL		
17. EURL to propose to ISO that the MPN calculator referenced in ISO 7218 be amended to include a bivalve-specific worksheet, to be made more user friendly, and to be accompanied by a user guide. The EURL also to request information from ISO on how the calculator was validated and to provide this information to NRLs	EURL		
18. In the short-term, the EURL to amend the generic <i>E.coli</i> protocol to provide guidance on the use of the calculator	EURL		
19. NRL Iceland to provide clarification on whether production of sea cucumbers in Iceland (for export to China) is subject to classification	NRL Iceland		
<b>Viruses</b>			
20. EURL to produce guidance document on approaches for laboratories to determine limit of detection and limit of quantification for in-house application of ISO 15216-1	EURL		
21. EURL to provide worked examples of quantification calculations for PT 55	EURL		
22. EURL to provide a calculation spreadsheet for ISO/TS 15216-1 by e-mail to NRLs and also on the website	EURL		
23. EURL to supply quantification standards with the next matrix PT for viruses. In addition information on in-house generation of quantification standards to be requested from participants	EURL		
24. NRLs from producer states to ask Competent Authorities to ask about funding for the EFSA baseline survey at the next restricted working group meeting	NRLS from producer states		
<b>Marine vibrios</b>			
25. EURL to request from Anna Newton (CDC, Atlanta USA) papers demonstrating the effectiveness of new food safety procedures (icing etc.) in reducing shellfish-related vibriosis and to inform NRLs	EURL		
26. EURL to collate results of the CEN mandate vibrio validation study and forward to all study participants	EURL		
27. EURL to clarify current status with regards to vibrio standardisation (particularly with regards to the new CEN TC275 WG6 TAG15) and inform NRLs	EURL		
28. EURL to organise a discussion of vibrio specialists from within the NRLs to agree future strategies for method	EURL		

improvement; <i>NOTE: following clarification of ISO responsibilities post workshop, EURL will progress this objective through TAG15</i>			
29. NRLs to share potentially useful in-house protocols for vibrio detection/quantification with the discussion group; <i>NOTE: see action 28 – protocols will be shared with TAG15</i>	NRLs		
30. EURL to request methodology used by participants in vibrio PT 58, to analyse the impact of methodology on results, and to include findings in PT report.	EURL		

## Resolutions of the 14<sup>th</sup> workshop of microbiological NRLs for bivalve molluscs, 20-22<sup>nd</sup> May 2015

### Official controls

1. The EURL briefed the workshop on pending legislative changes regarding classification and end-product criteria for *E.coli*. The EURL noted that they would amend Community Guidance appropriately following adoption of the Community legislation. NRLs agreed to notify the EURL of any other issues that should be considered in this revision.
2. The workshop noted the recent RASFF alerts regarding norovirus contamination in cooked products imported into the EU. It was agreed that the use of PCR to assess risk in cooked products was not appropriate. The workshop was informed that EFSA had received a mandate from the Commission to investigate the application of Community heat treatment requirements and would report in due course.
3. With respect to the potential application of measurement uncertainty to microbiological results, the EURL noted that this was due to be discussed between the Commission and Member States. The EURL would circulate a previous Commission discussion document on this topic and draft a section covering this issue in Community Guidance.
4. The workshop considered developments in land-based and enclosed production systems for bivalve molluscs, noted the lack of clarity regarding the legal basis for classification, and agreed that this needed to be clarified by Competent Authorities. Following this clarification the EURL agreed to develop monitoring guidance for incorporation within Community Guidance, but also noted that it was the responsibility of FBOs to incorporate risks from such systems into their HACCP plans.
5. Following discussion of approaches applied in Member States to disregard *E.coli* results associated with high rainfall the EURL agreed to review and further clarify the Good Practice Guidance on this issue.

### *E.coli* and Salmonella

6. NRLs identified that there was no appropriate guidance on the use of the MPN Calculator referenced in ISO 7218. The EURL undertook to propose to ISO that the MPN calculator be amended to include a bivalve-specific worksheet, to be made more user friendly, and to be accompanied by a user guide. The EURL also agreed to request information from ISO on how the calculator was validated and to provide this information to NRLs. In the short-term, the EURL would amend the generic *E.coli* protocol to provide guidance on the use of the MPN calculator.
7. The workshop considered a presentation on *E.coli* levels in echinoderms (*Paracentrotus lividus*) in the vicinity of a pollution source and noted surprisingly high levels of contamination. The implications for classification of this species were noted. The workshop noted a lack of data and experience on approaches to official control testing and classification for other non-bivalve species covered by EU legislation.

## Viruses

8. In the content of virus outbreaks in non-bivalve mollusc foods the workshop briefly discussed whether there were any plans for establishment of a EURL/NRL network for this issue. The importance of retaining virus responsibility within the network of bivalve microbiology NRLs was endorsed by all participants of the workshop. It was agreed that viruses in non-bivalve foods fell outside of the remit of the bivalve mollusc network but the group was interested in developments on viruses in other foods and exchange of information.
9. The workshop considered the performance characteristics of the virus method as determined by the validation of ISO/TS 15216-1 under mandate M/381. It was noted that, for accreditation purposes, laboratories would be required to generate their own limits of detection and quantification. The EURL agreed to produce guidance on approaches for laboratories to determine these characteristics.
10. The results of virus PT distributions 53 and 55 showed continued improvements in qualitative performance and method harmonisation amongst NRLs. However, further improvements to quantitative comparability would be desirable.
11. Further to the above the EURL agreed to assist NRLs with calculation of sample concentrations from Ct values, by provision of worked examples for PT 55 and by placing on the EURL website a calculation spreadsheet for ISO/TS 15216-1.
12. Further to resolution 10 the workshop also agreed that in-house generation of quantification standards could contribute to variability. The EURL agreed that, for the next matrix PT, quantification standards would be supplied and information on in-house generation of standards requested from participants. The EURL would analyse responses in relation to the standards generation methodology utilised, to identify potential sources of variability.
13. The EURL agreed to continue to develop accessible quantification standards to assist laboratories with the application of ISO/TS 15216-1.
14. It was agreed to continue virus PT with 2 distributions per year including one matrix distribution which should include at least one whole animal sample (shucked or unshucked).
15. The workshop considered presentations on recent hepatitis A virus outbreaks attributed to consumption of bivalve shellfish produced in the EU. It was recognised that with the low immune status of hepatitis A virus in the EU, and the low infective dose of this virus, that sporadic community cases of hepatitis A virus in coastal locations could present a high risk for shellfish contamination.
16. Further to the above, the workshop recommended that risk mitigation for hepatitis A virus needs to be improved and this would be achieved by better communication between health authorities and shellfish risk managers. In this context it was noted that laboratory analysis significantly assists risk management.
17. Further to resolution 15 the workshop considered resolutions 11 and 14 of 2012 in relation to risks posed by shellfish contamination with hepatitis A virus and

norovirus respectively. The workshop agreed that emerging data confirmed these resolutions and that they continued to be appropriate advice.

18. The workshop considered emerging plans for an EU-wide harmonised survey for viruses in oysters. EFSA presented considerations for the sampling plan. It was agreed that it was important to consider analytical aspects, volumes of shellfish production, logistics and cost in designing the sampling plan.

19. The workshop noted data on variation in norovirus levels between individual oysters and its implications for risk assessment. Furthermore, it was noted that the data reinforced the approach in ISO/TS 15216 of analysis of a pooled sample of 10 animals.

20. The workshop considered data on norovirus monitoring in oyster production areas associated with outbreaks. It was noted that outbreaks may reoccur in areas that remain virus contaminated but are reopened. The workshop noted the implications of this for risk management.

21. The workshop noted the application of ISO/TS 15216 in diverse Member States and that the method seemed to provide useful risk management information. However the lack of correlation between *E.coli* and norovirus contamination was noted in several studies.

#### Marine vibrios

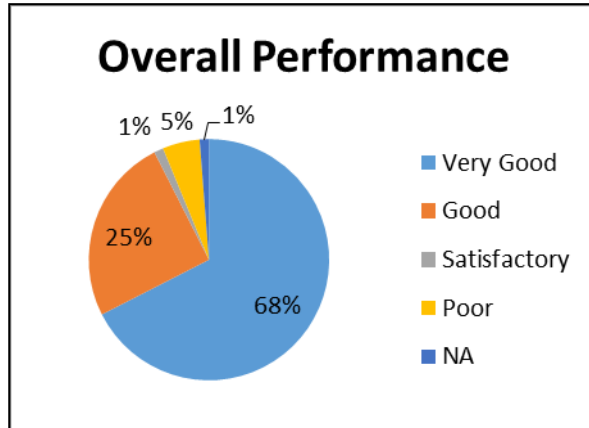
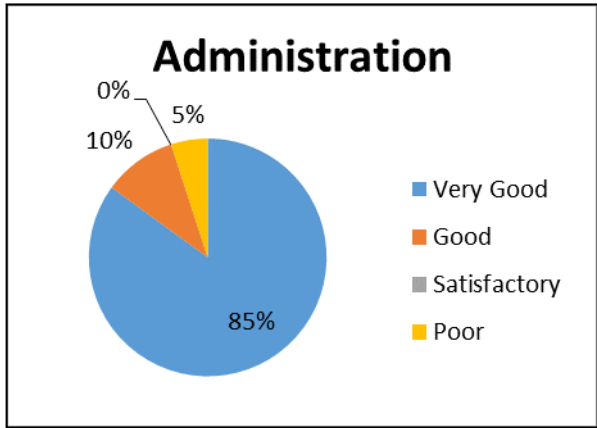
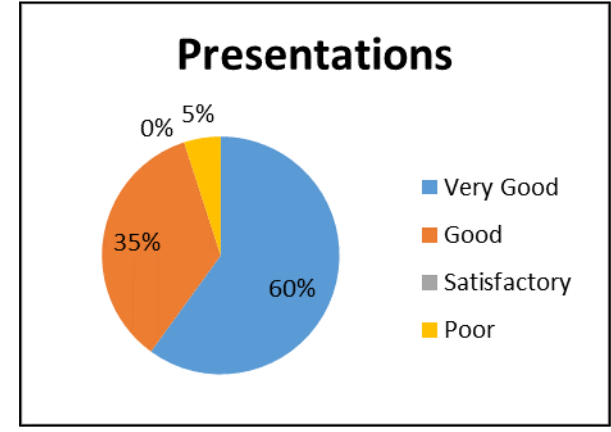
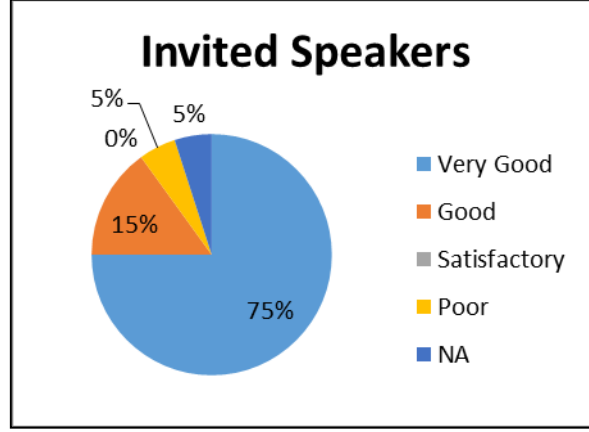
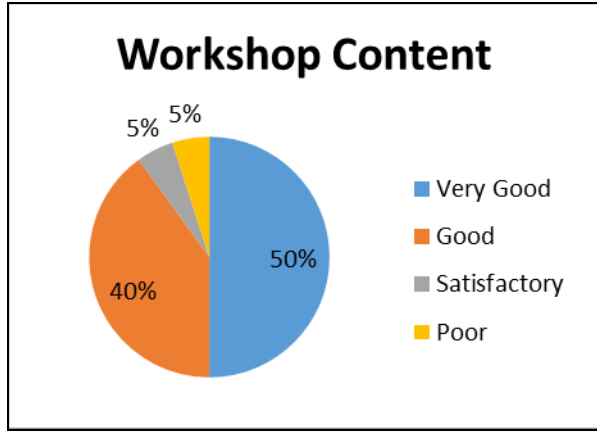
22. The workshop considered a presentation from CDC Atlanta USA giving a comprehensive overview of vibriosis in the USA. The workshop noted the significant underreporting of this disease in the USA, despite it being a notifiable illness, and considered that it was possible that vibriosis could be significantly underreported in the EU. More structured epidemiological reporting in the EU would improve understanding of the health burden from vibrios associated with shellfish consumption.

23. The workshop considered findings from the validation studies of the *Vibrio* method and agreed that aspects of the methodology were not yet satisfactory. The EURL agreed to organise a discussion of specialists to agree future strategies for method improvement. To assist this it was agreed that the detailed results of the validation study will be shared with NRLs. Furthermore it was agreed that NRLs would share relevant methods with the discussion group. Future EURL PT would be used to support such methodological improvements.

24. The EURL presented information on international microbiological criteria regarding vibrios in seafood. The workshop agreed that no international consensus had yet emerged and that it was therefore difficult to recommend appropriate microbiological criteria relevant to laboratory testing in the EU.

25. The next workshop will be held on 25<sup>th</sup>-27<sup>th</sup> May 2016 at the Federal Institute for Risk Assessment, Berlin, Germany.

**EURL Workshop 20<sup>th</sup> to 22<sup>nd</sup> May 2015, Nantes**  
**Confidential Participant Feedback Results**





### Comments :-

1. a. We were really out of the agenda. It is important to keep it.
2. a. More time for discussion.  
b. More participation of more laboratory.

### Comments from an observer

3. a. Very interesting (almost) on Wednesday (unfortunately I could not attend the end of the workshop).
- b. Too much topics and not enough discussion because we have not all presentations\* to prepare the discussion.  
Suggestion    1)     put apart “laboratories” topics (i.e. 3.1, 3.2, 3.4, 4.1...of agenda)  
                  2)     Please invite competent authorities to discuss about monitoring, management, guidance  
                          (at least 2 days twice a year!)  
                          (i.e. 2.2, 2.3, 2.5, 2.6, 2.7, 3.3 of the agenda +4.6, 4.7, 4.8...4.11 +5.1, 5.5...)

- \*These documents should contain
- Presentation (of the case on local situation)
  - Proposition for harmonising : through EU legislation or through guidance

## **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 Nantes 20th-22nd May 2015.

Dr David Lees

7<sup>th</sup> October 2015

EURL Director

Dr James Lowther

7<sup>th</sup> October 2015

EURL Co-ordinator