



Report of the 15th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs.

Berlin, Germany, 25th – 27th May, 2016.

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Foreword

This document summarises relevant information from the 15th workshop of National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs held at BFR, Berlin, Germany 25th – 27th May 2016. 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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AGENDA

15th Workshop of Microbiological NRLs, 25-27 MAY 2016, GERMANY

Venue: Room D146
Bundesinstitut für Risikobewertung (BfR)
Diedersdorfer Weg 1
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Day 1 - Wednesday 25 May 9:00 - 17:30

1 Introduction

- 1.1 Welcome to BfR
- 1.2 Introductions and apologies (paper WS 15/01)
- 1.3 Domestic arrangements including reclaim of expenses (papers WS15/02, WS15/03)
- 1.4 Actions arising from the 14th workshop 2015 (paper WS15/04)
- 1.5 Agreement of the agenda (paper WS15/05)
- 1.6 EURL work programme 2016-17 (EURL) (paper WS15/06)

2 Official controls

- 2.1 Update on the Commission Restricted Working Group (EURL)

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

- 2.2 EU/US trade negotiations
 - FVO audit of the US (EURL)
 - Equivalence of microbiological standards (EURL) (paper WS15/07)
 - FDA audit: The Netherlands (NRL Netherlands)
 - FDA audit: Galicia (Cristina Alvarez, INTECMAR, Spain)
- 2.3 Update on the Good Practise Guide (EURL) (papers WS15/08, WS15/09)
- 2.4 Mussel and oyster production in Germany: Production areas, Monitoring and Future Developments (Edda Bartelt, Lower Saxony State Office for Consumer Protection and Food Safety, Germany & Torsten Trumpf, Landeslabor Schleswig-Holstein, Germany)

Lunch break (13:00 – 14:00)

3 *E.coli and Salmonella*

- 3.1 *E.coli* and *Salmonella* methodology update (EURL) (paper WS15/10)
- 3.2 EURL proficiency testing for *E. coli* and *Salmonella* (EURL) (papers WS15/11, WS15/12)
- 3.3 Update on discussions regarding the ISO MPN calculator (EURL)
- 3.4 Method uncertainty (EURL) (paper WS15/13)
- 3.5 Summary of NRL organised PT schemes for Member State OCLs (EURL) (paper WS15/14)
- 3.6 Elevated *E. coli* results from a UK official control laboratory, July 2015 (NRL United Kingdom)

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

4 Marine vibrios

- 4.1 Validation of ISO TS 21872 under the CEN mandate (M/381) and revision of ISO 21872 (EURL) (paper WS15/15)
- 4.2 Update on activities of CEN/TC 275/WG6/TAG15 (vibrios) (EURL)
- 4.3 Vibrio proficiency testing (EURL) (paper WS15/16)

Day 2 - Thursday 26 May 9:00 - 17:30

- 4.4 Trh (tdh-/trh+) gene analysis of clinical, environmental and food isolates of Vibrio parahaemolyticus (NRL Italy)
- 4.5 Cold chain measures to reduce Vibrio incidence from the USA (EURL)

5 Viruses

- 5.1 EFSA Scientific Opinion on alternative heat treatment profiles for processing of live bivalve molluscs (Winy Messens, EFSA) (paper WS15/17)

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

- 5.2 Update on the progress of ISO 15216 (EURL)
- 5.3 EURL proficiency testing for norovirus and HAV – PT 59 (EURL) (paper WS15/18)
- 5.4 European baseline survey of norovirus in oysters
 - The sampling plan (Jane Richardson, EFSA) (paper WS15/19)

Lunch break (13:00 – 14:00)

- The method specification (EURL)
- Training and proficiency testing (EURL)
- Determination of LOD and LOQ (EURL) (paper WS15/20)
- Approaches to sampling in Member States (round-table discussion)

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

- 5.5 Optimisation of digital PCR and application to naturally contaminated oysters (NRL France)
- 5.6 The experience of the Belgian NRL for BMS on Norovirus detection, especially during outbreak investigations (NRL Belgium)
- 5.7 Hepatitis E virus; an overview (NRL Germany)

Day 3 - Friday 27 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

List of papers for 15th Workshop of Microbiological NRL's

WS15/00	List of papers
WS15/01	Delegates List
WS15/02	EURL Workshop Payment Details Form
WS15/03	EURL Workshop Expenses Claim Form
WS15/04	Actions from 14th workshop
WS15/05	Agenda
WS15/06	EURL Microbiological Contamination of Bivalve Molluscs Work Programme 2016-2017
WS15/07	Comparison of Current and Proposed European Union Classification Criteria for Category A Shellfish Harvesting Waters
WS15/08	Community Guide Microbiological Monitoring Bivalve Mollusc Harvesting Areas Issue 3 draft revision
WS15/09	Commission Regulation (EU) 2015-2285
WS15/10	Issue 12 EURL Generic SOP <i>E-coli</i>
WS15/11	Proficiency testing 60 – <i>E-coli</i>
WS15/12	Proficiency testing 63 - EQA
WS15/13	Determining Uncertainty of Measurement draft v2
WS15/14	Summary of NRL organised PT schemes for Member State OCLs
WS15/15	Validation of ISO TS 21872 under the CEN mandate (M 381) and revision of ISO 21872
WS15/16	Proficiency testing 58 - Vibrio
WS15/17	EFSA Scientific Opinion on alternative heat treatment profiles
WS15/18	Proficiency testing 59 - Virus
WS15/19	Baseline survey sampling plan
WS15/20	LOD and LOQ guidance note v1 Draft

Minutes of the 15th Workshop of Microbiological NRLs for Bivalve Molluscs, 25th – 27th May, 2016

Attendees

EURL Director	David Lees (DNL) (chair)	Cefas, Weymouth, UK
EURL Coordinator	James Lowther (JL)	Cefas, Weymouth, UK
EURL Administrator	Samantha Arkell (SA)	Cefas, Weymouth, UK
EURL	Rachel Hartnell (RH)	Cefas, Weymouth, UK
EURL	Louise Stockley (LS)	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	Gergana Krumova-Valcheva (GKV)	National Diagnostic and Research Veterinary Institute, Sofia
NRL Croatia	Eddy Listes (EL)	Croatian Veterinary Institute, Split
	Irena Listes (IL)	
NRL Denmark	Anna Charlotte Schultz (ACS)	Institute of Food Safety and Nutrition, Søborg
NRL France	Soizick Le Guyader (SLG)	Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER), Nantes
	Pascal Garry (PG)	
NRL Germany	Eckhard Strauch (ES)	Federal Institute for Risk Assessment, Berlin
	Reimar Johne (RJ)	
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 Myrnel (MM)	Norwegian School of Veterinary Science, Oslo
NRL Poland	Artur Rzezutka (AR)	National Veterinary Research Institute, Pulawy
	Magdalena Lopatek (MLo)	
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	Julia Jurovcikova (JJ)	State Veterinary and Food Institute, Dolny Kubin

NRL Slovenia	Urska Henigman (UH)	Institute for Food Hygiene and Bromatology, Ljubljana
NRL Spain	Juan Olmedo (JO)	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	Cristina Alvarez Alvarez (CAA)	Centro de Control da Qualidade do Medio Marino, Pontevedra
Invited expert	Edda Bartelt (EB)	Lower Saxony State Office for Consumer Protection and Food Safety, Cuxhaven
Invited expert	Thorsten Trumpf (TT)	Landeslabor Schleswig-Holstein, Neumünster
Invited expert	Jane Richardson (JR)	EFSA
Observer	Julia Uriol (JU)	FVO
Observer	Paolo Caricato (PC)	DG SANTE

Apologies

Elina Vatunen, NRL Finland

Acronyms

CEN	Comité Européen de Normalisation	MU	Method Uncertainty
DG SANTE	Directorate-General for Health and Food Safety	NoV	Norovirus
EQA	External Quality Assessment	NSSP	US National Shellfish Sanitation Program
EFSA	European Food Safety Authority	NRL	National Reference Laboratory
EU	European Union	OCL	Official Control Laboratory
EURL	European Union Reference Laboratory	PCR	Polymerase Chain Reaction
FDA	US Food and Drug Administration	PHE	Public Health England
FVO	Food and Veterinary Office	PT	Proficiency Testing
HAV	Hepatitis A Virus	TAG	Technical Advisory Group
HEV	Hepatitis E Virus	TS	Technical Specification
ISO	International Standards 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

<p>1. Introduction</p> <p>1.1. Welcome to BFR Prof. Dr. Reiner Wittkowski from the Federal Institute for Risk Assessment, Berlin welcomed the delegates to the institute.</p> <p>1.2. Introduction and apologies DNL opened the meeting, followed by round table introductions.</p> <p>1.3. Domestic arrangements including reclaim form Delegates were given instructions on electronic submission of forms and supporting information to enable payment of expenses.</p> <p>1.4. Actions arising from the 14th workshop All actions identified were either complete or covered separately as agenda items.</p> <p>1.5. Agreement of the agenda The workshop agenda (WS15/05) was agreed.</p> <p>1.6. EURL Work Programme 2015 JL presented the EURL work programme (WS15/06) agreed with DG SANTE for the calendar year 2016.</p>	
<p>2. Official Controls</p> <p>2.1 Update on the Commission Restricted Working Group DNL gave an overview on the developments within the Restricted Working Group particularly with regards to Regulation (EU) No. 2015/2285, the EU baseline survey for norovirus, method uncertainty, and classification and monitoring. JU clarified the position of the Commission with regards to closing/reclassification of non-compliant class A areas, whereby they would seek a consensus between MS, rather than specific legal advice on the matter. There was some discussion between the EURL/NRLs and PC and JU from the Commission as to whether the definition of production areas in EU legislation excludes artificial ponds on land. The issues of accumulation of tetrodotoxin and parasites (pathogenic to humans) within LBM were raised and it was agreed that further collaborations with relevant experts were desirable.</p>	<p>Resolution 1 Actions 1&4</p>
<p>2.2 EU/US trade negotiations</p> <p>a. FVO audit of the US DNL provided an update on the findings of the FVO audit of the US, following the publication of the audit report. It was clarified that the audit was to check compliance with US rules, not EU rules. It was noted, in particular, that the US control programmes based on the NSSP were comprehensive, and that the US states visited implemented them adequately. There were gaps identified in certain areas regarding compliance with the NSSP and the report included recommendations for rectification.</p> <p>b. Equivalence of microbiological standards RH presented a study looking at the equivalence of US approved and EU class A standards, in light of the forthcoming change to a 3 class standard (Regulation (EU) No. 2015/2285). No significant difference in equivalence of</p>	<p>Resolution 2</p>

the existing and new EU standards compared to the US standard was noted. However, there was some discussion on the number of discordant classifications under the EU and US systems amongst the sites in the data set. Further analysis of the agreed EU US dataset was underway prior to a physical technical meeting planned later in the year.

c. FDA audit: The Netherlands

IPH provided a comprehensive overview of the audit carried out by the US FDA on mussel and oyster production (6 areas) in the Netherlands. The audit generally found a high level of compliance with EU legislation and additional good practise, however a recommendation around discharge of human waste from commercial vessels was made by FDA, in addition to a request for sight of the validation data for the *E. coli* method used in the Netherlands.

d. FDA audit: Galicia

CAA provided a comprehensive overview of the audit carried out by the US FDA on bivalve production in the Xunta de Galicia, Spain. The audit generally found a high level of compliance with EU legislation and additional good practise, however recommendations on reopening following sewage discharges into growing waters, discharge of human waste from commercial vessels, co-purification of shellfish from different classifications and the performance of private laboratories were made by the FDA.

Following the audit presentations, PC confirmed that any additional areas within the EU (and the US) will not be subject to formal audits prior to approval for transatlantic trade.

2.3 Update on the Good Practice Guide

RH provided an update on the revision of the Good Practice Guide, identifying areas of the document that have undergone changes, and highlighting outstanding areas for discussion, particularly with regards to seasonal classification. The WS discussed the treatment of holding areas within the guidance and PC reiterated that it was important that the documents do not exceed what is included in EU legislation, nor modify the definitions therein. Additional required changes to the class A alert level were identified by the WS.

2.4 Mussel and oyster production in Germany

EB gave a presentation on bivalve production in the states of Lower Saxony and Schleswig-Holstein in Germany, detailing production areas and volumes, and the classification monitoring programmes, and presenting the results of microbial testing, including for non-regulatory determinands.

3. *E. coli* and *Salmonella*

3.1. *E. coli* and *Salmonella* methodology

LS updated the WS on the revisions of ISO standards 6579-1, 6887-3 and 16649-3, and detailed the current status of the EURL generic protocols for *E. coli* and *Salmonella* in terms of harmonisation with the standards.

The WS discussed the potential discrepancy in the number of animals comprising a sample in the period between the application of Regulation (EU) No. 2015/2285 on 1st January 2017, and the publication of the new revision of ISO 6887-3. The EURL reiterated that in such an event the number of animals comprising a sample remained 10, as in currently enacted legislation.

**Resolutions 3-4
Actions 2-3**

**Resolutions 5&7
Actions 6,7&11**

SPe noted a discrepancy between the loop size in ISO 16649-3 and the EURL generic protocol; the EURL agreed to amend the generic protocol to remove the discrepancy.

The WS discussed whether the use of proprietary *Salmonella* detection systems was permissible according to ISO 6579-1. The EURL indicated that it would investigate.

3.2. EURL proficiency testing for *E. coli* and *Salmonella*

LS presented the results of PT 60 and the EURL/PHE EQA scheme, with particular emphasis on the performance of NRLs and follow-up actions resulting from underperformance.

The EURL informed the WS that from 2017, it would cover the costs of participation for all NRLs in one EQA distribution annually (in February). It would therefore be mandatory for all NRLs to participate. The WS agreed that this was a positive development.

NRLs emphasised the importance of inclusion of *Salmonella* analysis in all PT distributions – the EURL agreed and promised to include this in all future distributions.

**Resolutions 8-9
Action 8**

3.3. Update on discussions regarding the ISO MPN calculator

RH provided the WS with an update on issues around the MPN calculator, including the production of guidance documents, and discussions with ISO TC34 SC9 WG2 (Food Microbiology - Statistics) to produce a bivalve specific worksheet incorporating <18, >18,000 scores etc., and to incorporate reference to the worksheet in ISO 7218. NRLs welcomed the developments and reiterated the importance of resolving this issue, as accredited labs are currently risking problems due to the discrepancy between ISO 7218 and the EURL guidance documents.

**Resolution 6
Action 5**

3.4. Method uncertainty

RH presented an update on the EURL guidance on determination of method uncertainty (MU) for *E. coli* analysis. Following questions from NRLs, the EURL confirmed that although MU is not referenced in ISO 16649-3, national accreditation bodies generally require labs to calculate MU in order to approve accreditation to ISO 17025.

**Resolution 11
Action 10**

3.5. Summary of NRL organised PT schemes for Member State OCLs

LS presented findings from a questionnaire distributed to NRLs to determine both their participation in PT schemes (both organised by the EURL and by others) and their oversight of the participation of OCLs within their MS in such schemes. Following a round-table discussion between the EURL and NRLs from producer countries, it was noted that in two countries (Bulgaria and Norway), OCLs did not participate in PT schemes under the supervision of the NRL. The EURL reiterated that it was a responsibility of NRLs to ensure that labs in their OCL network participated in PT schemes.

**Resolution 10
Action 9**

3.6. Elevated *E. coli* results from a UK official control laboratory, July 2015

CBA gave a presentation on a series of high *E. coli* results reported by a UK OCL during a short period in 2015. The WS discussed this interesting case and some NRLs reported some similar unusual *E. coli* results, but on a less significant scale.

<p>4. Marine Vibrios</p> <p>4.1 Validation of ISO TS 21872 under the CEN mandate (M/381) and revision of ISO 21872 (M/381) (EURL)</p> <p>RH gave a comprehensive overview of the CEN project to validate the vibrio ISO 21872, including presentation of the method characteristics determined. Following questions from NRLs, RH confirmed that under ISO 21872, there is a choice of conventional, real-time or biochemical confirmation tests. The WS suggested some modifications to improve the new draft of 21872, including the clarification of definitions of “fresh” and “frozen” seafood, and the inclusion of information regarding the strains used for contamination.</p>	<p>Resolution 12 Action 15</p>
<p>4.2 Update on activities of CEN/TC 275/WG6/TAG15 (vibrios)</p> <p>CBA updated the WS on the activities of TAG15 relating to the development of new PCR based methods for the detection and quantification of pathogenic vibrios. It was noted that a focus of work would be directed at quantitative methods for <i>V. parahaemolyticus</i>. The call for relevant national experts to participate in, and share methods with TAG15 was reiterated, particularly from countries where vibrio testing is currently widespread and routine.</p>	<p>Resolutions 13&14 Actions 12&17</p>
<p>4.3 Vibrio proficiency testing (EURL)</p> <p>LS presented the findings of PT 58 distribution for vibrios. The WS discussed and agreed the need for future EURL vibrio PT rounds to focus on method development, to assist the activities of TAG15.</p>	<p>Resolution 15</p>
<p>4.4 <i>Trh</i> (<i>tdh</i>-/<i>trh</i>+) gene analysis of clinical, environmental and food isolates of <i>Vibrio parahaemolyticus</i></p> <p>FL updated the WS with the results of genome sequencing efforts for <i>V. parahaemolyticus</i> isolates carried out by NRL Italy, highlighting in particular the possible use of <i>trh</i> gene characterisation for assessment of potential pathogenicity. The EURL agreed to share relevant strains with NRL Italy to assist in further research in this area.</p>	<p>Action 16</p>
<p>4.5 Cold chain measures to reduce <i>Vibrio</i> incidence from the USA</p> <p>CBA presented epidemiological data supplied by colleagues in the US on the spread of <i>Vibrio parahaemolyticus</i> cases to new States and on the effectiveness of new cold chain measures (particularly the use of ice slurries) to reduce shellfish-related vibriosis. The WS acknowledged that the epidemiological data suggested these measures were potentially effective however there were possible problems (mortality, organoleptic issues etc.). CBA suggested that more epidemiological data was required to fully determine the efficacy of these control measures, and further noted that given the potential risk of <i>Vibrio</i> contamination of oysters in the US, and the new approval for importing oysters from approved areas within the US, that efforts should be taken to collate information on shellfish-related vibriosis cases within the EU. It was further agreed that it was important to share all information on this issue whether linked to EU-US trade or not, and mechanisms for capturing the information were discussed. The WS was informed that the EU and US had agreed to keep bivalve shellfish related vibriosis, potentially associated with traded products, under review and to revisit trade agreements if necessary.</p>	<p>Resolutions 16&17 Actions 13&14</p>

<p>5. Viruses</p> <p>5.1 EFSA Scientific Opinion on alternative heat treatment profiles for processing of live bivalve molluscs</p> <p>Winy Messens of EFSA gave a presentation by telephone detailing the preparation and findings of the scientific opinion of alternative heat treatments. The WS discussed the significance of the conclusions with regards to the existing legal heat treatment criteria, and agreed that the experimental evidence broadly supported the existing criteria, and that it would be difficult to recommend heat treatment at <90°C, due to the wide confidence intervals on the appropriate length of time at lower temperatures. DNL, who was part of the EFSA WG formed to address this question, further emphasised that considerations of organoleptic qualities were important.</p> <p>5.2 Update on the progress of ISO 15216 (EURL)</p> <p>JL provided a brief update on the status of both parts of the virus ISO; 15216-1 (quantification) had passed the first vote and a final draft based on the comments received had been prepared. Publication was anticipated by the end of 2016. Further, the CEN parent committee had requested that a new revision of 15216-2 (detection) was prepared to harmonise with the revised part 1, and to incorporate method characteristics.</p> <p>5.3 EURL proficiency testing for norovirus and HAV – PT 59 (EURL)</p> <p>LS provided an overview of PT 59 for viruses in LENTICULE samples, with particular emphasis on the participation and performance of NRLs.</p> <p>5.4 European baseline survey of norovirus in oysters</p> <p>a. The sampling plan</p> <p>JR gave a presentation on the development of the sampling plan for the baseline survey, detailing the input parameters used in the model, and presenting the total samples per MS required.</p> <p>b. The method specification (EURL)</p> <p>JL presented the baseline survey method specification to the WS, explaining the development of the document and its relationship to ISO 15216-1. The additional technical support in terms of control materials offered by the EURL was also detailed.</p> <p>The WS engaged in considerable discussion on the use of archived test sample materials for additional purposes e.g. HAV or HEV analysis. It was noted that there were some discrepancies in the baseline survey protocol as to how priority for use of the archive was decided, and the WS agreed that clarification on this point would be important.</p> <p>c. Training and proficiency testing (EURL)</p> <p>LC presented the EURL plans for two training workshops for laboratories designated for analysis in the baseline survey, and in addition presented a first draft of a proposal for competence scoring of labs, following the planned PT distribution, prior to approval to take part in the baseline survey.</p> <p>d. Determination of LOD and LOQ (EURL)</p> <p>JL gave an overview of an EURL guidance note for determination of LOD and LOQ for the quantification of viruses in LBM. It was reiterated that although the method included in the guidance note was not mandatory, it was necessary for all designated laboratories undertaking analysis for the</p>	<p>Resolution 18</p> <p>Actions 22&23</p> <p>Action 24</p> <p>Resolutions 19-20 & 22-24</p> <p>Actions 18-21</p>
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baseline survey to report LOQ values to EFSA.

e. Approaches to sampling in Member States (round-table discussion)

NRLs from those MS involved in the baseline survey presented what they knew of their national plans to collect samples for the survey. In the majority of cases sampling was due to be carried out by Competent Authority officials.

5.5 Optimisation of digital PCR and application to naturally contaminated oysters

SLG gave a presentation on application of the digital PCR quantification method to oysters. The WS discussed the positive aspects and limitations of this technology compared with real-time PCR.

5.6 The experience of the Belgian NRL for BMS on Norovirus detection, especially during outbreak investigations

NB gave an overview of food and waterborne outbreaks of norovirus in Belgium, with diverse sources and epidemiologies described.

5.7 Hepatitis E virus; an overview

RJ provided a comprehensive overview on HEV, covering its virology, epidemiology, occurrence in LBM and other foods, and methods for its culture and detection. The WS discussed the growing emergence of this pathogen and agreed that it should be a priority for shellfish hygiene research in the short and medium term. The WS identified that archived samples from the norovirus baseline survey presented a good opportunity to gather information on the occurrence of HEV in molluscs.

6. Any other business

No other business was raised.

7. Agreement of Workshop resolutions

The workshop resolutions were agreed by the delegates.

8. Date and venue for next meeting

The workshop agreed with the proposal that the next meeting would be held on 3rd - 5th May 2017, in Split, Croatia (subject to confirmation by the EURL).

Resolution 21

Resolution 25

Actions of the 15th Workshop of Microbiological NRLs for Bivalve Molluscs, Bundesinstitut für Risikobewertung (BfR), Berlin 25th – 27th May, 2016

Action	Owner	Completed	Notes
Official controls			
1. EURL to investigate the possibility of a collaboration with the EURL on marine biotoxins on tetrodotoxin.	EURL		
2. EURL to update guidance documents to address comments on holding areas and alert levels for class A areas, then to circulate to NRLs for comment prior to presentation at the meeting of the Commission Restricted Working Group.	EURL		
3. EURL to consider the complications with respect to seasonal classification within the expert Working Group and report back at the next workshop.	EURL		
4. EURL to write to relevant contacts at EURL Parasites to ask whether they have any data on the occurrence of parasites (<i>Giardia</i> , <i>Cryptosporidium</i> etc.) in bivalve shellfish, particularly with respect to human illness. EURL to report at next WS.	EURL		
<u>E.coli and Salmonella</u>			
5. EURL to contact the relevant expert (Cordelia Wilrich) to progress the bivalve specific MPN calculator and the convenor of ISO/TC 34/SC 9/WG 7 “General requirements and guidance for microbiological examinations” to discuss inclusion of the calculator within the revision of ISO 7218.	EURL		
6. EURL to consider the scope of ISO 6579-1, to determine if it is possible to use proprietary <i>Salmonella</i> detection systems as a screen.	EURL		
7. Subject to the findings from the previous action, EURL to undertake an evaluation of any available validation data relating to bivalves/seafood.	EURL		
8. EURL to liaise with PHE to make necessary arrangements and determine details re: registration and payment etc. for participation of NRLs in the February 2017 EURL/PHE <i>E. coli</i> and <i>Salmonella</i> External Quality Assessment distribution at the expense of the EURL.	EURL		

9. EURL to finalise the report on the responses to the questionnaire on proficiency testing and distribute to NRLs, to assess the findings and decide on further actions, and to report back at the next WS.	EURL		
10. EURL to complete a final version of the guidance on Method Uncertainty and to publish on the website. Final version to incorporate comments received, in addition to the EURL in-house MU value.	EURL		
11. EURL to amend <i>E. coli</i> generic protocol to change recommended loop size in body text to 10µl to align with ISO 16649-3, and to add a footnote allowing the use of other loop sizes (e.g. 1µl) subject to appropriate verification.	EURL		
Marine vibrios			
12. All NRL members with expertise in vibrios to consider joining TAG 15 through nomination by their National Standardisation Bodies and in addition to propose other relevant experts in their Member States to seek nomination.	NRLs		
13. EURL to develop a simple database, to be held on the restricted area of the website, to capture details of shellfish-related vibriosis across the EU.	EURL		
14. NRLs to share any data that they may have on shellfish-related vibriosis, through the mechanism of the database mentioned in the previous action.	NRLs		
15. EURL, as project leader for validation of ISO 21872, to add relevant <i>Vibrio</i> strain details to the forthcoming FDIS.	EURL		
16. EURL to send trh+ <i>Vibrio</i> strains to IZS to facilitate research.	EURL		
17. EURL to clarify whether <i>Vibrio</i> methods that are currently in routine use for large number of samples in the US, e.g. Washington State, have been shared with TAG15.	EURL		
Viruses			
18. EURL to contact EFSA with a view to producing a joint note clarifying the approach regarding use of archived materials from the baseline survey for EU-wide and national studies for distribution to NRLs and to MS survey coordinators.	EURL		

19. EURL to contact EFSA to ask them to consider adding a reporting parameter on whether a sanitary survey has been carried out on the production area for samples collected in the baseline survey	EURL		
20. EURL to circulate the proposal for competence scoring for use in the virus proficiency testing prior to the baseline survey to NRLs for comment.	EURL		
21. EURL to finalise the non-mandatory EURL guidance on determination of a Limit of Detection (LOD) and Limit of Quantification (LOQ) for the norovirus method and distribute to NRLs.	EURL		
22. EURL to update generic protocol for quantification of viruses to harmonise with final draft of ISO 15216-1	EURL		
23. EURL to clarify with CEN TC 275 WG6 secretariat whether it is allowed to share precision characteristics from the validation of ISO 15216-1 with NRLs prior to publication of the new version of the ISO	EURL		
24. EURL to circulate a lenticule-specific calculation spreadsheet to participants in PT 65	EURL		

RESOLUTIONS OF THE 15th WORKSHOP OF MICROBIOLOGICAL NRLs, 25-27th MAY 2016, BERLIN, GERMANY

Official controls

1. The commission reported to the workshop the emerging situation with tetrodotoxin contamination of LBMs. The possible connection with *Vibrio* sp. was noted and the workshop supported the need for further collaborative research with algal toxin scientists.
2. The commission informed the workshop that consequential on trade discussions with the US FDA, an audit of additional MS would not be required prior to approval of that MS for export. The EURL reminded the workshop of the need for MS seeking approval to comply with the Good Practice Guide including Annex 4 on export to the USA.
3. The EURL presented draft version 6 of the Good Practice Guide and draft issue 3 of the Community Guide. The EURL further agreed to address comments on holding areas and alert levels for class A areas, and to recirculate the updated drafts amongst NRLs by June 17th 2016. NRLs agreed to provide any additional comments by July 15th 2016.
4. Further to the above, the EURL informed the workshop of the complications with respect to seasonal classification and proposed to further consider this within the expert Working Group and report back at the next workshop.

E.coli and Salmonella

5. The EURL reiterated the requirement to test 10 animals for *E.coli* analysis and acknowledged the current discrepancy within ISO 6887-3;2003 (cross-referenced in regulation 2015/2285) and informed the workshop that this will be resolved with the forthcoming publication of the revised ISO 6887-3 (currently at FDIS stage).
6. The EURL agreed to progress the development of a bivalve-specific MPN calculator (including reporting values of <18, >18,000 and >180,000 *E.coli* MPN/100g) and to endeavour to incorporate reference to this calculator within the forthcoming revision of ISO 7218.
7. The EURL agreed to review ISO 6579-1 and undertake an evaluation of any available validation data for proprietary *Salmonella* detection systems to determine whether such methods can be used within the scope of the ISO as a screen.
8. The EURL informed the workshop that for the February 2017 EURL/Public Health England *E.coli* and *Salmonella* External Quality Assessment distribution, and for future annual distributions, the EURL would meet the costs of NRLs, and that participation would therefore be mandatory.
9. The EURL agreed to include both *E.coli* and *Salmonella* analysis in future whole animal proficiency testing distributions to assist NRLs in their accreditation requirements.
10. Following analysis of responses to an EURL questionnaire, the workshop noted that all EU Official Control Laboratories performing analysis for *E.coli* and *Salmonella* in Member States with production areas participated in proficiency testing for those determinands, under the supervision of their NRL, with the exception of Official Control Laboratories in Bulgaria and Norway.
11. The workshop noted the guidance on determination of measurement uncertainty for MPN methods provided by the EURL. The importance of sub-sampling prior to the homogenisation stage, for tests where replicate sample test measurements are required, was reinforced.

Vibrios

12. The workshop noted the need for a clearer definition of “fresh” or “frozen” seafood within ISO 21872 in relation to the selection of the initial incubation temperatures. NRLs were encouraged to submit comments through their National Standardisation Bodies to facilitate this.
13. The TAG 15 group leader informed the workshop that the primary objective of TAG 15 was to establish robust quantitative methods for pathogenic *Vibrio parahaemolyticus*. The workshop agreed that the primary focus of this methodological development should be an MPN format with sufficient precision to enable robust quantification, confirmed using molecular methods.
14. The TAG 15 group leader encouraged NRLs with expertise in vibrios to join TAG 15 through nomination by their National Standardisation Bodies. In addition NRLs were encouraged to propose other relevant experts in their Member States to seek nomination.
15. The workshop agreed that EURL ring trials for vibrios organised during 2016-17 would focus on assisting method development and standardisation in collaboration with TAG 15 rather than assessment of laboratory competence.
16. In relation to EU/US trade, the workshop noted the increasing risk of shellfish-related vibriosis in the USA however data indicates that recently-introduced cold-chain measures may mitigate this risk.
17. The workshop agreed the need to share information regarding vibriosis associated with bivalve mollusc consumption, including any associated with US imports, in order to inform future review of risk.

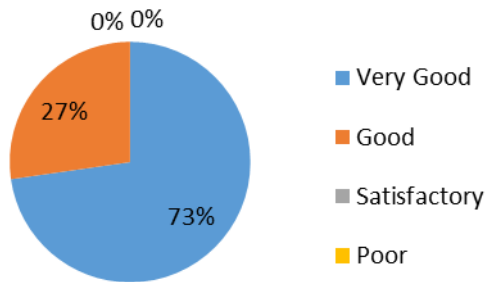
Viruses

18. The workshop commended the excellent work conducted by European Food Safety Authority regarding alternative heat treatments for bivalve molluscs and noted the further steps necessary to revise the criteria if appropriate.
19. Regarding the EU-wide baseline survey for norovirus in oysters, the workshop noted an inconsistency within the European Food Safety Authority protocol between the body text (section 2.3.3) and the method specification (Appendix B) regarding use of archived materials for EU-wide and national studies and sought clarification from European Food Safety Authority on this issue. The workshop recommended that Member State Competent Authorities should be free to decide the most appropriate use of archived samples collected in their Member State without the need for consultation of Standing Committee on Plants, Animals, Food and Feed.
20. Further to the above, the workshop agreed the importance of analysis of the baseline survey samples for HAV as a priority, considering the severity of this pathogen and the lack of data on the prevalence of HAV in oysters in the EU.
21. The workshop noted the growing awareness and importance of Hepatitis E virus as a foodborne pathogen and acknowledged the lack of data regarding the potential role of bivalve molluscs as a transmission vehicle for this virus within the EU. The workshop agreed the possibility to address this data gap through analysis of the archive from the baseline survey.
22. Following discussion, the EURL agreed to circulate the proposal for competence scoring for use in the virus proficiency testing prior to the baseline survey by the end of June 2016 for comment by NRLs by end of July 2016.

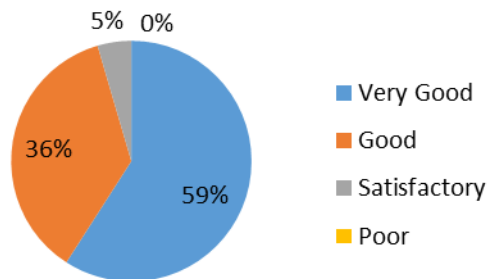
23. Following discussion on the non-mandatory EURL guidance on determination of Limit of Detection (LOD) and Limit of Quantification (LOQ) for the norovirus method, the EURL agreed to incorporate comments on the rationale for the assigned maximum standard deviation value, and to incorporate a provision for user laboratories to use appropriate methods for interpolation of LOQ between tested levels. A revised draft would be circulated by end of June 2016 for comment by NRLs by end of July 2016.
24. Based on information supplied by NRLs, the workshop noted that in most Member States samples for the baseline survey were planned to be collected by official samplers for both production areas and dispatch centres.
25. The next workshop will be held 3rd-5th May 2017, in Split, Croatia (subject to confirmation by the EURL).

EURL Workshop 25th to 27th May 2016, Berlin Confidential Participant Feedback Results

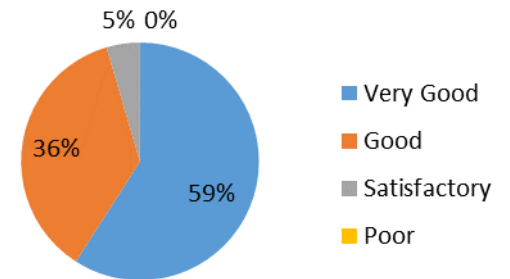
Invited Speakers



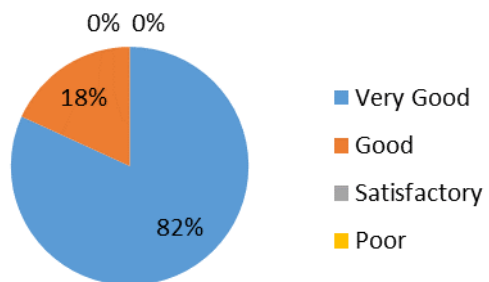
Workshop Content



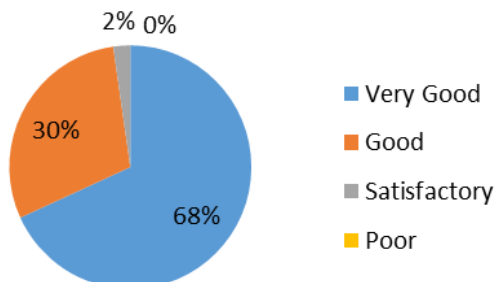
Presentations



Administration



Overall Performance



Comments :-

1. When you have no production area's you are out of the scope of this workshop.
2. First day too long.
3. Organised beautifully. Thank you!
4. Excellent content, could do with moving or suggesting that questions and discussions are continued during tea and lunch breaks to help keep to agenda.

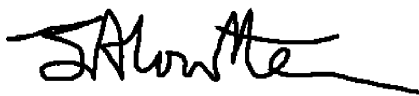
Workshop declaration

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 Berlin 25th-27th May 2016.

A handwritten signature in black ink, consisting of a large, stylized 'D' and 'L' intertwined, with a long horizontal stroke extending to the right.

Dr David Lees
EURL Director

06/09/2016

A handwritten signature in black ink, appearing to read 'J Lowther' in a cursive style, with a long horizontal stroke extending to the right.

Dr James Lowther
EURL Co-ordinator

06/09/2016