Report on the 12th Workshop of National Reference Laboratories for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs

Rome, Italy, 7th – 9th May 2013

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Contents

Forward

This document summarises relevant information from the 12th workshop of National Reference Laboratories for monitoring bacteriological and viral contamination of bivalve molluscs held at the Istituto Superiore di Sanita Rome, Italy on 7th - 9th May 2013. 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.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

12th Workshop of Microbiological NRLs, 7 – 9 May 2013

Venue: Istituto Superiore di Sanita,
Viale Regina Elena No. 299,
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Italy

Tel : +39 06 49 90 36 46 (contact Dr Elisabetta Suffredini)

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Day 1 - Tuesday 7th May 9:30 - 17:30

1 Introductory meeting

1.1 Welcome, introductions and apologies (paper WS12/01).

1.2 Domestic arrangements including reclaim of expenses (papers WS12/02, WS12/03, WS12/03A).

1.3 Actions arising from the 11th workshop 2012 (paper WS12/04).

1.4 Agreement of the agenda (paper WS12/05).

1.5 EURL work programme 2013 (EURL) (paper WS12/06).

1.6 Revisions to EURL website (EURL).

Coffee/tea break (10:30 am)

2 Official controls - Microbiological monitoring and classification

2.1 Feedback from the 2nd International Workshop on Molluscan Shellfish Area Classification and Management, USA (paper WS12/07) (EURL).

~~**2.2** Feedback from the latest round of MS audits on LBM (Telmo Valinhas, DG Sanco FVO).~~ CANCELLED

2.3 Feedback from the Commission Restricted Working Group - Codex *E. coli* standards (paper WS12/08) (EURL).

2.4 Good Practice Guidance

2.4.1 Publication of Community Guide issue 1 (EURL).

2.4.2 Revisions to Technical Good Practice Guide issue 4 (paper WS12/09) (EURL).

2.4.3 Practices on waiving results of monitoring programmes (roundtable).

Lunch break (1:00 – 2:00 pm)

2.5 Data management, spatial analysis and epidemiological investigation: a task force in evaluating risk factors for microbiological shellfish contamination – (Francesca Barchiesi, NRL Italy, Ancona).

2.6 Feedback on status of equivalency negotiations with USA (EURL).

2.7 Possible approaches to buffer (prohibition) zones around sewer pipes.

2.7.1 Introduction and US FDA requirements (EURL).

2.7.2 Considerations from Italy?

2.7.3 Considerations from France?

2.7.4 Discussion (roundtable).

Coffee/tea break (3:30 pm)

3 Official Controls - Proficiency Testing

3.1 Whole animal distribution for *E. coli* and *Salmonella*, (PT 45) (paper WS12/10) (EURL).

3.2 Shellfish EQA scheme for *E. coli* and *Salmonella*, (PT 48) (paper WS12/11) (EURL).

Dinner –7.30pm

Day 2 - Wednesday 25 April 9:15 - 17:00

4 Marine vibrios

4.1 Investigation of the presence of type III secretory systems 2 (T3SS2) in *V. parahaemolyticus* strains isolated in Italy (Francesca Leoni, NRL Italy, Ancona).

4.2 *Vibrio parahaemolyticus* outbreak, Northwest Spain, August 2012 (EURL).

4.3 Review of the practical methods workshop July 4-5th 2012

4.4 Revision of ISO TS 21872 and progress with the validation under the CEN mandate (M/381) (paper WS12/12) (EURL).

4.5 Bivalve trade discussion between the EU and USA (paper WS12/13) (EURL).

Coffee/tea break (11:00am)

5 Virus monitoring

5.1 Korean Experience with NoV monitoring and the Korean Shellfish Programme (Hongsik Yu, NRDRI, Republic of Korea).

5.2 Enteric viruses regional surveillance: detection of Norovirus and Hepatitis A in bivalve shellfish farmed and marketed in Sardinia Region - 2009/2012 **–** (Riccardo Bazzardi, Food Hygiene Dept. IZS Sardegna – Sassari).

5.3 A two year study of enteric viruses in Scandinavian blue mussels (Anna Charlotte Schultz, NRL Denmark, Mette Myrmel, NRL Norway and Magnus Simonson, NRL Sweden).

5.4 Assessment of viral contamination in Italy: qualitative and quantitative data (NRL Italy, Roma).

5.5 HEV bioaccumulation in shellfish species and environmental investigations (Soizick LeGuyader, NRL France).

Lunch break (1:00 – 2:00 pm)

6 Virus methods

6.1 Technical progress on the application of the methods for virus analysis in Italy (NRL Italy, Roma).

6.2 EURL proficiency test norovirus and HAV (PT 46) (paper WS12/14) (EURL).

6.2.1 Discussion on next steps.

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

7 Virus outbreaks

7.1 Norovirus virus outbreaks associated with oysters in 2012/13 in the UK (UK NRL).

7.2 *Anything else from NRLs on outbreaks.*

7.3 Discussion on management of outbreaks (eg minimum closure periods, reopening criteria, virus testing policy, etc) (roundtable).

Coffee/tea break (3:30 pm)

8 Virus controls

8.1 Discussion paper on live bivalve molluscs and human enteric virus contamination: options for improving risk management in EU food hygiene package (paper WS12/15) (EURL).

8.1.1 Feedback from discussion at Member State Working Group (EURL).

8.1.2 Discussion and opinions of NRLs on setting virus criteria (roundtable).

Day 3 - Thursday 9 May 9:30 - 12:00

9 Agreement of Workshop resolutions.

10 Any other business.

11 Date and venue for next meeting.

Meeting close

Delegate List

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Korea	(observer)	Hongsik Yu	Invited speaker	yhspknu@korea.kr
		Dr Kim	Invited speaker	
Sardinia	(observer)	.Riccardo Bazzardi	Invited speaker	riccardo.bazzardi@gmail.com
		Margherita Pisanu	Invited speaker	margherita.pisanu@izs-sardegna.it

Minutes of the 12th Workshop of Microbiological NRLs for Bivalve Molluscs, Cefas, Rome, 7th - 9th May, 2013.

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.
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.
Reimar Johne (RJ)	NRL Germany	Federal Institute for Risk Assessment, Berlin.
Ntina Vasileiadi (NV)	NRL Greece	Institute of Food Hygiene of Athens, Athens.
Zsuzsanna Sreterne Lancz (ZSL)	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 (GT)	NRL Latvia	National Diagnostic Centre of Food & Veterinary Service (FVS), Riga.
Aurelija Kraucelyte (AK)	NRL Lithuania	National Food and Veterinary Risk Assessment Institute, Lithuania
Irene Pol-Hofstad (IPH)	NRL Netherlands	National Institute of Public Health and the Environment (RIVM), Bilthoven.
Ewelina Bigoraj (EB)	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.
Urska Henigman (UH)	NRL Slovenia	National Veterinary Laboratory, Ljubljana.
Cristina Acebal (CA)	NRL Spain	Agencia Espanola de Seguridad Alimentaria, Majadahonda, Madrid.
Cristina Alvarez Alvarez CAA)	Invited expert	Centro de Control da Calidade do Medio Marino Pontevedra
Magnus Simonsson (MS)	NRL Sweden	National Food Administration, Uppsala.
Ronnie Eriksson (RE)	NRL Sweden	National Food Administration, Uppsala.
Ron Lee (RL)	NRL UK	Cefas, Weymouth.
James Lowther (JL)	NRL UK	Cefas, Weymouth.
Craig Baker-Austin (CBA)	NRL UK	Cefas, Weymouth.
Ines Skoko	Croatia	Croatian Veterinary Institute, Split.
Irena Listes (IL)	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.
Hongsik Yu (HY)	Invited Speaker	NFRDI, Korea
Director Dr Kim (DK)	Invited Speaker	NFRDI, Korea
Riccardo Bazzardi (RB)	Invited Speaker	Istituto Zooprofilattico Sperimentale della, Sardinia
Margherita Pisanu (MP)	Invited Speaker	Istituto Zooprofilattico Sperimentale della, Sardinia

Observers Chelvi Leonard (CL) FSA.

Apologies
Paolo Caricato DG SANCO, European Commission.
Telmo Valinhas, FVO, DG Sanco, European Commission

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

Note. All presentations can be viewed in the Restricted documents section of the EURL website www.eurlcefas.org

Acronyms

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

1 Welcome meeting

1.1 Welcome and introduction

DNL opened the meeting and invited all NRL representatives to introduce themselves to the group. The EURL thanked NRL Rome for hosting the workshop.

1.2 Domestic arrangements including reclaim form (WS12/02, WS12/03, WS12/04)

Delegates were informed that it was their responsibility to ensure all expense receipts were copied and returned to SA by the 24th May 2013.

1.3 Actions arising from the 11th workshop

The resolutions and report from the 11th workshop (Weymouth 2012) were reviewed (WS12/04). All actions had been completed; any major findings are covered separately as agenda items.

1.4 Agreement of the agenda (WS12/05)

All NRLs agreed to the workshop agenda (WS12/05).

1.5 EURL Work Programme 2013 (WS12/06)

RH presented the main themes of the EURL WP agreed with DG Sanco. The WP for 2013 included provision of technical advice and training for NRLs, Accession country NRLs and others in relation to official and non-statutory control analyses (WS12/06). [WS12_Pre 1.5]

1.6 Revisions to EURL website

LS gave a demonstration of the new EURL website. All documentation except the EURL workshop presentations and papers would be available as public domain information documents to enable easier access. LS recommended that all NRLs re-registered on the website once live. [WS12_Pre 1.6]

2 Official Control - Microbiological monitoring and classification **2.1. Feedback from 2nd International Workshop on Molluscan Shellfish Area classification and Management, US (WS12/07)** RH presented paper WS12/07 and summarized the findings from the international workshop, outlining the differences between the EU and US systems. Within the EU testing is performed on flesh, whilst the US analyse water. It was noted that third countries wishing to export to both the EU and US are required to run both systems. DNL stated that it had been proposed that international guidance was elaborated. The FAO/WHO had been approached to support the development and NRLs would be informed of any progress. The role of Codex was discussed, although the general views were that any guidance must be produced through elaboration of the Codex Code of Practice format rather than Codex *per sé*. It was envisaged that the WG will comprise members from outside the EU and US. Following a discussion it was agreed that individuals or institutes wishing to participate should seek agreement from the WG. RH would act as the contact point. [WS12_Pre 2.1]	
2.2. Feedback from the latest round of MS audits on Live bivalve molluscs Due to prior engagements TV was unable to attend the meeting so the agenda item was not included.	
2.3. Feedback from the Commission Restricted Working Group – Codex *E. coli* standards (WS12/08) DNL gave an overview from the MS restricted WGs held since the last NRLs workshop. Progress with respect to the Codex *E. coli* criterion for end-product testing, standards for Class A production areas and continuation of *Salmonella* spp. testing were discussed. Following a brief discussion, NRLs offered their continued support for the Codex criterion proposed by the EURL and would liaise with their CA's with regard to this issue **(Resolution 1).** [WS12_Pre 2.3]	All NRLs
2.4. Good Practice Guide **2.4.1. Publication of Community Guide issue 1** DNL informed the group of the new CG on Good Practice with respect to the monitoring of LBM production areas. The CG documents are to assist MS in interpretation of Community legislation. The guidance was derived from the EURL GPG and as an official Commission document has a legal basis. DNL informed all NRLs that the CG was available on the EURL and DG Sanco websites and urged all NRLs to read. DNL also noted that this would be the basis of trade agreements with the FDA. [WS12_Pre 2.4.1]	
2.4.2. Revisions to Technical Good Practice Guide (GPG) issue 4 (WS12/09) RL presented paper WS12/09 outlining the proposed changes to the new revision of the GPG which included the introduction of an executive summary, extension of the review period for SS's from three to six years, harmonisation of terms with the CG, removal of statistical stability assessment and clearer links between the sampling plan and the extent of the classified zone. NRLs were requested to send any comments to RL which he would then forward to the WG. RL presented a stability assessment on data obtained from 12 Class A "stable" sites. Geometric means showed no support for either the original or revised stability criteria. A larger data set would assist in development of production area stability assessments. NRLs agreed to provide additional data, the EURL would provide detailed criteria to assist this **(Resolution 2)**. Whilst additional data were being analysed, an agreement to assess 3 years of data would continue with no result exceeding 230 for class A areas and 4,600 for class B areas **(Resolution 3).** [WS12_Pre 2.4.2]	NRLs EURL
2.4.3. Practices on waiving results of monitoring programmes (roundtable discussion) DNL chaired a roundtable discussion on the practices for waiving results from monitoring programme assessments. Several NRLs stated the GPG had been adopted in full or part and that data may be excluded based on STW failure or non-compliance with protocols. A summary of all NRLs responses would be collated by the EURL **(Resolution 4).** (See Appendix 1 – Details of criteria for waiving results of monitoring programmes).	EURL

2.5. Data management, spatial analysis and epidemiological investigation: a task force in evaluating risk factors for microbiological shellfish contamination

Francesca Barchiesi (NRL Italy, Ancona) presented on the complexity of performing a risk analysis. All sources of environmental and analytical data were collated to produce an unbiased and efficient risk analysis. [WS12_Pre 2.5]

2.6. Feedback on status of equivalency negotiations with USA

DNL gave an update on the EU and US trade negotiations, outlining the issues raised at the trade negotiation meeting held in Washington DC in December 2012. FDA will accept only EU 'class A' (equivalent to US 'Approved') sites following completion of an FDA audit basis on both the Community and Technical Guidance. To date only the UK, Netherlands and Spain have expressed an interest in trading with the US. Any MS wishing to trade can register with the Commission. [WS12_Pre 2.6]

2.7. Possible approaches to buffer (prohibition) zones around sewer pipes

2.7.1a Introduction and US FDA requirements

The EURL presented paper (2.7.1a) Molluscan Shellfish Equivalence Project – paper for workshop discussion on harvesting area/buffer zones. Essentially this set out the FDA requirements for consideration of buffer zones included in the NSSP with respect to wastewater discharges, managements plans, zones around marinas, It also included some additional points for clarification with respect to the practices across the EU. [WS12_Pre 2.7]

2.7.1 Evaluating the dilution of wastewater treatment plant effluent and viral impacts on shellfish growing areas in Mobile Bay, Alabama

RL (EURL) gave a presentation summary on behalf of the FDA showing possible scenarios following a WWTP failure. These illustrated a zoned approach moving from prohibited - restricted - conditionally approved - approved and various combinations thereof. US FDA guidance was that a 1000:1 dilution should be considered as a minimum near outfall, together with use of notification times e.g. the speed at which harvest can be suspended. Following discussion, it was noted that in the EU it would not be possible to demonstrate that US faecal coliform standards were met on the perimeter of buffer zones without testing water. NRL Italy noted that the absence of a category in the EU corresponding to "conditionally approved" made management of rainfall events problematic, even with the use of prohibition zones.

2.7.2 Considerations from Italy

NRL Italy, Ancona (ML) presented on the use of prohibition zones around known pollution sources. The prohibition zones were determined by coast guard coordinates and not based on scientific data. In some areas prohibition zones were set to 500 metres from pollution source. [WS12_Pre 2.7.2]

2.7.3 Considerations from France

NRL France (PG) presented on the use of shoreline surveys to determine buffer zones around sewer pipes. [WS12_Pre 2.7.3]

2.7.4 Prohibition zones (roundtable discussion)

NRLs were asked to provide information on their countries practices relating to prohibition (buffer) zones. From the information provided it was noted that practices varied both between countries and within regions. Most NRLs supported the principle of prohibition zones and agreed to further develop the use of geographical or dilution approaches **(Resolution 5).** A summary of all NRLs responses would be collated by the EURL **(Resolution 6).** (See Appendix 2 – Prohibition zones). EURL

3 Official controls – Proficiency Testing

3.1 Whole animal distribution for *E. coli* and *Salmonella* spp. (WS12/10)

The EURL (LS) presented results for the whole animal PT (PT 45) which consisted of 2 samples, sample A, Pacific oyster (*C. gigas*) and sample B, Common mussels (*Mytilus edulis*).

Fifty laboratories (26 NRLs) participated in the distribution (WS12/10). [WS12_Pre 3.1] *E. coli*: Forty-two laboratories returned results within ±3 SD of participants' median for sample A (Oyster) with 37 laboratories returning results within ±3 SD of participants' median for sample B (Mussels). Four laboratories for sample A and 8 laboratories for sample B reported results which fell outside ±3 SD of participants' median. Five laboratories had score deductions for MPN value(s) not consistent with guidance in ISO 7218 for interpretation of 5 x 3 MPN tables. *Salmonella* spp.: Forty-five laboratories returned a result of not detected for sample A (Oysters) with 46 laboratories returning a result of not detected for sample B (Mussels). Three laboratories (2 for sample A and 1 for sample B incorrectly reported the presence of *Salmonella* spp. in a single sample. Over the last 3 whole animal distributions, 19 NRLs scored 100%. 5 NRLs had not participated in any distribution. The EURL would follow the Commission protocol with respect to non participation **(Resolution 7)**. **3.2 Shellfish EQA scheme for *E. coli* and *Salmonella* (WS12/11)** The EURL (LS) presented the results reported by NRLs for the EURL/PHE EQA scheme for *E. coli* and *Salmonella* spp. detection PT 48 (WS12/11). Samples consisted of LENTICULE™ discs containing fully characterised bacterial isolates. Twenty-two NRLs participated in one, two or all (12 NRLs) of the distributions. All laboratories achieved a cumulative total of >70% for the EURL whole animal and 1 or more EURL/PHE distributions. [WS12_Pre 3.2] **3.3 Liaison with ISO and CEN** RH updated the network on changes to ISO standards following the last SC9 and WG6 plenary meetings in Brussels in 2012. **4 Marine Vibrios** **4.1 Investigation of the presence of type III secretory systems 2 (T3SS2) in *V. parahaemolyticus* strains isolated in Italy** NRL Ancona (FL) presented on *Vibrio parahaemolyticus* strains isolated in Italy, highlighting the 5 variants of the *tdh* and the 2 subgroups of *trh* gene, with O3:K6 serotype having emerged as a major cause of illness. There followed a brief discussion on traditional pathogenic markers not being reliable. CBA explained in the UK a small scale surveillance project looking at clinical isolates would be starting shortly. **4.2 *Vibrio parahaemolyticus* outbreak, Northwest Spain, August 2012** The NRL (CBA) gave a presentation on the *V. parahaemolyticus* outbreak which occurred in Galicia, Northwest Spain in August 2012. The outbreak was linked to the consumption of shellfish at a banquet on a local boat, where epidemiological indicated locally produced ice used to cool the shellfish as a likely route of exposure. It was confirmed that shrimp produced (cooled on ice) was the most risky food item consumed, although several other food items (e.g. lobster and crab meat) had been implicated. This was the largest foodborne *Vibrio* outbreak ever to be reported in Europe involving seafood. **4.3 Review of the practical methods workshop - July 2012** The EURL presented on the practical methods workshop held at Cefas in July 2012. The main objective of the meeting was to provide practical insight into methods being developed at Cefas for the detection and quantification of *Vibrio* spp.. The EURL assembled a range of presentations from experts in their respective fields that encompassed many relevant aspects to vibrios in a European context, these included Dr Dominique Hervio-Heath (IFREMER) and Professor James D. Oliver (University of North Carolina)Several important outputs from the meeting were agreed including; the requirements for - greater collaborative research efforts across the EU and internationally; greater efforts to share strains by providing a central resource for *Vibrio* isolates; -provision of *Vibrio* data on a range of shellfish matrices other	EURL All NRLs

than Pacific oysters and, the need to establish vibriosis as a regionally notifiable disease within Europe as in the US. A second workshop has provisionally been scheduled for January 2014 and anyone interested in attending should contact CBA.	All NRLs
4.4 Revision of ISO TS 21872 and progress with the validation under the CEN mandate (M/381) (WS12/12) The EURL (RH) presented paper WS12/12 highlighting the proposed merging of ISO 21872 parts 1 and 2, although this was not yet formally approved. RH stated the revision would include modifications of the requirements for growth in salt peptone water, the removal of saline triple sugar iron agar and ornithine decarboxylase test and the optional use of PCR for identification of species and pathogenic markers. RH stated preliminary studies had indicated performance was enhanced in terms of specificity of isolate identification through use of PCR compared with biochemical test identification. Comments from the group on the draft were welcome, and these would be fed back to the CEN working group. At present the EURL were not planning any Vibrio PT distributions, although the PHE provides a Vibrio scheme in which NRLs can participate. RH and DNL suggested that the issue of a Vibrio PT should be revisited at next year's workshop **(Resolution 8).** [WS12_Pre 4.4]	EURL
4.5 Bivalve trade discussion between the EU and USA (WS12/13) CBA presented paper WS12/13 on behalf of the EURL, marine vibrios - summary of food safety concerns regarding trade of LBM from the US to EU. The main focus of the negotiations was with regard to the establishment of a reciprocal trade agreement for LBM produce. The negotiations involved representation from the EU (EURL), DG Sanco and the US (FDA). It was identified during preliminary discussions that there were various different human health risks associated with trade of these commodities originated from the different regions. In particular, this related to the issue of potentially pathogenic vibrios from US LBM produce and the norovirus risks from EU LBM The EURL provided a summary of potential concerns and a position paper(WS12/13) which had been drafted and submitted to the FDA prior to initial discussions (May 2013). There followed a discussion on the effectiveness of the US control plans, and the impact on public health if products from an at risk area in the US were exported to the EU *V. vulnificus* was recognised as the primary public health concern from imported US produce, it was further noted that currently across the EU indigenously acquired *V. vulnificus* infections were almost exclusively wound infections and were not associated with consumption of LBM. [WS12_Pre 4.5]	
5 Virus Monitoring **5.1 Korean experience with NoV monitoring and the Korean shellfish programme** The invited speaker from NFRDI, MOF Korea (HY) gave a presentation on the KSSP Growing Area Management for mitigation of NoV pollution sources, problems and solutions. Emergency closures, re-opening procedure. The use of SS's for reclassifying an area were described. Where pollution events had occurred LA's were responsible for dealing with issues associated with sewage treatment works **(Resolution 10)**. [WS12_Pre 5.1]	
5.2 Enteric viruses in the local surveillance: Detection of norovirus and Hepatitis A virus in bivalve molluscs farmed and marketed in the Sardinia region from 2009 to 2012 RB, an invited speaker from the Isituto Zooprofilattico Sperimnetale della Sardegna - IZS, Italy presented on an enteric virus surveillance study outlining the monitoring plan and action taken following virus detection (non-compliance). [WS12_Pre 5.2]	
5.3 A 2 year study of enteric viruses in Scandinavian blue mussels The Danish NRL (ACS) gave a presentation on a collaborative study (NRLs Denmark, Norway and Sweden) using mussels as indicators for viral risk in raw water. Samples were located 10km from away sewage outlets. Data collected showed a negative correlation between NoV levels air temperature i.e. higher levels of viruses were detection when air temperatures were lower. Unusually higher levels of GI compared to lower levels of GII were demonstrated in mussels. It was concluded that mussles could be used as an indicator species for the accumulation of viruses in raw waters**.** [WS12_Pre 5.3]	

5.4 Assessment of viral contamination in Italy: Qualitative and quantitative data

NRL Rome (LC) presented data (2008-2012) taken from harvesting areas (class A and B) representative of major production areas around the Italian coast and Sardinia. Samples analysed included imported products and samples relating to an outbreak in January 2013. Data identified prevalence in all harvesting areas, lower in class A areas. Import products showed prevalance levels comparable to class B products **(Resolution 9).** [WS12_Pre 5.4]

5.5 HEV bioaccumulation in shellfish species and environmental investigations

NRL France (SLG) presented details of the ANR HEVECODYN project which proposed a transversal understanding of HEV epidemiology, from pork farms, wild animal and environmental samples through to LBM contamination. The objectives were to determine HEV presence in various environments, study pork farm infection dynamics and evaluate HEV adaptation to different hosts. It was suggested that HEV could bioaccumulate in different shellfish species. There was an indication that mussels and clams were more susceptible to contamination. Sample analysis was based on the CEN approach for detection **(Resolution 9).** Surveillance of areas did not demonstrate detection of naturally accumulated HEV in shellfish. [WS12_Pre 5.5]

6 Virus Methods

6.1 Technical progress on the application of the methods for virus analysis in Italy

ES (NRL Italy, Rome) presented data from the 2012 and 2013 Italian PT scheme. Samples included synthetic dsDNA and shellfish extracts from NoV-naturally contaminated shellfish (*Mytilus galloprovincialis*). Eleven regional laboratories took part in the scheme. Results showed evidence of participants' improvement for all parameters analysed. Future plan was to use whole shellfish. [WS12_Pre 6.1]

6.2 EURL proficiency test norovirus and HAV - PT 46 (WS12/14)

The EURL (JL) presented the results for the NoV and HAV PT (PT 46) which consisted of 6 samples (4 shellfish (3 x Pacific oyster (*C. gigas*) and 1 Common mussel (*Mytilus edulis*)) and 2 LENTICULE^{TM}). Forty-two laboratories (16 NRLs) participated in the distribution (WS12/14). Performance improvements (qualitative) were noted for all samples and determinands. Overall accuracy was 94% (22 laboratories obtained 100%) and overall specificity was 98% (based on total 6 positive results). Sensitivity for shellfish was 89%; GI 83%, GII 92% and HAV 93% (27 laboratories obtained 100%) and LENTICULE^{TM} 90%. Increased harmonisation of methods was shown to be a key factor for improvement, with methods based on the CEN TAG4 / ISO 15216 demonstrating improved accuracy. Further developments were discussed including: sample homogeneity, extraction efficiency, and accurate quantification through the provision of standardised control material **(Resolution 11)**. The EURL agreed to develop material to assist NRLs in implementation of ISO 15216 and supply starter stocks **(Resolution 12).** Further virus PT distributions would be organised in 2013/14 by the EURL and will include a scoring scheme to assist laboratories in monitoring their performance **(Resolution 13).** [WS12_Pre 6.2]

All NRLs
EURL

6.3 Update on the progress of CEN validation

JL (EURL) gave a brief update on the CEN virus validation. ISO CEN/TS 15216 for the detection of virus in foods (including shellfish) has been published (May 2013) and available on the internet. Completion of the validation (quantitative) will enable the TS 15216 to become a full standard.

7 Virus outbreaks

7.1 Norovirus virus outbreaks associated with oysters in 2012/13 in the UK

JL (NRL UK) gave a presentation on 4 outbreaks that had occurred in the UK between October and March 2013. In each case illness was strongly associated with the consumption of bivalve shellfish (all oysters) although neither clinical data (stool samples) nor oysters from restaurants were available in all cases. Following discussion, it was agreed that good information on sample traceability and availability were vital in outbreak investigations. Additionally in the absence of clinical confirmation of NoV and detection in the potential food vehicle actions

after outbreaks such as closures, product recalls/destruction were difficult to implement. **(Resolution 14).** [WS12_Pre 7.1] **7.2 a Oysters implicated in Danish norovirus outbreaks since 11th NRL meeting** ACS (NRL Denmark) presented on 4 restaurant outbreaks that had occurred between December and March 2013. [WS12_Pre 7.2] **7.2 b Winter norovirus protocol** NRL France (SG) presented the DGAI-Ifremer Winter Norovirus protocol details are provided in Appendix 3 - Management of outbreaks. [WS12_Pre 7.3] **7.3 Management of outbreaks (roundtable discussion)** NRLs were asked to provide information on their countries management ofoutbreaks with respect to minimum closure times, reopening criteria and virus testing policies. From the information provided it was noted that formal procedures were in place in a number of MS but were not uniform. A summary of all NRLs responses would be collated by the EURL **(Resolution 15).** (See Appendix 3 – Management of outbreaks). **8 Virus controls** **8.1 Discussion paper on live bivalve molluscs and human enteric virus contamination: options for improving risk management in EU food hygiene package (WS12/15)** DNL (EURL) presented paper WS12/15. The discussion paper had been produced Following a request from DG Sanco relating to LBM contamination, requesting possible options for introducing NoV and HAV controls in European food legislation. It was noted that discussions at the restricted working group on bivalve molluscs were at a very early stage. To date concerns had related to: where in the food chain to apply controls, which species to monitor and what NoV limits to set. Following discussion on the possible introduction of virus controls several NRLs noted that setting quantitative compliance criteria for LBM required would require careful consideration with respect to the analytical methods used to monitor against those criteria. NRLs further noted that prior to the completion of the validation of the putative reference method ISO TS 15216-1 performance characteristics of the methodology were not well established. **(Resolution 16).** [WS12_Pre 8.1] **9 Next meeting** The workshop accepted that the next meeting would be held at Cefas Weymouth, the dates being 13-15 May 2014 **(Resolution 17)**.	EURL

Resolutions of the 12th workshop of NRLs for bacteriological and viral contamination of bivalve molluscs, 7-9th May 2013.

Official Controls – Microbiological monitoring

1. NRLs supported the application of the Codex criterion applied over time (i.e. 80% of results ≤230 *E. coli* MPN/100g with no results >700 *E. coli* MPN/100g) to Class A classification. NRLs supported the approach to classification described in Community Guidance. NRLs agreed to communicate with their Member State Competent Authority with regard to this issue.

2. NRLs expressed continued support to the principle of development of production area stability assessments for inclusion in the good practice guide to enable reduced monitoring frequency of stable areas. NRLs agreed to provide additional datasets to enable further analysis. The EURL would provide instructions to NRLs detailing criteria for these datasets.

3. Further to the above, as an interim measure, the minimum criterion for assessing the variability of *E. coli* results over the previous 3 years (Section 3.11, item iii, of the Good Practice Guide Technical Application) was; for Class A areas - no result more than 230 *E. coli* MPN/100g; for Class B areas – no result more than 4,600 *E. coli* MPN/100g.

4. NRLs provided information on practices regarding excluding monitoring results from classification assessments (Section 7.3.7 of the Good Practice Guide - Technical Application). The EURL agreed to summarise the information and place on the EURL website.

5. NRLs agreed that the introduction of prohibition (buffer) zones around significant point source human faecal discharges (e.g. municipal sewage discharge pipes) would improve health protection against enteric viruses and other anthropogenic pollutants. It was agreed that further work was required to develop criteria (e.g. based on geographic or dilution approaches) for such zones.

6. Further to the above, several NRLs provided information on current practices regarding buffer zones in their countries. The EURL agreed to place a summary of the information on the EURL website.

Official Controls –Proficiency testing statutory determinands

7. The workshop noted that performance of all NRLs in proficiency testing for statutory determinands *E. coli* and *Salmonella* was satisfactory. However, it was identified that several NRLs were either not participating or were not participating at the agreed frequency (1 matrix and at least 1 EURL non-matrix distribution per year – Resolution 8, workshop 2012). The EURL identified that it intended to follow-up lack of acceptable participation in proficiency testing through the Commission Protocol for Lack of Collaboration with EURLs.

Marine Vibrios

8. The workshop noted the progress of the revision of ISO TS 21872 (Detection of potentially pathogenic *Vibrio* spp.) and the method validation under the CEN mandate (M/381). The EURL thanked NRLs for their support in these initiatives. It was agreed that proficiency testing distributions for *Vibrio* spp. would be organised following the conclusion of the validation exercises due for completion by the end of 2013.

Viruses

9. Several NRLs reported the results from surveillance studies for enteric viruses in a variety of bivalve species in their Member State. Studies confirmed the high prevalence of norovirus in both production areas and products placed on the market. However hepatitis A virus was either

not detected or detected at a very low frequency. Studies by NRL France showed the suitability of the CEN approach for detection of hepatitis E virus (HEV) in bivalve molluscs. Surveillance studies using this approach did not detect HEV in bivalve molluscs in that Member State.

10. A third country presented data on the importance of control of faecal pollution sources in production areas for reducing norovirus contamination. NRLs agreed that this was an important focus of attention for improving water and bivalve shellfish quality.

11. The EURL noted the trend from proficiency testing indicating that performance had improved substantially over recent distributions. Method harmonisation (i.e. use of ISO TS 15216 type methodologies including reagents recommended by CEN TAG4) was a key factor in this improvement. Regarding assessment of quantification performance, possible areas for improvement were identified including: ensuring sufficient sample homogeneity; considering extraction efficiency; ensuring accurate quantity calculations; use of standardised control materials.

12. Further to the above, NRLs noted the importance of the availability of commercial quantitative standards and controls for implementation of virus testing. The EURL agreed to continue to progress the development of materials to assist in the implementation of ISO TS 15216. In the interim starter stocks of dsDNA (and mengo virus) would be made available to NRLs on request.

13. NRLs supported continuing virus proficiency testing on the same basis for 2013/14. NRLs were encouraged to communicate the availability of the PT scheme to other interested laboratories. It was agreed to introduce scoring of performance for qualitative detection for the next distribution.

14. NRLs reported information on outbreaks associated with bivalve mollusc consumption occurring in their Member State. For effective outbreak investigation NRLs identified that it remained important to secure good evidence on sample origin (traceability), clinical aetiology, and epidemiological association with the food vehicle. It was noted that without this evidence risk managers may experience difficulty in implementing effective controls.

15. NRLs provided information on practices regarding management of norovirus outbreaks associated with bivalve molluscs. The EURL agreed to summarise the information and place it on the EURL website. It was identified that procedures for management of outbreaks varied across Member States and would benefit from improved harmonisation.

16. NRLs noted the current discussion regarding possible introduction of virus controls and identified that it was important to ensure alignment of analytical techniques with any limits defined in legislation e.g. performance characteristics for quantification of total norovirus content (GI plus GII).

Date and time of next meeting

17. The next workshop would provisionally (subject to confirmation of costs) be held at Cefas in Weymouth, UK 13th, 14th and 15th May 2014.

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

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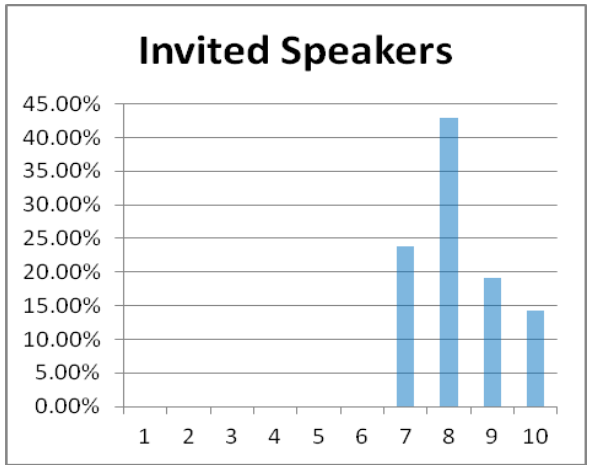
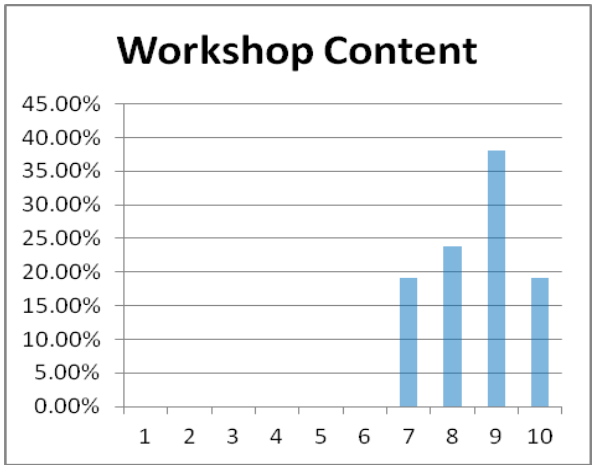
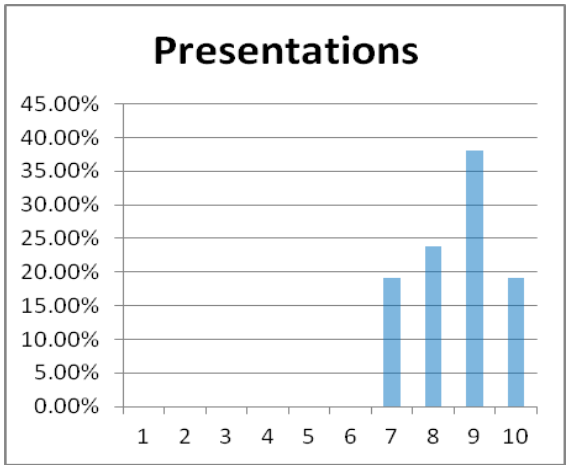
List of papers for 12th Workshop of Microbiological NRL's

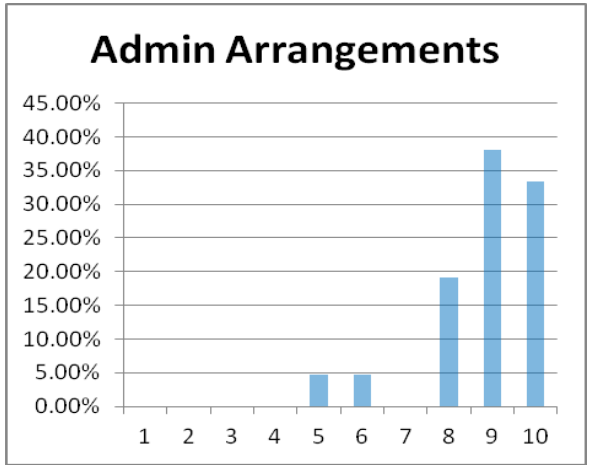
WS12/00	List of papers
WS12/01	Delegates List
WS12/02	Instructions on how to complete your expenses claim form
WS12/03	Expenses claim form
WS12/03A	Example of claim form
WS12/04	Report on the 11th Workshop of National Reference Laboratories for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs
WS12/05	Agenda
WS12/06	EURL microbiological contamination of bivalve molluscs final work programme 2013
WS12/07	Feedback from the 2nd International Workshop on Molluscan Shellfish Area Classification and Management, USA
WS12/08	Feedback from the Commission Restricted Working Group - Codex *E.coli* standards
WS12/09	Revisions to Technical Good Practice Guide issue 4
WS12/10	Whole animal distribution for *E. coli* and *Salmonella*, (PT 45)
WS12/11	Shellfish EQA scheme for *E. coli* and *Salmonella*, (PT 48)
WS12/12	Revision of ISO TS 21872 and progress with the validation under the CEN mandate (M/381)
WS12/13	Bivalve trade discussion between the EU and USA
WS12/14	EURL proficiency test norovirus and HAV – PT 46
WS12/15	Discussion on virus risk management

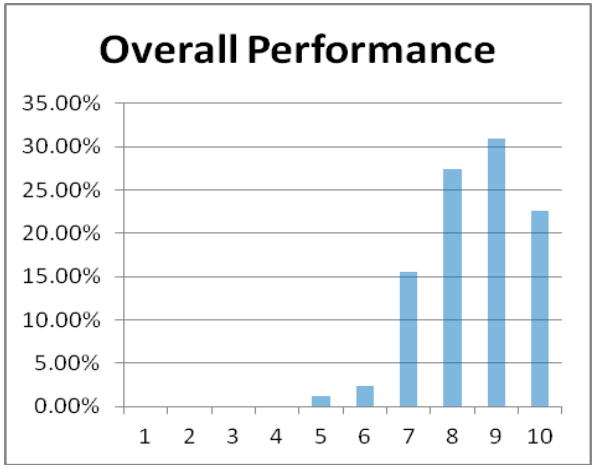
Confidential Participant Feedback Results











Comments : -

1. To be considered : Day 1 - Restricted to MS having harvesting areas or planning to.
   Day 2, 3(½) - All MS, including those without harvesting areas.

2. Lunch : Not enough warm food for the latest arrived to taste.

3. Primary focus should be the requirements of Reg 882 with respect to bacteria and viruses.

4. It would be great to have access to internet.

5. Too many discussions on control issues, which are in the responsibility of the Competent Authority.

6. a. - More scientific presentations.
   b. - More participation from different NRLs.

Appendix 1

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

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Report of the 12th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs

Details of criteria for waiving results of monitoring programmes – EU situation

Background

Community guidance for microbiological classification and monitoring of bivalve mollusc production areas with regard to Regulation 854/2004 (European Communities, 2012) sets out criteria which if met may enable identification of anomalous *E. coli* results and permit exclusion of those data from classification datasets. Criteria are:

- Failure to comply with sampling protocols (e.g. time or temperature requirements) or,
- Failure of sewerage or sewage treatment works that has been rectified and is not expected to reoccur or,
- A rainfall event with a return period of 5 years or greater.

The guidance includes recommendations on additional measures to be taken following the occurrence of one or more of the above, e.g. taking additional random samples, investigations in the harvesting area and short-term control measures.

The EURL Good Practice Guide – Technical Application, section 7.3 provides some additional explanatory text (EURL 2010).

With the aim of improving harmonisation throughout the EU and, in recognition that recent climatic anomalies may be increasing the frequency of extreme rainfall events, NRLs were asked to provide information with regard to waiving monitoring results. This paper summarises information provided by laboratories at the 12th workshop (Table 1).

In summary, not all NRLs were able to supply information. Where data were available and exclusion was permitted, several broadly similar approaches were described. A few NRLs stated that the Good Practice Guide – Technical Application had been adopted in full or part. Others reported a more *ad hoc* approach with less formalised case by case decisions. Several NRLs reported that data may be excluded on the basis of sewage treatment works failures or non-compliance with sampling protocols but less frequently following extreme weather events.

The workshop agreed the following resolution:

Resolution 4

NRLs provided information on practices regarding excluding monitoring results from classification assessments (Section 7.3.of the Good Practice Guide – Technical Application). The EURL agreed to summarise the information and place o the EURL website.

Table 1. Summary information of by NRLs with production areas on criteria for waiving results of monitoring programmes [where information was provided]

NRL	Requirements/guidelines
Bulgaria	The NRL reported that the responsible authority was in the process of elaborating guidance but that currently no formal policy was in place.
Croatia	**The NRL reported that all results from monitoring programmes were sent to the Competent Authority (CA), no data were excluded. All results exceeding 230 *E. coli* MPN/100g in a zone with class A status would result in a downgrade of the zone to class B, by the national regulation and on the order of the CA inspector. Re-sampling would take place after 7 and 10 days. If both results of the additional (investigative) sampling were ≤230 *E. coli* MPN/100g the area could return to class A status. If the results exceeded 230 *E. coli* MPN/100g over the next three months during the regular monitoring programme, additional - investigative re-sampling is not implemented and the zone automatically becomes class B.**
Denmark	The NRL reported that data were not excluded from classification datasets, although climatic information may be used to provide additional explanatory information in the event of unusual results. A temporary downgrade of the area to B (or C depending on the results) could take place if results in relation to the verification of classification show that this is necessary. Recalls of harvested batches may also be implemented in such cases. Extra samples were taken for several weeks until results show that the original classification of the area had been reached.
France	The NRL reported that the recommendations set out in Community Guidance and Good Practice Guide – Technical Application were followed.
Germany	The NRL reported that data were not excluded from classification datasets.
Norway	The NRL reported that data were not generally excluded from classification datasets however; unusually high results may trigger an investigation and subsequent exclusion if causation was proven.
Portugal	No formal procedures were in place, but waivers were used in the event of sewerage or sewage treatment works failures. Results were not excluded on the basis of high rainfall.
Romania	No information was available to the NRL.
Slovenia	No information was available to the NRL.
Spain (Galicia)	Information available for Galicia only. Results of monitoring programmes are not excluded from classification datasets, but can be waived following the recommendations set out in Community Guidance and Good Practice Guide – Technical Application. Following a scheduled sewage spill due to maintenance operations in a water treatment plant (WTP), a protocol had been developed for weekly monitoring in order to verify the affect on the harvesting area. In this case, samples are taken weekly and, based on Good Practice Guide criterion; it was considered that some monitoring results must be excluded from classification datasets to avoid the bías of the data.
Sweden	The NRL reported that the recommendations set out in Community Guidance and Good Practice Guide – Technical Application were followed.

United Kingdom	The NRL reported that in general the recommendations of the Community Guidance and Good Practice Guide – Technical Application were followed. It was noted however the Competent Authority may not always have relevant information in order to justify exclusions, particularly if this was held by another agency (e.g. rainfall data). Furthermore the relevance of the 1 in 5 year rainfall criterion (in the Community Guidance) with regard to climatic change and predicted increase in extreme weather events was currently being questioned

Appendix 2

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Report of the 12th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs

Prohibition zones – EU situation

NRLs for bacteriological and viral contamination of bivalve molluscs were asked to provide information on in country practices with regard to the use of prohibition (buffer) zones in bivalve mollusc production areas e.g. around sewage discharge pipes, for discussion at the 12th workshop. This paper summarises the information provided by laboratories.

Summary of discussion

Prohibition zones are implemented in EU Member States[1], in a few Member States guidelines for implementation via national legislation were in force. Where information was available most reported closures around harbours, marina, river mouths and discharges for class A areas following the recommendations of the good practice guide. Some Member States were able to give detailed information on their application, although the information was not uniformly available to NRLs (Table 1). Following discussion, it was clear that practices varied both between countries and within regions, although fixed zones established by geographical distance from the contamination sources were most frequently used. The radii of zones were based upon a mixture of historic, local knowledge with additional information from sanitary surveys, risk assessments undertaken by the food business operator and some bathymetry, microbiological testing and modelling.

Management scenarios described by US FDA were presented by the EURL illustrating a zoned approach moving from prohibited – restricted - conditionally approved - approved and various combinations thereof. US FDA guidance is that a 1000:1 dilution should be considered as a minimum near outfall, together with use of notification times e.g. the speed at which harvest can be suspended. It was noted that in the EU it would not be possible to demonstrate that US faecal coliform standards were met on the perimeter of buffer zones without testing water. NRL Italy noted that the absence of a category in the EU corresponding to "conditionally approved" made management of rainfall events problematic, even with the use of prohibition zones.

A number of NRLs supported the principle of prohibition zones (Resolution 5) It was agreed that the most practical solution within the EU may be to consider zones of fixed geographical distance from the contaminating source based upon impact, alternately dilution factors, travel times (of viruses) and die off could be assessed, another approach to be used either in combination or to verify the efficacy of a prohibition zone would be virus testing in bivalve molluscs. The workshop passed the following resolution:

[1] Only applies to MS with production areas

<u>Resolution 5.</u> NRLs agreed that the introduction of prohibition (buffer) zones around significant point source human faecal discharges (e.g. municipal sewage discharge pipes) would improve health protection against enteric viruses and other anthropogenic pollutants. It was agreed that further work was required to develop criteria (e.g. based on geographic or dilution approaches) for such zones.

Table 1. Summary information of application of prohibition zones supplied by NRLs representing countries with bivalve mollusc production areas, [where information was available]

NRL	Requirements/guidelines
Denmark	Historically in Denmark no production area could be sited in a harbour or marina. Currently, the food business operator is required to undertake an initial risk assessment of an area, although there are no formal (written) procedures for conducting risk assessments or acceptance criteria, each is assessed by the Competent Authority on a case by case basis.
France	Prohibition zones are implemented in France, EU regulations are transcribed into French Regulations which recognise 4 classification categories A, B, C and D (prohibited). An example from the Bretagne region showed the modelling approach to delineation of D areas (≡prohibition zones). The models were informed by data collected during the shoreline survey comprising *in situ* measurement of river flows, wastewater treatment plant flows and levels of *E. coli* in bivalves. The models were then used to predict flow and contamination as various states of river flow and tide and used to predict the extent of category D zones.
Iceland	The Icelandic Competent Authority have a general requirements/guidelines (a working guideline implemented for harvesting areas of kúfskel (Iceland cyprine) and also valid for contamination from harbour areas.) for growing and harvesting areas not to be situated within a 500m of a discharge outlet or pipe. In spite of this decision on location or limits of production areas is based on results from sanitary surveys
Italy	Prohibition zones are implemented in regions in Italy with some regional differences in application. Detailed examples with GPS co-ordinates and radii of zones within harbours, areas around harbour channels and around estuaries in the Marche region were available, the sizes of these were based upon historic and local information. In the Venice lagoon prohibition zones surrounding offshore discharge pipes were fixed and mapped. In Napoli guidance stated that in the absence of data on the bathymetry and tidal cycles that would enable assessment of the circulation of pollutants, that water bodies located within a 500 m radius of each contaminating source should not be classified for production or relay of bivalve molluscs.
The Netherlands	The requirements for prohibition zones around waste water discharges or other known sources of faecal contamination are set out in national legislation (Verordening van het Productschap Vis van 13 oktober 2005 tot vaststelling van nadere regels inzake de productiegebieden voor levende tweekleppige weekdieren (Verordening productiegebieden levende tweekleppige weekdieren 2006) (Regulation of Fish Product of 13 October 2005 laying down detailed rules for the production areas for bivalve molluscs (Regulation production of live bivalve molluscs in 2006) (Anon, 2006). Prohibition zones are specified for discharges, ports and river mouths based on geographical distance. The radii of zones ranged from 100m to 1,500m.
United Kingdom	There are no specific requirements for the application of prohibition zones in the UK. The Competent Authority do however recognise that proximity to wastewater treatment discharges is a public health risk and works with Cefas scientists through the sanitary survey programmes to exclude significant discharges from production areas. It was noted that modelling carried out at Cefas using a 1:1000 dilution factor for a selected discharge in the U.K. predicted a requirement for an exclusion zone of >2km.

Appendix 3

Cefas

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Report of the 12th workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs

Management of outbreaks– EU situation

NRLs for bacteriological and viral contamination of bivalve molluscs were asked to provide information on in country practices with regard to the management of outbreaks, associated predominantly with noroviruses. This paper summarises the discussion at the 12th workshop and gives information provided by laboratories.

Summary
Table 1 describes information on existing practices with regard to management of virus (norovirus) outbreaks in MS, where this information was available to the NRL. It was noted that whereas in a very few MS formal procedures were in force, this was not the norm.

Further to Table 1, several NRLs provided additional data on outbreaks (norovirus) in 2012-2013, including virus levels in bivalve shellfish where these data were complete (Table 2). Approximately, 35 separate clusters each involving between 5 and over 130 individuals were reported.

It was concluded that decisions after bivalve shellfish associated gastroenteritis were not uniform or harmonised across EU MS.

Following discussion issues emerged which were considered impairments to formal action after outbreaks:

- The lack of legislative virus controls – Provision within Regulation (EC) No 178/2002 did not appear to be sufficient,
- The lack of robust epidemiological linkage between the commodity and the patient meant that enforcement action was often problematic, even in the presence of high virus levels (when tested using appropriate methods). The burden of proof is often uncertain and open to challenge by producer organisations (in some cases),
- The lack of traceability of product traded both within and between MS has the capacity to create additional problems when conducting outbreak investigations and recommending enforcement action.

The workshop agreed the following resolution:

Resolution 15, **NRLs provided information on practices regarding management of norovirus outbreaks associated with bivalve molluscs. The EURL agreed to summarise the information and place it on the EURL website. It was identified that procedures for management of outbreaks varied across Member States and would benefit from improved harmonisation.**

Use of the RASSF system

Actions resulting from RASSF for norovirus were discussed, these varied between MS. NRLs reported that requirement for mandatory actions/investigation were in place following alerts based upon regulatory excedences (elevated *E. coli*, presence of *Salmonella* etc). Where action was considered following alerts for norovirus it was identified that methodology should be examined to ensure use of methods derived from the putative reference method (ISO TS 15216-1). It was further suggested that the absence of requirements for Official Control testing for norovirus across the Community was likely to bias RASFF. The RASSF for norovirus associated with bivalve molluscs between 2008 and 2012 is provided for information (Table 3).

Table 1. Summary information of by NRLs regarding management of norovirus outbreaks associated with bivalve molluscs [where information was provided]

NRL	Requirements/guidelines
Belgium	The NRL reported that there were no known virus (norovirus) outbreaks associated with bivalve shellfish in Belgium. General provision in Belgium legislation required that the Competent Authority (CA) (would take action in the event of food borne outbreaks.
Bulgaria	The NRL reported that they had no data on virus (norovirus) outbreaks associated with bivalve shellfish in Bulgaria. The NRL were not aware of a formal policy for management of outbreaks.
Croatia	The NRL reported that there were no known virus (norovirus) outbreaks associated with bivalve shellfish in Croatia. The NRL were not aware of a formal policy for management of outbreaks.
Denmark	The NRL was not aware of outbreaks associated with indigenously produced bivalve shellfish. However, actions after detection of norovirus in the production area was considered the responsibility of the Food Authority. Risk was assessed on a case by case basis and if significant action would be taken under Regulation (EC) No. 178/2002 [laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety].
France	The French Food Authority Ministry (DGAI) Winter Norovirus Protocol required that following an outbreak (unequivocally linked to bivalve shellfish) area would be closed for 28 days. Norovirus analysis in the production area undertaken at a frequency of two-weekly, and the area may be reopened if norovirus negative results are obtained. In addition, in the period November – April, the protocol required actions after REMI alert (elevated *E. coli* result i.e. result outwith classification status), heavy rain or failure of sewage treatment works (STW). Recommended actions were 28 days closure if positive samples are obtained; reopening criteria were either no further incident in the 28 day period (i.e. no climatic event, *E. coli* levels return to baseline and no STW failure). If norovirus analysis was carried out and results were negative closure period of less than 28 days was permissible.
Germany	The NRL reported that there were no known virus (norovirus) outbreaks associated with bivalve shellfish in Germany. The local CA would take action in the event of food borne outbreaks; larger food borne outbreaks are managed by a special task force. However, no formal policy for management of shellfish-related outbreaks is in place.
Greece	The NRL reported that there were not aware of virus (norovirus) outbreaks associated with bivalve shellfish in Greece. Actions following outbreaks were the responsibility of the CA. The NRL were not aware of a formal policy for management of outbreaks.
Hungary	The NRL reported that there were not aware of virus (norovirus) outbreaks associated with bivalve shellfish in Hungary. A documented formal policy for food borne outbreaks was in place but this did not explicitly identify bivalve molluscs.

Iceland	The NRL reported that there were not aware of virus (norovirus) outbreaks associated with bivalve shellfish in Iceland. The NRL were not aware of a formal policy for management of outbreaks.
Ireland	In Ireland Management Control Plans (MCP) in place for harvesting areas. Following norovirus associated outbreak the area is closed, no minimum closure period. It is the responsibility of the Food Business Operator (FBO) to demonstrate norovirus levels of <200 genome copies/g for product placed on the market.
Italy (Roma)	The NRL reported that no formal policy for management of outbreaks was in place. CA has coordinated an expert working group (with NRL representation) to discuss the issue
Lithuania	The NRL reported that there were no production areas in Lithuania. The NRL were not aware of a formal policy for management of outbreaks.
The Netherlands	The NRL reported that no formal policy for management of outbreaks was in place. In practice, if product placed on the market is tested for and positive for norovirus it is withdrawn.
Norway	The NRL reported that no formal policy for management of outbreaks was in place. Norovirus outbreak in 2009-10 resulted in area closure; reopening criterion was absence of norovirus. The FBO now depurates from this area and continues to monitor norovirus in the production area.
Poland	The NRL reported that there were no production areas in Poland. The NRL were not aware of a formal policy for management of outbreaks.
Portugal	The Portuguese Department of Health is responsibility for food borne outbreaks, general procedures for foodstuffs are in place but nothing specific is included for bivalve shellfish.
Romania	The NRL reported that they have no data on virus (norovirus) outbreaks associated with bivalve shellfish and that there was no formal policy for management of outbreaks.
Slovenia	The NRL reported that no formal policy for management of outbreaks was in place. One reported incident related to presence of HAV in the production area resulted in an FBO voluntary closure.
Spain (Galicia)	Information was only available from Galicia. It is the FBO responsibility to demonstrate consideration of norovirus in the HACCP. For oysters Galician Health Authority require absence of norovirus for foods placed on the market. CA for the official control of production areas runs a monthly monitoring of norovirus in oyster harvesting areas to gather information of norovirus levels in order to establish action measurements. It was noted that no information was available for other autonomous regions of Spain
United Kingdom	A minimum closure period is not applied in response to virus outbreaks. Decisions on closures of beds will be taken during the management of each incident/multiple reports of human illness and the criteria used to open and close beds will depend on the circumstances of each incident and the conditions in the bed/harvesting areas that are potentially involved.

Table 2. Summary data describing levels of norovirus in outbreak associated bivalve shellfish reported at the 12th workshop of NRLs [not exhaustive]

Batches tested related to outbreaks in 2012-13	Estimated level of norovirus in bivalve shellfish (genome copies/g) (sum of genogroup I and II)	Clinical linkage between bivalve shellfish and patient[1]
1	891	yes
2	<70	no
3	920	yes
4	556	no[2]
5	1,265	no[2]
6	5,396	yes
7	4,786	no[2]
8	10,029	no[2]
9	7,869	yes
10	4,713	yes
11	≈500	no[2]

[1]means that stool samples were tested for and were positive for norovirus, or very strong epidemiological linkages were confirmed
[2] stool samples not tested

Table 3. Summary of RASSF associated with norovirus and bivalve molluscs or products thereof (2008-2012)

Classification	Notified by	Origin	Bivalve mollusc
Information	Italy	France	oysters
Alert	Denmark	France	oysters
Alert	The Netherlands	Ireland	oysters
Alert	France	UK	clams
Alert	Norway	Ireland via France	oysters
Information	Denmark	Ireland via The Netherlands	oysters
Alert	The Netherlands	The Netherlands	oysters
Alert	The Netherlands	The Netherlands	oysters
Information	Denmark	France	oysters
Information	Denmark	Ireland	oysters
Alert	France	UK, Ireland and The Netherlands	mussels
Alert	Norway	The Netherlands	oysters
Information	Italy	France	oysters
Information	Italy	France	oysters
Border rejection	France	Peru	scallops
Alert	France	Chile	scallops
Alert	Norway	France	oysters
Information	Denmark	France	oysters
Information	Finland	Ireland via France	oysters
Alert	Denmark	France via Ireland	oysters
Alert	Ireland	Ireland	oysters
Alert	Denmark	France	oysters
Alert	Denmark	France	oysters
Alert	Denmark	France	oysters
Alert	Ireland	Ireland	oysters
Border rejection	France	Peru	scallops
Information	Norway	Sweden	oysters
Information	Norway	Ireland	oysters
Alert	Norway	UK	oysters
Alert	Norway	UK	oysters
Alert	Norway	UK	oysters
Alert	The Netherlands	France	oysters
Information	France	Spain	oysters

Workshop declaration

This technical report is submitted in accordance with the requirements of Commission Implementing Regulation (EC) No 926/2011 laying down detailed rules for the granting of Community financial assistance to Community reference laboratories for feed and food and the animal health sector, following the workshop of National Reference Laboratories for bacteriological and viral contamination of bivalve molluscs held in Weymouth 7th - 9th May 2013.

Dr David Lees 18th October 2013
EURL Director

Dr Rachel Hartnell 18th October 2013
EURL Co-ordinator

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