



**Report of the 11th workshop of NRLs for
monitoring bacteriological and viral
contamination of bivalve molluscs.**

Weymouth, UK, 24th – 26th April, 2012.

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Forward

This document comprises relevant information arising from the 11th workshop of National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs held at the EURL in Weymouth, UK on 24th - 26th April 2012. It includes the workshop agenda, delegate contact information, workshop minutes, lists of associated papers, and the resolutions agreed by the meeting. All supplementary supporting information identified in this report can be accessed in full via the information centre of the website of the European Union Reference Laboratory www.crlcefas.org or may be supplied on request by the EURL. All requests should be made to the EURL co-ordinator.

Dr Rachel Hartnell
EU RL Co-ordinator
Cefas Weymouth Laboratory,
Barrack Road,
The Nothe,
Weymouth, Dorset
DT4 8UB, United Kingdom

Telephone: +44 (0) 1305 206600

Direct line: +44 (0) 1305 206707

Fax: +44 (0) 1305 206601

E-mail : <mailto:rachel.hartnell@cefas.co.uk>

General e-mail: fsq@cefas.co.uk

EURL Website: www.crlcefas.org

AGENDA

11th Workshop of Microbiological NRLs, 24 – 26 April 2012

Venue: Cefas Weymouth Laboratory
Barrack Road
The Nothe
Weymouth
DORSET
DT4 8UB
UK

Main reception Tel: +44 (0) 1305 206600

Main reception Fax: +44 (0) 1305 206601

Enquiries : Prior to the workshop enquiries should be directed to Rachel Hartnell or
Samantha Arkell.

EURL for monitoring bacteriological and viral contamination of bivalve molluscs,
Cefas Weymouth Laboratory,
Barrack Road,
The Nothe,
Weymouth,
Dorset,
DT4 8UB,
UK

Direct line : Rachel Hartnell +44 (0) 1305 206707

Samantha Arkell +44 (0) 1305 206698

E-mail : rachel.hartnell@cefas.co.uk or
samantha.arkell@cefas.co.uk

Day 1 - Tuesday 24 April 9:30 - 17:30

1 Introductory meeting

- 1.1 Welcome, introductions and apologies.
- 1.2 Domestic arrangements including reclaim of expenses (papers WS11/01, WS11/02).
- 1.3 Actions arising from the 10th workshop 2010 (paper WS11/04).
- 1.4 Agreement of the agenda (paper WS11/05).
- 1.5 EURL work programme 2012 (EURL) (paper WS11/06).

Coffee/tea break (10:30 am)

2 Official Controls - Microbiological monitoring and classification

- 2.1 Feedback from the Commission Restricted Working Group on Classification and Monitoring of Bivalve Molluscs (EURL).
- 2.2 Harmonisation of Codex and EU standards (paper WS11/07) (EURL).
- 2.3 Community Guide to the Principles of Good Practice for the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas with regard to Regulation 854/2004 (*provisional* paper WS11/08) (EURL).
- 2.4 Update on application of sanitary surveys across Member States (paper WS11/09, paper WS11/10) (All – roundtable)
- 2.5 Proposal for a revision to the stability assessment for monitoring frequency in the good practice guide (EURL).
- 2.6 Second International Workshop on Molluscan Shellfish Area Classification (paper WS11/11, paper WS11/11a) (EURL).
- 2.7 Temporal trends of *E. coli* level in clams (*Chamelea gallina*) harvesting areas of the Marche Region (NRL Italy, Ancona).

Lunch (1:00 pm)

3 Liaison with ISO and CEN

- 3.1 Update on activities at ISO SC9 and CEN WG6 groups (EURL).

4 Official Controls - Proficiency Testing

4.1 *E. coli* and *Salmonella* spp.

1. Proficiency testing among Spanish Official Control Laboratories for bivalve molluscs (NRL Spain).
2. NRLs participation and performance assessment in the EURL/HPA Shellfish EQA scheme for *E. coli* and *Salmonella* (RT 44) (paper WS11/12) (EURL).
3. NRLs participation and performance assessment in the whole animal distribution for *E. coli* and *Salmonella* (RT 41) (paper WS11/13) (EURL).

4. Ring trial on fresh shellfish: an overview on several years experience (NRL France)
5. Supervision of Official Control Laboratories by NRLs (paper WS11/14) (All – roundtable)
6. EURL draft guidance on the level and frequency of proficiency testing participation (paper WS11/15) (EURL).

Coffee/tea (3:30 pm)

5 Marine vibrios

- 5.1 Characterisation of *Vibrio cholerae* isolated in Italy (NRL Italy, Ancona).
- 5.2 Outcomes of *Vibrio* ring trial (RT38) and ongoing revision of ISO TS 21872 (paper WS11/16) (EURL)
- 5.3 Progress at CEN TAG3 – direct quantitative determination of *Vibrio* spp. and proposals for practical methods workshop July 4-5th 2012 (EURL).
- 5.4 The research consortium VibrioNet (NRL Germany).
- 5.5 Proposals for a COST initiative (EURL).

Dinner – Sense Restaurant and Wine Bar for 7.30pm

Day 2 - Wednesday 25 April 9:15 - 17:30

- 6 Impact of chronic microbial pollution on shellfish – (EURL).
- 7 **Viruses - Hepatitis A virus**
 - 7.1 Hepatitis A virus, a somewhat neglected shellfish-borne pathogen (Prof. Albert Bosch-University of Barcelona).
 - 7.2 Looking for HAV in a shellfish production area over 2 years (NRL France).
 - 7.3 **Any more contributions on HAV? Please**

Coffee/tea (10:30am)

8 Viruses - Noroviruses

- 8.1 Tracing norovirus in oysters from Ireland to Denmark through France (NRLs Denmark and France).
- 8.2 Norovirus surveillance in the UK – prevalence and levels of norovirus in *Crassostrea gigas* and *Ostrea edulis* in England, Wales and Scotland (UK NRL).

9 Viruses - Towards virus controls

- 9.1 EFSA Panel on Biological Hazards (BIOHAZ) - Scientific Opinion on Norovirus (NoV) in oysters: methods, limits and control options (paper WS11/17) (NRL Ireland)

9.2 Discussion – Control options for norovirus (paper WS11/18)(all)

Lunch 12:30 pm

10 Viruses - Proficiency testing for norovirus and hepatitis A virus

10.1 The first ring trial for the detection of HAV and NoV (GI and GII) in bivalve mussels organized in Italy (NRL Italy, Roma).

10.2 EURL norovirus and hepatitis A virus proficiency testing – PT43 (EURL) (Paper WS11/19).

10.3 Proposals for:

10.3.1 Proficiency testing,

10.3.2 EURL harmonised protocol (Paper WS11/20),

10.3.3 Training requirements.

Coffee/tea (3:30 pm)

10.4 Discussion (all)

10.5 Update on the progress of CEN validation (M/381) (EURL).

Day 3 - Thursday 26 April 9:30 - 12:00

11 Agreement of Workshop resolutions.

12 Any other business.

13 Date and venue for next meeting.

Meeting close

Delegate List

Country	Country Status	Delegate	Specialist area	E-mail
Austria	Member State	Johann Ladstaetter	NRL Representative	johann.ladstaetter@ages.at
Belgium & Luxembourg	Member State	Sarah Denayer	NRL Representative	Sarah.Denayer@wiv-isp.be
Bulgaria	Member State	Vanya Chikiova	NRL Representative	vchikova@abv.bg
Denmark	Member State	Anna Charlotte Schultz	NRL Representative	acsc@food.dtu.dk
France	Member State	Soizick Le Guyader	NRL Representative	Soizick.Le.Guyader@ifremer.fr
		Pascal Garry	NRL Representative	pascal.garry@ifremer.fr
Germany	Member State	Eckhard Strauch	NRL Representative	Eckhard.Strauch@bfr.bund.de
Greece	Member State	Ntina Vasileiadi	NRL Representative	iyt@otenet.gr
Hungary	Member State	Erzsebet Adrian	NRL Representative	adriane@oai.hu
Ireland	Member State	Bill Dore	NRL Representative	bill.dore@marine.ie
Italy	Member State	Elisabetta Suffredini	NRL Representative	elisabetta.suffredini@iss.it
		Luciana Croci	NRL Representative	luciana.croci@iss.it
		Francesca Leoni	NRL Representative	f.leoni@pg.izs.it
		Mario Latini	NRL Representative	m.latini@izsum.it
Latvia	Member State	Gita Tupe	NRL Representative	gita.tupe@bior.gov.lv
Netherlands	Member State	Irene Pol	NRL Representative	Irene.pol@rivm.nl
Poland	Member State	Ewelina Bigoraj	NRL Representative	ewelina.bigoraj@piwet.pulawy.pl
		Magdalena Lopatek	NRL Representative	magdalena.tataarcz@piwet.pulawy.pl
Portugal	Member State	Sonia Pedro	NRL Representative	spedro@ipimar.pt

Delegate List (cont.)

Country	Country Status	Delegate	Specialist area	E-mail
Romania	Member State	Alina Popescu	NRL Representative	Popescu.Alina@IDAH.RO
Slovakia	Member State	Miriám Filipova	NRL Representative	filipova@svpudk.sk
Slovenia	Member state	Andrej Kirbis	NRL Representative	Andrej.Kirbis@vf.uni-lj.si
Spain	Member State	Cristina Alvarez	NRL Representative	calvarez@intecmar.org
		Albert Bosch	Invited Speaker	abosch@ub.edu
		Oscar Vilariño Bermúdez	Invited	ovilarino@msssi.es
		Cristina Acebal	NRL Representative	cacebal@msssi.es
Sweden	Member State	Magnus Simonsson	NRL Representative	magnus.simonsson@slv.se
United Kingdom	Member State	Ron Lee	NRL Representative	Ron.lee@cefasc.co.uk
		James Lowther	NRL Representative	James.lowther@cefasc.co.uk
EU-RL	EU-RL	Rachel Rangdale	EU-RL Co-ordinator	Rachel.hartnell@cefasc.co.uk
		Louise Stockley	EU-RL Representative	Louise.stockley@cefasc.co.uk
		David Lees	EU-RL Director	David.lees@cefasc.co.uk
		Samantha Arkell	EU-RL Administrator	Samantha.arkell@cefasc.co.uk
Croatia	Accession country	Ines Škoko	NRL Representative	i.skoko.vzs@veinst.hr
Iceland	EFTA	Franklin Georgsson		franklin.georgsson@matis.is
Norway	EFTA	Mette Myrmel	NRL Representative	Mette.Myrmel@nvh.no
		Liv Marit Rørvik	NRL Representative	LivMarit.Rorvik@nvh.no

Minutes of the 11th Workshop of Microbiological NRLs for Bivalve Molluscs, Cefas, Weymouth, 24th - 26th April, 2012.

Attendees

David Lees (DNL) (chair)	EURL Director	Cefas, UK.
Rachel Hartnell (RH)	EURL Coordinator	Cefas, UK.
Louise Stockley (LS)	EURL	Cefas, UK.
Samantha Arkell (SA)	EURL	Cefas, UK.
Craig Baker-Austin (CBA)	EURL	Cefas, UK.
Simon Kershaw (SK)	EURL	Cefas, UK.
Johann Ladstaetter (JL)	NRL Austria	Austrian Agency for Health and Food Safety, Wien.
Sarah Denayer (SD)	NRL Belgium and Luxembourg	Scientific Institute of Public Health, Brussels.
Vanya Chikiova (VC)	NRL Bulgaria	Pencho Slaveikov, Sofia.
Anna Charlotte Schultz (ACS)	NRL Denmark	Institute of Food Safety and Nutrition, Soborg.
Soizick Le Guyader (SG)	NRL France	Institut Français de Recherche pour L'Exploitation de la Mer (IFREMER)
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.
Bill Doré (BD)	NRL Ireland	Marine Institute, Galway.
Luciana Croci (LC)	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
Elisabetta Suffredini (ELS)	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	NRL Latvia	National Diagnostic Centre of Food & Veterinary Service (FVS), Riga.
Irene Pol (IP)	NRL Netherlands	National Institute of Public Health and the Environment (RIVM), Bilthoven.
Ewelina Bigoraj (EB)	NRL Poland	National Veterinary Research Institute, Pulawy.
Magdalena Lopatek (ML)	NRL Poland	National Veterinary Research Institute, Pulawy.
Sonia Pedro (SP)	NRL Portugal	Instituto de Investigacao das Pescas e do Mar (IPIMAR), Lisbon.
Alina Popescu (AP)	NRL Romania	Institute of Diagnosis and Animal Health, Buharest.
Miriam Filipova (MF)	NRL Slovakia	State Veterinary and Food Institute, Dolny Kubin.
Andrej Kirbis (AK)	NRL Slovenia	National Veterinary Laboratory, Ljubljana.
Cristina Acebal (CA)	NRL Spain	Agencia Espanola de Seguridad Alimentaria, Majadahonda, Madrid.
Cristina Alvarez	Invited expert	Centro de Control da Qualidade do Medio Marino Pontevedra
Albert Bosch	Invited expert	University of Barcelona, Barcelona
Oscar Vilarino Bermudez	Liaison with EURL biotoxins	EURL biotoxins, Vigo
Magnus Simonsson (MS)	NRL Sweden	National Food Administration, Uppsala.
Ron Lee (RL)	NRL UK	Cefas, Weymouth.
James Lowther (JL)	NRL UK	Cefas, Weymouth.
Ines Skoko	Croatia	Croatian Veterinary Institute, Split.
Franklin Georgsson (FG)	EFTA Iceland	Matis, Reykjavik.
Liv Marrit Rorvik (LMR)	EFTA Norway	The Norwegian School of Veterinary Science, Oslo.
Mette Myrmel (MM)	EFTA Norway	The Norwegian School of Veterinary Science, Oslo.

Apologies

Paolo Caricato DG SANCO, European Commission.

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

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

Acronyms

CA	Competent Authority	LBM	Live Bivalve Molluscs
CCFH	Codex Committee of Food Hygiene	LOQ	Limit of Quantification
CEN	Comité Européen de Normalisation	MS	Member State
CPA	Cumulative Performance Assessment	NoV	Norovirus
DG Sanco	Directorate General for Food and Consumers	NRL	National Reference Laboratory
EFSA	European Food Safety Authority	OCL	Official Control laboratory
EQA	External Quality Assessment	PT	Proficiency Testing
EU	European Union	SOP	Standard Operating procedure
EURL	European Union Reference Laboratory	SS	Sanitary Survey
ECDC	European Centre for Disease Prevention and Control	SCFCAH	Standing Committee on the food chain and animal health
FDA	Food and Drug Administration	TAG	Technical Advisory Group
GPG	Good Practice Guide	TBX	Tryptone Bile X-glucuronide
HAV	Hepatitis A Virus	US	United States
HPA	Health Protection Agency	WP	Work Programme
ISO	International Standard Organisation	WG	Working Group

<p>1. Welcome meeting</p> <p>1.1. Welcome and introduction</p> <p>DNL opened the meeting and invited all NRL representatives to introduce themselves to the group. RH explained the reasoning behind the change of venue (from Slovenia to Weymouth), this was due to budgetary constraints. The EURL thanked NRL Slovenia for offering to host the workshop. Delegates were informed that it was their responsibility to ensure all expense receipts were copied and returned to SA by the 25th May 2012. The workshop agenda was agreed (WS11/05).</p> <p>1.2. Actions arising from the 9th workshop</p> <p>The resolutions and report from the 10th workshop (Weymouth 2011) were reviewed (WS11/04). All actions had been completed; any major findings are summarised below or covered separately as agenda items.</p> <ul style="list-style-type: none"> Two alternative methods for official control analysis of LBM for <i>E. coli</i> were accepted by SCFCAH in June 2011. Subsequently, EU harmonised SOPs covering their use (BacTrac 4300 impedance method and TBX (based upon ISO 16649-2) have been completed and were available on the EURL website. The methods were included in the draft Commission guidance (2.3). DNL presented briefly the findings of the 5 year audit of EURLs undertaken on behalf of the Commission in 2011. The EURL had performed very well overall particularly with respect to PT. One finding was the lack of formal feedback from NRLs with respect to workshops, to address this issue NRLs were supplied with feedback questionnaires and asked to complete and return after the workshop. <p>1.3. EURL work programme 2012</p> <p>RH presented the main themes of the EURL WP agreed with DG Sanco. The WP for 2012 included provision of advice to DG Sanco and NRLs, representation of the network on scientific committees/working groups (e.g. EFSA, ISO and CEN), provision of PT and assistance with development of alternative methods.</p>	<p>Action</p> <p>All NRLs reclaiming expenses</p> <p>All NRLs</p>
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2. Official Control - Microbiological monitoring and classification

2.1. Harmonisation of Codex and EU standards

RH presented on the continuing development with respect to harmonisation of the Codex STAN 292-2008 (3 class plan (3CP)) and the EU regulation (2 class plan (2CP)) for the microbiological *E. coli* criteria (WS11/07). The EURL showed data and analysis on a direct comparison with the two proposed plans for end products and emphasised that a 3CP would be likely to detect non compliant samples from more contaminated areas more quickly than a 2CP approach. The workshop had previously agreed that a 3CP for end product (i.e. product placed on the market) was scientifically justified and should be supported – see resolutions and report of workshop 2011

http://www.crcefas.org/InformationCentre/documents.asp?action=list&Section_ID=17. The implications of adopting the 3CP approach overtime were discussed. Further to the consideration and analysis of real dataset supplied form NRLs in 2011, the EURL had evaluated the potential effect on pathogen content (*Salmonella*) and impact on passing end product standard of LBM subject to the 3CP approach overtime. It was considered that there would be a marginal effect on pathogen content which would not substantially increase public health risk. But it was noted that this approach did inevitably increase the possibility of end product failures. Paper WS11/07 provided a description of the analyses undertaken; this had been provided to the Commission and would be considered by MS. The importance of standardising tolerances (currently applied unilaterally by some MS) was stressed as was the discontinuity between permissible exceedences for class A and B areas. The implications and resultant problems (see above and previous workshop report 2011) of applying the same standards for end product and environmental standards were discussed. EURL would report any progress to NRLs in due course (**Resolution 1**).

EURL

2.2. Feedback from the Commission restricted working group on classification and monitoring of bivalve molluscs

DNL gave an overview of the topics discussed at the most recent Commission restricted WG. The main discussion points were areas were:

- Codex 3 class plan (covered in 2.1)
- Class A areas - Issues regarding compliance and the need for separate end-product standards from environmental standards (covered in 2.1).
- Microbiological guidelines for monitoring of harvesting areas (2.3)
- USA/EU equivalence – Ongoing equivalency negotiations between EU and US with respect to trade in LBM. US audit programme of the EU was ongoing, awaiting translation of EURL GPG to a formal EU guidance document (2.3). In principal equivalence of US and EU systems with respect to approved and class A areas with SS had been accepted. It was noted that individual MS wishing to trade to the US would require an audit from the US.

2.3. Community guide to Good Practice Guide

DNL informed the group of the new draft Community Guidance on good practice with respect to monitoring LBM production areas, this document was circulated to NRLs as paper WS11/08. The intent of Community Guidance documents was to assist MS in interpretation of Community legislation; the guidance was derived from the EURL Good Practice Guide and would as an official Commission document have a legal basis. NRLs were asked to provide any comments to the EURL. The EURL would report progress to NRLs in due course (**Resolution 2**).

EURL

2.4. Update on application of sanitary surveys across Member States

Information obtained from the SS questionnaire distributed to NRLs was presented by RH. From the data provided it was noted that most NRLs returning questionnaires had carried out SS to some extent. The majority were performed in accordance with the GPG. NRLs that had not provided information were asked to return completed questionnaires to the EURL by 18th April 2012 (**Resolution 3**). Once all information had been received the finalised document would be circulated to NRLs before being sent to the Commission and placed on the EURL website.

NRLs

2.5. Proposal for a revision to the stability assessment for monitoring frequency in the good practice guide

The EURL presented proposed revisions to stability assessments for monitoring frequency included in the GPG. Following discussion NRLs were asked to assist the EURL in the development of new criteria and were asked to provide additional datasets where possible to assist in this work. In the mean time a revised issue of the guide would be available on the EURL website (**Resolution 4**).

NRL
EURL

2.6. Second international workshop on molluscan shellfish area classification

RH informed the group of the 2nd International Workshop on Molluscan Shellfish Area Classification (WS11/11, WS11/11a) to be held between the 24 - 28th September 2012 in Rhode Island, US. The objectives of the workshop was to help expand technical understanding of the systems for classifying molluscan shellfish areas, and elaborate approaches for unifying regulatory requirements among countries. The focus would be on comparing the EU and US systems, it was anticipated that the workshop would assist with the ongoing US/EU equivalency negotiations. NRL's and CA's from MS interested in exporting to the US were encouraged to attend via notification of either the Commission or the EURL (**Resolution 5**). Further information would be available in due course from the EURL.

EURL

2.7. Temporal trends of *E.coli* level in clams harvesting areas of the Marche Region

NRL Italy (ML) presented on work evaluating annual and seasonal changes of *E. coli* levels in naturally contaminated clam beds. From the data presented it was evident that the levels of *E. coli* were higher during autumn and winter periods. Further work was being planned to continue the study with the CA to identify where the pollution originates from. This will be performed region by region. Seasonal classification was not carried out in Italy.

3. Liaison with ISO and CEN

A presentation on the activities at ISO and CEN was given by RH. These included:

ISO 16649-3 - *E. coli* enumeration by MPN

- New revision to translate from a technical specification to a full standard
- The inclusion of a detection clause
- Minor text modifications

ISO 6887-3 – initial preparation and dilutions

- Amendment to LBM sample size to be in line with legislation
- The sampling of production areas for LBM
- Alternative initial suspensions included to reflect ISO 16649-2 TBX method
- Major revision to text

ISO 6579 – detection of Salmonella

- ISO to be split into 3 parts to include detection, enumeration and serotyping of *Salmonella* spp.
 - Horizontal detection method
 - Miniaturized enumeration method
 - Guidance on serotyping

Developments at CEN and ISO regarding norovirus, hepatitis A virus and *Vibrio* spp. are included in latter sections

4. Official controls – Proficiency Testing

4.1. *E. coli* and *Salmonella* spp.

4.1.1. Proficiency testing among Spanish Official Control Laboratories for bivalve molluscs

NRL Spain (CA) presented on the PT scheme organised for Spanish OCL for *E. coli* enumeration and *Salmonella* spp. detection. The PT scheme was started in 2004 using LBM with 1 distribution per year. Participants include OCL's responsible for end product testing and the control of production areas with around 31 OCL's participating from across

autonomous regions of Spain. Both naturally and artificially contaminated shellfish samples were used and prepared using an external commercial company. Shellfish material was homogenised prior to freezing and distributed on dry ice. The majority of OCLs used the MPN method for enumeration with only 2 laboratories using the pour plate method. Z-scores were used to assess participants' performance. The scheme was not compulsory for OCLs', but was used by most for accreditation purposes. Follow-up procedures and advice were given where required.

4.1.2. Ring trial on fresh shellfish: An overview on several years experience

PG (NRL France) gave an overview of the ring trial scheme organised by the French NRL for the enumeration of *E. coli* in live oysters. The scheme was started in 2009 and consisted of 2 distributions each year to approximately 35 laboratories. Samples comprised of bio-accumulated (with fresh sewage) Pacific oysters and mussels. Each distribution comprised of 5 samples to be tested in duplicate. Points were allocated for reported results for both *E. coli* and *Salmonella* spp., this enabled performance assessments for each laboratory to be calculated. Satisfactory performance was identified as >80%.

4.1.3. NRLs participation and performance assessment in the EURL/HPA Shellfish EQA scheme for *E. coli* and *Salmonella*

The EURL (LS) presented the results reported by NRLs for the EURL/HPA EQA scheme for *E. coli* and *Salmonella* spp. detection. RT 44 (WS11/12). Twenty-one NRLs participated in 1 or more distributions during 2011 with 19 NRLs returning results for 2 or more distributions. Of those, 15 achieved a maximum CPA of 100% for both *E. coli* and *Salmonella* spp. detection. Ongoing performance assessment generated from data obtained from the last 3 years indicated that all NRLs participating in the scheme achieved >70% for *E. coli* analysis.

4.1.4. NRLs participation and performance assessment in the whole animal distribution for *E. coli* and *Salmonella*

The EURL (LS) presented results for the whole animal ring trial (RT 41) which consisted of a bio-accumulated Pacific oyster (*C. gigas*) sample. Twenty-nine laboratories (18 NRLs) participated in the distribution (WS11/13). Twenty-five laboratories returned replicate results within the expected range for *E. coli* and anticipated result for *Salmonella* spp.. One laboratory reported both replicate results outside -5 SD of participants' median and 3 laboratories reported high censored MPN results. Score deductions were incurred due to MPN values not being consistent with 5 x 3 MPN tables in ISO 7218 or 5 x 3 MPN tables previously supplied by the EURL and incorrect dilutions being prepared as instructed. An ongoing performance assessments was carried out on 18 NRLs who participated in the last 3 distributions achieved a CPA of >70%.

4.1.5. Supervision of Official Control Laboratories by NRLs

Information obtained from the PT questionnaire distributed to NRLs prior to the meeting was presented to the group. From the information provided it was noted that the majority of MS's stated that it was compulsory for OCLs to participate in PT schemes relating to LBM. NRL's who had not provided information were asked to return completed questionnaires to the EURL by 18th April 2012 (**Resolution 6**). Once all information had been received a summary document would be circulated prior distribution to the Commission and placing on the EURL website.

NRL
EURL

4.1.6. EURL draft guidance on the level and frequency of proficiency testing participation

RH gave an overview of the guidance document (WS11/15) written to assist NRLs on the level of frequency laboratories should participate in PT schemes along side other quality assurance procedures. It was noted that under EC Reg 882/2004 it is the responsibility of the NRL to supervise the performance of OCL's through PT schemes and access to OCL ID numbers should be available (**Resolution 7**). It was noted that this was not the case with all MS with Belgium identifying that the CA was responsible for assessing OCL's. No set criterion for participation frequency was given in ISO standards or guidance documents or in legislation. Guidance in the form of an advisory document from the European co-operation for accreditation was available which recommended laboratories should set their own criteria based essentially on risk. The EURL had undertaken some evaluation of PT frequency and

which inevitably showed that poor performance was identified more quickly with more frequent participation. It was stressed that participation in PT should be part of laboratory quality assurance procedures and that exclusive reliance on PT performance should not replace internal routine QC. Following a discussion NRLs agreed that participation in 2 PT distributions per year (1 EQA and 1 matrix) should be considered an absolute minimum if PT performance had been satisfactory over previous rounds (**Resolution 8**).

5. Marine Vibrios

5.1. Characterisation of *Vibrio cholerae* isolated in Italy

A brief overview on the work carried out on *V. cholerae* from clinical samples was presented by FL (NRL Italy). Six clinical cases of *V. cholerae* non-O1 non-O139 infection had been reported during 2006 – 2009 causing gastroenteritis and necrotizing fasciitis. Of those infections two were associated with the consumption of raw local mussels. In a clinical surveillance study in 2006, 2 out of 58 stool samples causing acute diarrhoea were positive for *V. cholerae* non-O1 non-O139; in a seafood study which analysed 230 samples, 4.7% of the samples were positive. It was noted that even though illness relating to *Vibrio* spp. were not generally notifiable in the EU, having better quality epidemiological information was vital to inform risk assessment, this was of particular relevance considering the potential increase in *Vibrio* exposure related to changing climate. The EURL would contact the ECDC regarding this issue (**Resolution 9**).

EURL

5.2. Outcomes of *Vibrio* ring trial (RT 38), ongoing revision of ISO TS 21872 and the mandate

The EURL presented the outcome of the *Vibrio* ring trial RT 38 and the progress to the revision of ISO TS 21872-1 and 2. Data generated from the PT had assisted in the selection of appropriate PCR targets (informative) for *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* for the ongoing revision of the ISO 21872 series. Relatively better (more accurate identification had been achieved using PCR ID compared with biochemical gallery based approaches on reference strains. It was noted that the data produced supported the inclusion of PCR based approaches for identification purposes. The proposal for an informative annex specifically for quantitation of toxigenic *V. parahaemolyticus* using MPN was discussed and would be proposed at the forthcoming SC9/WG6 plenary meeting. The next steps were discussed – prior to the main interlaboratory study (planned for Summer 2013) a pre-validation trial examining the use of PCR screening for 6-18 hour broths would be undertaken. The EURL would inform interested laboratories in due course.

EURL

5.3. Progress at CEN TAG3 – direct quantitative determination of *Vibrio* spp. and proposals for practical methods workshop July 4 – 5th 2012

The progress at CEN TAG3 on the development of a quantitative molecular detection method for *Vibrio* spp. was presented by CBA (EURL). The main focus has been to develop a method which can be applied directly to shellfish matrix. Currently two methods (colony hybridisation and real-time PCR) have been put forward. To date real-time PCR has shown to be easy to use, and extremely fast with extraction and real-time PCR being performed in less than 5 hours, compared to 4-5 days for culture-based approaches. To help with the application of these proposed methods the EURL announced a *Vibrio* methods workshop on the 4 - 5th July 2012 for which NRLs were encouraged to consider attending (**Resolution 10**).

NRLs

5.4. The research consortium Vibronet

ES (NRL Germany) informed NRLs of a research network being established for climate warming and the emergence of seafood and waterborne vibrios. The aim of the group is to establish a network of scientists to assess the occurrence of *Vibrio* spp. in the environment, identify problematic food processes and transportation, develop host-specific marker genes and a rapid and affordable method for detection.

5.5. Proposals for a COST initiative

CBA (EURL) highlighted several publications associated to *Vibrio* outbreaks in Europe. In several countries the reporting of outbreaks associated with *Vibrio* was good but not consistent. It was agreed that a need existed for a centralised system to record information. ECDC records outbreaks relating to toxigenic *V. cholerae* but others are not included. To address this, a proposal has been put forward to COST (European Cooperation in Science

and Technology) for funding. A separate objective of the COST proposal would be to develop a pan-European network of researchers currently engaged in *Vibrio* research, as some work in this field is fragmentary, and lacks cohesion both within and between regions in the EU, limiting its usefulness. NRLs interested in joining the group were welcomed.

NRLs

6. Pollution impacts

Summary findings from the impact of continuous sewage discharges on the microbiological quality of shellfisheries were presented by SK (UK NRL). The aim of the project was to obtain a better understanding of the accumulation and removal kinetics of various shellfish species exposed to continuous low level faecal contamination and to relate this to levels in water.

7. Viruses – Hepatitis A virus

7.1. Hepatitis A virus, a somewhat neglected shellfish-borne pathogen

Prof. AB (University of Barcelona) gave an excellent overview of Hepatitis A virus and the role of bivalve shellfish in disease transmission. Two notable hepatitis A virus outbreaks had occurred in Valencia (Spain) associated with the consumption of frozen clams imported from Peru which complied with all EU standards. The first outbreak occurred in 1999 with 189 tons of clams involved. The second outbreak occurred in 2008. The amount of clams involved in the outbreak was 3,766 tons. In the US 100,000 cases per year are reported. Vaccines are available and improved hygiene conditions help to reduce infection but it is still the most common viral hepatitis worldwide. The presentation is available to view in the Information Centre of the EURL website.

7.2. Looking for HAV in a shellfish production area over 2 years

SG (NRL France) described a study that was carried out over a 2 year period on a 500 Ha wild shellfish area. The presentation is available to view in the Information Centre of the EURL website. No evidence of hepatitis A virus in the production areas demonstrated, similarly in Denmark and Italy where semi-systematic studies on hepatitis A virus had been undertaken, no evidence of contamination was shown. Following discussion, it was concluded that considering the severity of infection and reported rare occurrence of hepatitis A virus in LBM production areas across the EU an absence of the virus in product was sensible (**Resolution 11**).

8. Viruses – Noroviruses

8.1. Tracing norovirus in oysters from Ireland to Denmark through France

ACS (NRL Denmark) and SG (NRL France) presented on several outbreaks that had occurred in France, Denmark and Norway between December 2011 and February 2012. Information provided with the oysters gave a French producer as the supplier. Oysters received in Denmark and Norway tested positive for NoV. The French authority informed the producer who stated that the oysters tested in Norway originated directly from Ireland. BD stated that large shipments of shellfish from Ireland were not always depurated before leaving the country. After a discussion relating to the transporting of shellfish between MS and outbreaks, the workshop recommended that the Commission should be informed of this issue (**Resolution 13**). The EURL would progress. Methodology used can also have a major impact on virus results and questions were asked as to whether reference material was included where negative results were obtained. The EURL informed NRLs that reference material was available from the HPA and requested that NRLs contact commercial laboratories testing for NoV and HAV recommending that they obtained reference material and participated in future PT (**Resolution 12**).

EURL

NRLs

8.2. Norovirus surveillance in the UK – Prevalence and levels of norovirus in *C. gigas* and *O. edulis* in England, Wales, Scotland and Northern Ireland

JL (UK NRL) presented a summary of a 2 year study to assess NoV contamination in UK oysters. The work was funded by the UK FSA and covered 39 sites. The aim was to determine NoV levels in UK oysters, assess seasonal and geographical variation, examine the relationship with *E. coli* and provide evidence for risk management. Both Pacific and native oysters were analysed for NoV (GI and GII) and *E. coli*. From the study 76.2% of samples were positive for NoV with levels varying widely (NoV GI maximum 16,507 copies/g; NoV GII maximum 18,024 copies/g) with a clear increase in autumn and decrease in spring. A large proportion of positive results were below the LOQ (<100copies/g). The study showed

good correlation between NoV and *E. coli* on a site-by site basis as well as between NoV and water temperatures. NRLs discussed the possible introduction of control limits for NoV in shellfish. It was agreed that an absence standard would impact on producers where unsuitable epidemiological evidence available (**Resolution 14**).

9. Virus – Towards virus controls

9.1. EFSA panel on Biological Hazards (BIOHAZ) – Scientific opinion on norovirus (NoV) in oysters: methods, limits and control options

BD (NRL Ireland) presented on the EFSA BIOHAZ WG on NoV in oysters (WS11/17). The FSA Ireland had asked the WG for a scientific opinion on the use of real-time PCR for detection and quantification of NoV, with a view to obtaining recommendations on levels of NoV that did not pose a risk to consumers. In addition treatment regimes to reduce NoV in oysters were requested. It was concluded that the use of real-time PCR was a reliable and robust method. Further work was needed to establish relationships between genome copies per g and human health risks. Post harvest treatment of oysters was not always effective for reducing NoV. The recommendations given were to place harvested oysters in non polluted production areas, to establish an acceptable limit for NoV in oysters and to optimise sampling strategies. The presentation is available to view in the Information Centre of the EURL website.

9.2. Discussion – Control options for norovirus

Suitable control strategies for NoV reduction in LBM were discussed. A number of potential approaches were identified including risk based monitoring in the harvesting areas. The need for harmonisation of methods to enable comparability of results between MS and laboratories was stressed. To help address the latter, the EURL will produce a generic EURL protocol based on the CEN standard and distribute it to NRLs and place on the EURL website (**Resolution 16**). Following discussion, on potential control measures. NRLs agreed that for NoV standards may be different for raw and cooked product. It was noted that few existed with respect to efficacy of cooking and/or commercial purification (mainly depuration). Substantial data gaps were identified in the knowledge base for species other than oyster (**Resolution 15**). Where possible NRLs should influence research programmes to provide additional datasets.

EURL

NRLs

The EURL presented paper WS11/18 - a summary of EFSA recommendations. Major discussion points included:

- Methodology standardisation (covered above).
- Interpretation of PCR results – problems associated with nonviable detection of non-infectious virus.
- Approach to setting a virus standard –risk management should include both end-product standards and harvesting areas.
- Sampling strategies – require investigation.
- Pollution reduction strategies – NRLs agreed that prohibition zones (dilution or size) around sources of pollutants was potentially valuable and worthy of further investigation. The EURL would investigate and propose a dedicated session at the next workshop (**Resolution 20**).
- Class B criteria – Following discussion, NRLs agreed that the current class B criterion of 10% of samples up to 46,000 *E. coli* MPN/100g was too high and may allow highly contaminated product to be placed on the market. It was agreed that the upper tolerance should be based upon practical performance of the method; the EURL would communicate this opinion to the Commission and the EFSA WG (**Resolution 21**).

EURL

EURL

10. Viruses – Proficiency Testing for norovirus and hepatitis A virus

10.1. The first ring trial for the detection of HAV and NoV (GI and GII) in bivalve mussels organised in Italy

ELS (NRL Italy) presented on the 1st virus PT distributed organised by NRL Italy in which material was distributed to 12 laboratories. Prior to distribution the qualitative real-time PCR method and practical training was provided. Material was prepared and QC performed. The distribution comprised dsDNA and RNA, viral suspensions, matrix samples and process controls. A variety of methods were reported by participants with a 100 fold difference in

<p>concentration levels. A large proportion of false negatives were reported for matrix samples.</p>	
<p>10.2. EURL norovirus and hepatitis A virus proficiency testing – PT 43 A summary of participants' performance in the EURL virus PT RT 43 (WS11/19) was given by JL. The distribution included LENTICULES and shucked oyster matrix distributed to 26 laboratories. Thirty-eight percent of participating laboratories obtained 100% for all samples. The false positive reporting rates for GI, GII and HAV were 0%, 12% and 2% respectively. The false negative reporting rates for GI, GII and HAV were 31%, 27% and 4% respectively. Seventeen laboratories returned quantitative real-time PCR data which was an increase from last years PT. From the information on the methods used it was evident that laboratories using predominantly CEN methodology tended to have better results than those using predominantly non-CEN methodology (Resolution 17). The EURL would offer further virus PT in 2012, the programme of EURL organised PT was available on the EURL website. NRLs should express interest in participation to LS.</p>	<p>NRLs</p>
<p>10.3. Proposals for PT, EURL harmonised protocol and training requirements – Discussion The EURL described the draft EURL generic protocol (WS11/20). Further consideration should be given to training to assist laboratories wishing to implement virus methods. Several NRLs were keen that training should be made available at the EURL. The EURL would propose a virus training workshop for early in 2013 further information would be disseminated to NRLs in due course (Resolution 19).</p> <p>Further to the above substantial work was still needed to improve comparability of quantitative results for NoV. The EURL agreed to continue the development of standards to assist in accurate quantitation and would organise further PT using matrix samples and dsDNA standards (Resolution 18).</p>	<p>EURL EURL</p>
<p>10.4. Update on the progress of CEN validation JL gave a brief update on the CEN validation (M/381) for viruses. The mandate was signed in January 2011 by DG Enterprise. Performance characterisation is due to take place at the end of 2012 with the Inter-laboratory study expected to take place during 2013. NRLs would be informed of progress</p>	<p>EURL</p>
<p>11. Next meeting NRL Italy (Rome) offered ISS as the next meeting venue; the workshop accepted provisionally pending assessment of the finances by the EURL. Proposed dates were 7-9 May 2013 (Resolution 22).</p>	<p>EURL</p>

Resolutions of the 11th workshop of Microbiological NRLs for Bivalve Molluscs, Cefas, Weymouth, 24 - 26th April 2012

Official controls – microbiological monitoring and classification

1. The final recommendations to the Commission concerning harmonisation of Codex Standards (292-2008) and EU hygiene regulations for *E. coli* criteria were presented to NRLs. Recommendations include adoption of the Codex 3 class plan as an end product microbiological criteria and introduction of the Codex criteria applied 'over time' as criteria for classification of A grade production areas. These recommendations were now with Member States (MS) for consideration and the EURL would report progress to NRLs in due course.
2. Issue 3 (incorporating MS comments) of the proposed Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Harvesting Areas was presented to NRLs. The guide was now with MS for comment prior to submission to SCFCAH. It was noted that adoption as an official Community guide would confer legal status. The EURL would report progress to NRLs in due course.
3. The EURL thanked NRLs for the updated information on sanitary surveys conducted in their MS. Outstanding information would be supplied within 3 weeks of the workshop. The finalised document would be circulated to NRLs prior to distribution to the Commission and publication on the EURL website (public domain).
4. The EURL presented recommendations for revision of the stability assessment included in the Microbiological Monitoring Good Practice Guide (a reduced frequency of monitoring is acceptable for 'stable' areas). The EURL proposed additional work to develop new criteria and, following definition of requirements, will request monitoring datasets to assist evaluation. In the interim a revised revision of the Guide (issue 5) will be distributed to NRLs and published on the EURL website (public domain).
5. The EURL announced the 2nd international workshop on molluscan shellfish area classification on 24th – 28th September 2012 at Newport, Rhode Island, USA jointly organised by the EURL and the US FDA. It was noted that this workshop would focus on comparison of EU and US systems and was regarded as important for informing the ongoing US/EU equivalency negotiations. NRLs and Competent Authorities from MS interested in exporting to the US were encouraged to attend via notification of either the Commission or the EURL.

Official control – Comparative testing

6. The EURL thanked NRLs for the updated information on official control laboratories (OCL) and proficiency testing in their MS. Following supply of outstanding information (within 3 weeks of the workshop) a summary document would be circulated to NRLs prior to distribution to the Commission and publication on the EURL website (public domain).
7. Supervision of the quality of OCL testing through proficiency testing and other measures is a legislative responsibility of NRLs under EC Reg 882/2004. NRLs require access to OCL proficiency test results to discharge this responsibility. Competent Authorities have an obligation to work with NRLs to ensure access to this information.
8. Four annual proficiency testing distributions for *E. coli* and *Salmonella* are available (3 HPA/ EURL non-matrix and 1 EURL matrix based distribution). It was agreed that the minimum requirement for satisfactorily (>70% score) performing NRLs was participation in the EURL matrix distribution and at least one other non matrix distribution. This was in accordance with the minimum participation frequency specified in the Good Practice Guide. NRLs with <70% scores should participate in all available distributions.

Marine vibrios

9. NRLs noted that, with the exception of O1/O139 *V. cholerae*, illness related to *Vibrio* spp. were not generally notifiable in the EU. Considering the potential impact of climate change on *Vibrio*

spp. occurrence, better quality epidemiological information was vital to inform risk assessment. The EURL would contact the European Centre for Disease Prevention and Control (ECDC) regarding this issue.

10. The EURL announced a *Vibrio* methods workshop in July 4-5th 2012, NRLs were encouraged to consider attending the workshop. Expressions of interest should be sent to the EURL.

Viruses

11. NRLs considered control limits for hepatitis A virus (HAV) in bivalve molluscs. Given the severity of the infection and the rare occurrence in mollusc production areas in the EU, the workshop recommended a bivalve mollusc microbiological criterion of HAV absence (non detectable) when tested by the ISO CEN standard method.
12. It was agreed that to improve the quality of viral analysis, and hence risk management support available to producers, NRLs should write to commercial laboratories undertaking testing of live bivalve shellfish for norovirus and HAV and recommend the use of virus reference materials (available from the UK HPA) and participation in available, appropriate proficiency testing.
13. Several cross border EU outbreaks related to norovirus in oysters were reported by NRLs. It was noted that traceability of batches related to outbreaks was often problematical. This was particular relevant to trans-shipment of product between MS for further processing prior to final packaging and issue of health marks. The workshop recommended that this issue be brought to the attention of the Commission.
14. Following the recent EFSA reports, and data available from outbreaks and EU production area surveillance, NRLs considered possible control limits for norovirus in bivalve molluscs. NRLs agreed that an absence standard, whilst conservative for public health, would have a high impact on producers that would not seem justified by available epidemiological evidence.
15. The workshop agreed that for norovirus differential quantitative standards for products intended to be eaten raw, and cooked in the home or restaurant may be appropriate. Data gaps were the protection afforded by home and restaurant cooking, commercial depuration and on norovirus levels in species other than oysters.
16. NRLs were informed that the ISO CEN standard method - ISO TS 15216-1 Microbiology of food and animal feed: Detection of norviruses and hepatitis A virus, part 1: Quantitative determination would be published in 2012/13. An EURL harmonised protocol for molluscs based upon the standard was distributed and will be available on the EURL website (public domain).
17. The workshop noted encouraging performance in virus proficiency testing, particularly with respect to an increasing number of laboratories applying quantitative methods. NRLs noted that performance of laboratories using methods closely derived from the CEN standard method (ISO TS 15216-1) tended to perform better in proficiency testing than those using alternative methods.
18. Further to the above NRLs noted that further work was still required to improve comparability of quantitative results for norovirus prior to the introduction of quantitative legislative criteria. The EURL agreed to continue to develop quantitative reagents (standards) to help underpin accurate quantitation and to organise further proficiency testing distributions using matrix samples and dsDNA standards.
19. NRLs requested that the EURL provide technical training on quantitative determination of norovirus in bivalve shellfish. A formal training workshop would be proposed for early 2013.
20. NRLs agreed with the EFSA recommendation on pollution reduction strategies (e.g. prohibition zones) and agreed to have a dedicated session on this aspect at the next workshop.
21. NRLs agreed with the EFSA opinion that the current class B criterion of 10% of samples up to 46,000 *E. coli* MPN/100g was too high and may allow highly contaminated product to be placed

on the market. It was agreed that the upper tolerance should be based upon practical performance of the method.

Date and time of next meeting

22. The next workshop would provisionally (subject to confirmation of costs) be held at ISS in Rome, Italy 7 – 9th May 2013.



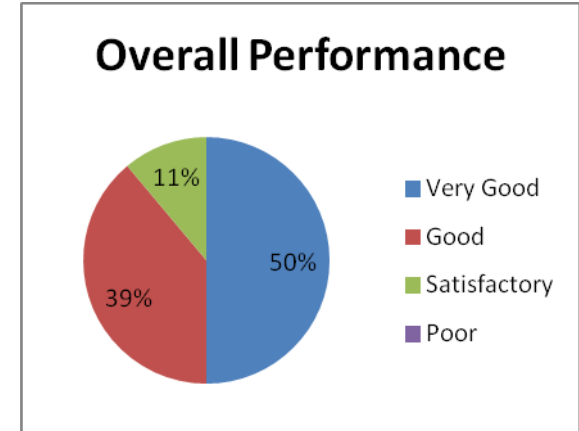
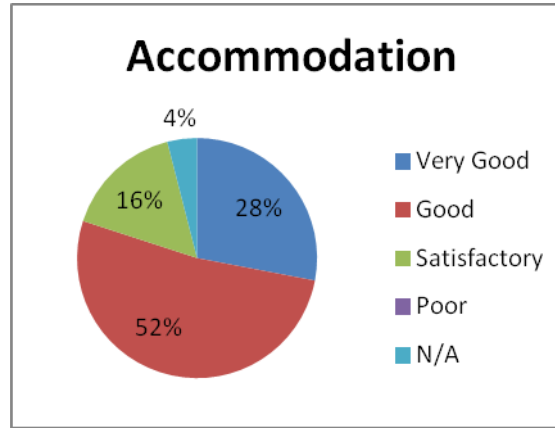
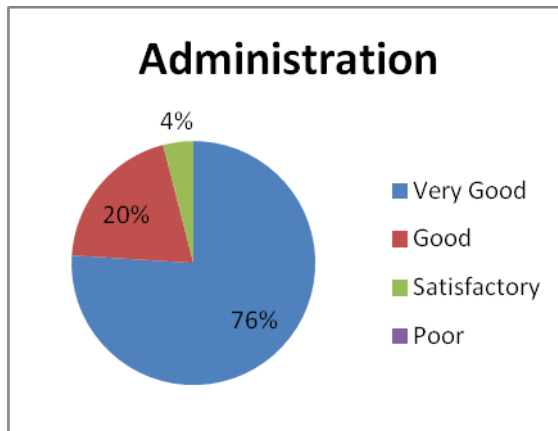
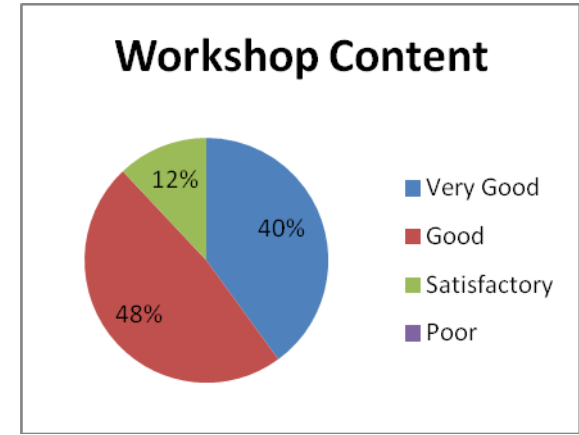
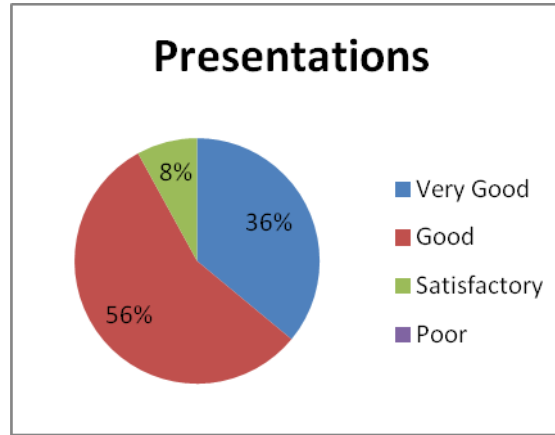
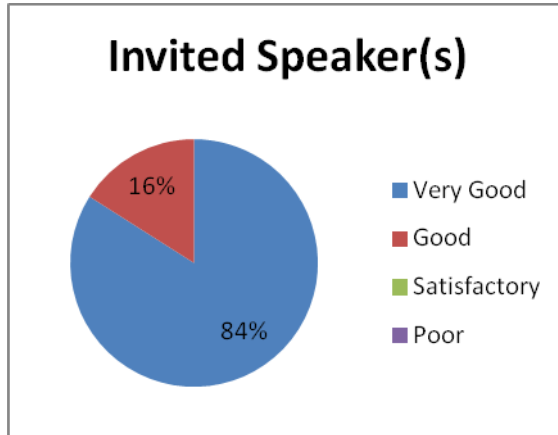
European Union Reference laboratory for monitoring bacteriological and viral contamination of bivalve molluscs

The Centre for Environment, Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road,
The Nothe,
Weymouth,
Dorset DT4 8UB UK
Tel: +44 (0) 1305 206600, Fax +44 (0) 1305 206601
Email: fsq@cefass.co.uk <http://www.crlcefass.org>

List of papers for 11th Workshop of Microbiological NRL's

WS11/00	List of papers
WS11/01	Instructions on how to complete your expenses claim form
WS11/02	Expenses claim form
WS11/03	Delegates List
WS11/04	Report on the 10 th Workshop of National Reference Laboratories for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs
WS11/05	Agenda
WS11/06	EURL microbiological contamination of bivalve molluscs final work programme 2012
WS11/07	EU-RL regarding possible harmonisation of EU and Codex standards for LBM
WS11/08	Community guide microbiological monitoring bivalve mollusc harvesting areas
WS11/09	The Application of Sanitary Surveys in EU Member States
WS11/10	Application of sanitary surveys in EU Member States (including EFTA and accession countries) version 1 (020412) Sanitary survey template
WS11/11	Second International Workshop on Molluscan Shellfish Area Classification
WS11/11a	Agenda FDA EU shellfish workshop draft v3
WS11/12	NRLs participation and performance assessment in the EURL/HPA Shellfish EQA scheme for <i>E. coli</i> and <i>Salmonella</i> (RT 44)
WS11/13	NRLs participation and performance assessment in the whole animal distribution for <i>E. coli</i> and <i>Salmonella</i> (RT 41)
WS11/14	Supervision of Official Control Laboratories by NRLs
WS11/15	EURL draft guidance on the level and frequency of PT participation
WS11/16	Inter-laboratory trial for generation of additional data for the revision of ISO TS 21872 series 2011
WS11/17	EFSA Panel on Biological Hazards (BIOHAZ)- Scientific Opinion on Norovirus (NoV) in oysters: methods, limits and control options
WS11/18	Control Options
WS11/19	EURL norovirus and hepatitis A virus proficiency testing – PT43
WS11/20	Quantitative detection of Norovirus and Hepatitis A virus

Confidential Participant Feedback Results



Comments:

1. The support in advance by EU-RL of funding travelling and accommodation is very helpful.
2. Many interesting discussions. However, for few there is no outcome on decision made.
3.
 - a. The first day content was somewhat too long.
 - b. Is it possible to have the workshop on 2 days only?
4.
 - a. Lower part of sheets are not well visible from seats in the back.
 - b. Discussions are very long, with loose ends.
 - c. Lot of extra time needed every day perhaps extend program?
5. Better weather!

Workshop declaration

This technical report is submitted in accordance with the requirements of Commission Regulation (EC) No1754/2006 laying down detailed rules for the granting of Community financial assistance to Community reference laboratories for feed and food and the animal health sector, following the workshop of National Reference Laboratories for bacteriological and viral contamination of bivalve molluscs held in Weymouth 24 - 26th April 2012.

Dr David Lees
EURL Director

22nd June 2012

Dr Rachel Hartnell
EURL Co-ordinator

22nd June 2012

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Head office

Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK

Tel +44 (0) 1502 56 2244
Fax +44 (0) 1502 51 3865
Web www.cefas.co.uk

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Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601

