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

Technical report for calendar years 2016-2017

FINAL report

(version 3)

23.02.2018

Contract Reference: Cefas ref (C6927)

Document approved by:	**James Lowther**	**Review date:**	**Not applicable**
Document checked by:	**Rachel Hartnell**	**Classification:**	**Official**
Document prepared by:	**James Lowther, Louise Stockley**	**Location**	**CRL$**

Contents

European Union Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs, Cefas, Weymouth

Technical Report for Calendar Years 2016 - 2017

Note: the work programme and all EURL reports mentioned in this technical report are available on the EURL website at https://eurlcefas.org

Legal functions and duties

The functions and duties of the EURL during the reporting period are specified in Article 32 of Council Regulation (EC) 882/2004 (Official Journal of the European Communities No L165).

Introduction

The two-year work programme for the EURL for 2016 - 2017 was approved by the European Union in December 2015. This report details activities of the EURL according to the work programme (available on the EURL website), additional tasks described under the resolutions and reports of the 15th workshop of microbiological NRLs held in Berlin, Germany and 16th workshop held in Split, Croatia, and other responsibilities outlined in Commission Regulation (EC) 882/2004 for the calendar years 2016 - 2017.

1. Scientific advice and support

To the European Union

The EURL has provided the following advice and support to the European Commission (DG Sante, Sante F, and EFSA) through participation in expert working groups, provision of briefing documents, guidance and audits, specifically in 2016 and 2017 this has comprised:

- Provision of advice to the Commission and Member States in the restricted working group on bivalve molluscs. Three working groups attended in 2016 (January, March, September) and four in 2017 (January, June, September and December). Comment, advice and presentations, including preparation for meetings and post meeting follow up were provided to the Commission and Member States on aspects of LBM including but not restricted to, development of Regulation 2015/2285 amending class A classification, MS input into consequential redrafts of the European guidance, the development of the EU wide norovirus baseline survey, the developing EU-US trade agreement, plans for the EURL and NRL network after EU exit, measurement uncertainty for the *E. coli* MPN reference method and classification requirements for echinoderms.
- Support, national expertise and follow-up to the two week audit mission to Peru (25th September – 5th October) to the EU Directorate Sante F to evaluate whether the official controls in place for bivalve molluscs destined for the EU guaranteed that their production conditions were in line with the requirements laid down in EU legislation; to verify the extent to which the guarantees and corrective actions submitted to the Commission services in response to the recommendations of previous Commission reports had been implemented and enforced; and to assess the measures taken following the El Niño climate event occurred in the first months of 2017.
- Support to the Commission regarding EU-US trade: This activity included correspondence and a report to the US FDA regarding the implications of adoption of Regulation 2015/2285 for trade, further

statistical analysis of EU and US datasets and a full report. Attendance of 3 EURL staff (including statistical support) at a meeting in Washington DC in September 2016 to present our report to the FDA, to scrutinise the corresponding analysis and presentation from the FDA, and to support the Commission in various other trade aspects under discussion including issue on *Vibrio* controls. Post meeting finalisation of technical documents, including redrafted Guidance annexes, and publication of material on the EURL website.

- Attendance at a meeting of EURLs in September 2017 to discuss the new rearrangements as regard the tasks for the microbiological and viral contamination of bivalve molluscs EURL after December 2018. The EURL presented the annual work programme and provided examples of the provision of advice, comparative testing and training to RLs for *E. coli*, *Salmonella*, viruses and marine biotoxins.
- Working with EFSA on the harmonised EU wide baseline survey of noroviruses in oysters, including development of the study plan, hosting a meeting of the protocol working group and analytical sub-group in Weymouth, 13th - 14th January 2016, provision of advice on technical issues as they pertain to the data received by EFSA from designated testing laboratories, and attendance at the meeting of the Working Group on Norovirus in Oysters to plan analysis of the baseline survey data, held in Parma, Italy, on 7th - 8th December 2017. Further support to the Commission on the baseline survey including provision of data relevant to financial arrangements.
- Advice on viruses in non-animal matrices, including maintenance of a database including details of laboratories in EU Member States with capacity for determination of noroviruses and hepatitis A virus in soft fruits (and detailing ISO IEC 17025 accreditation status).
- Redrafting and reissuing (in January 2017) the EURL Guide to Good Practice: Technical Application (issue 6) and the EU Community Guide to the Principles of Good Practice (issue 3) to update principally sections regarding implementation of Regulation 2015/2285 for classification and monitoring and also amending the annexes regarding EU exports to the USA linked to the trade negotiations. This activity included several rounds of technical discussion with the EURL steering group, tabling and presentation of draft proposals to NRLs, the Commission and MS, and consequential redrafting of the guidance according to comments made.

The Good Practice Guide Working Group subsequently met on 30th November in Nantes to discuss the next round of revisions, specifically in relation to seasonal classifications and possible changes that might be needed arising from a new norovirus standard in shellfish.

<u>Advice to other entities and contributions to standardisation activities under ISO or CEN working groups</u>

The EURL led the CEN/TC 275/WG6/TAG4 programme on work on viruses in foods, including:

- Preparation of the FDIS and publication versions of ISO 15216-1, Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR - Part 1: Method for quantification (published March 2017)
- Preparation of an initial draft of ISO 15216-2, Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR - Part 2: Method for detection, revised to harmonise with the newly published ISO 15216-1 and including method characteristics
- Attendance at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6, 9th - 13th May 2016, Paris, France and 19th – 23rd June 2017, Tokyo, Japan

The EURL led the CEN/TC 275/WG6/TAG15 programme on work on food borne pathogenic vibrios using molecular approaches, including:

- Preparation of the DIS, FDIS and final publication versions of ISO 21872, Microbiology of the food chain -- Horizontal method for the detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* (published July 2017).
- Preparation of a supplementary report on the vvHA *V. vulnificus* real-time PCR assay to assist in interpretation of the validation data in ISO 21872.
- Further work on the development of new PCR-based methods for quantification of potentially enteropathogenic *Vibrio* spp., including a meeting of TAG4 to discuss the issue in London, February 2017.

The EURL led the ISO/SC9/WG14 expert group on the EU standard reference method for enumeration of *E. coli* in LBM (ISO TS 16649-3). In 2016 a technical corrigendum was developed to harmonise incubation controls and performance testing of media with ISO 11133 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

The EURL led the ISO/SC9/WG8 expert sub-group on the revision of the ISO 6887-3, sample preparation and preparation of initial dilutions to include harmonisation of the ISO standard with the requirements of EU Regulation (EC (No.) 2073/2005) for live bivalve molluscs. This standard was published in March 2017.

The EURL participated in the expert working group ISO TC34 SC9 WG7 revising ISO 7218 Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.

The EURL provided comments on the draft of ISO/DIS 16140-3 'Method verification' prior to the working group meeting in September 2017.

The EURL provided advice to FAO/WHO on the theoretical LOD for the determination of FRNA bacteriophage in LBM.

The EURL presented the EURL work programme on reference materials to the annual meeting of the Biological Resource Centre Network meeting in NCIMB, Aberdeen, UK.

The EURL produced a short report summarising the application of seasonal classifications to bivalve mollusc production areas in Member States from information provided at and following the annual workshop of NRLs in Split.

The EURL produced a short report summarising bivalve shellfish associated outbreaks in Member States from information provided at and following the annual workshop of NRLs in Split.

The EURL produced a short report summarising classification changes due to Commission Regulation (EU) 2015/2285 from information provided at and following the annual workshop of NRLs in Split.

2. Project management and co-ordination of activities of NRL network

The EURL promotes full and active participation by NRLs in the activities of the network as outlined in Article 32 of Commission Regulation (EC) No. 882/2004. To assist in co-ordination activities a list of designated NRLs is updated annually. This information is published on the EURL website https://eurlcefas.org (Table 1).

Table 1. Designated NRLs in Member States, EFTA and Accession states in calendar years 2016 - 2017.

Member State	Laboratory
Austria	Austrian Agency for Health and Food Safety, Institute for Food Control, AGES-LMU Wien, Abt. Mikrobiologie, Spargelfeldstraße 191, A-1226 Wien
Belgium and Luxembourg	Scientific Service of Food-borne Pathogens, Operational Directorate of Communicable and Infectious Diseases, Juliette Wytsmanstraat 14, 1050 Brussels
Bulgaria	National Diagnostic and Research Veterinary Institute, Pencho, Slaveikov, 15 BG - 1606 Sofia
Croatia	Croatian Veterninary Institute, Regional Veterinary Laboratory Split, Poljicka Cesta 33, 21000 Split
Cyprus	No NRL designated
Czech Republic	No NRL designated
Denmark	National Food Institute, The Technical University of Denmark, Kemitorvet, Building 204, Room 227, 2800 Kgs. Lyngby
Estonia	No NRL designated
Finland	Finnish Customs Laboratory, Tekniikantie 13, FI-02150, Espoo
France	IFREMER, Departement Microbiologie et phycotoxines, Centre de Nantes, Rue de I'lle de'Yeu, BP 21105, 44311 Nantes Cedex 3
Germany	Bundesinstitut fur Risikobewertung (BfR), Federal Institute for Risk Assessment, Diedersdorfer Weg, D-12277, Berlin
Greece	Institute of Food Hygiene of Athens, Neapoleos 25, 15310 Ag. Paraskevi, Attiki, Athens
Hungary	Central Agricultural Office, Food and Feed Safety Directorate, Mester u. 81, H-1095 Budapest
Iceland[1]	Matís ohf. / Icelandic Food and Biotech R&D. Vínlandsleið 12, 113 Reykjavík
Ireland	Marine Institute, Rinville, Oranmore, Co. Galway
Italy[2]	Istituto Zooprofilattico Sperimentale Umbria e Marche, Via Cupa di Posatora 3, 60100, Ancona (NRL for bacteriology) Instituto Superiore di Sanità, Dipartmento di Sanita Pubblica Veterinaria e Sicurezza Alimentare, Viale Regina Elena 299, 00161, Rome (NRL for virology)
Latvia	Institute of Food Safety, Animal Health and Environment (BIOR), Lejupes iela 3, LV-1076 Riga
Lithuania	National Food and Veterinary Risk Assessment Institute, J.Kairiukscio Str. 10, LT-08409, Vilnius
Malta	No NRL designated
Netherlands	National Institute for Public Health and the Environment (RIVM), PO Box 1 A van Leeuwenhoeklaan 9, 3720 BA, Bilthoven
Norway[1,2]	NIFES - The National Institute of Nutrition and Seafood Research, Box 2029 Nordnes, NO-5817, Bergen (NRL for bacteriology) NMBU – Campus Adamstuen, Department of Food Safety and Infection Biology, P.O. Box 8146 Dep., 0033 Oslo (NRL for virology)
Poland	National Veterinary Research Institute, Partyzantów 57, PL - 24-100 Pulawy
Portugal	Portuguese Institute of Sea and Atmosphere, I.P. (IPMA)/Department of Sea and Marine Resources, IPMA-Alges, Avenida de Brasilia, 1449-006 Lisboa

Member State	Laboratory
Romania	Institute for Diagnosis and Animal Health, 63 Dr. Staicovici Street, Sector 5, Code 76202, Bucharest
Slovakia	State Veterinary and Food Institute, Janoskova 1611/58 Ministry of Agriculture, SK - 02601 Dolny Kubin
Slovenia	Institute for Food Hygiene and Bromatology, Veterinary Faculty, Gerbiceva 60, SI – 1000, Ljubljana
Spain	Centro Nacional de Alimentacion, Agencia Española de Seguridad Alimentaria, E-28220 Majadahonda, Madrid
Sweden	National Food Agency, P.O. Box 622, 751 26 Uppsala
United Kingdom	Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB

[1] Member of EFTA

[2] 2 NRLs established (one for bacteriology and one for virology)

EURL website

The EURL website https://eurlcefas.org continues to provide a very useful repository for information for NRLs and other stakeholders.

Annual workshops of NRLs

The 15th workshop of NRLs was hosted at BfR, Berlin, Germany between 25th – 27th May 2016. Forty-two delegates representing 23 EU Member States, 2 EFTA countries and the EURL alongside invited experts from EFSA, the Lower Saxony State Office for Consumer Protection and Food Safety, Germany, National Laboratory of Schleswig-Holstein, Germany and the Centro de Control da Calidade do Medio Marino of Galicia, Spain and observers from DG SANTE and the FVO. The workshop covered official controls, *E. coli* and *Salmonella*, marine vibrios and viruses. Twenty-five resolutions were agreed by the workshop.

The 16th workshop of NRLs was hosted at the Radisson Blu Hotel, Split, Croatia between 3rd – 5th May 2017. Forty-five delegates attended representing 23 EU Member States, 2 EFTA countries and the EURL alongside invited experts from ECDC, JRC, the University of North Carolina and the Centro de Control da Calidade do Medio Marino of Galicia, Spain and observers from DG SANTE and the FVO. The workshop covered the future of the network, official controls, marine vibrios, *E. coli* and *Salmonella* and viruses. Twenty-eight resolutions were agreed by the workshop. Reports of the workshops of NRLs are available to download from the EURL website (https://eurlcefas.org). Anonymised feedback from delegates attending the workshops showed overwhelmingly good to very good performance, feedback breakdown is provided within the relevant annual workshop report.

EURL Director's meeting

The EURL Director designate attended the meeting of Directors of EURLs in the field of Animal Health, Food and Feed in Brussels on December 2nd 2016.

3. Provision of technical advice and training

Methods, protocols and guidance notes

The EURL updated its generic protocols for detection of *E. coli*, *Salmonella* spp. and viruses in shellfish during 2016 and 2017 to reflect to reflect the developing ISO standards in these areas. Guidance on derivation of MPN values for official control testing was produced/updated. Further assistance for laboratories undertaking virus testing was provided in the form of an updated spreadsheet for quantity calculations, a guidance note on the determination of limits of detection and quantification and guidance for troubleshooting problematic results in virus proficiency testing. The EURL contributed to the survey protocol for the EFSA European baseline survey of norovirus in oysters, including leading the sub-group responsible for generation of the method specification detailing the analytical methods for use in the survey.

The EURL finalised and published the electronic MPN calculator for derivation of *E. coli* MPN in bivalve shellfish, this harmonises the *E. coli* reference method ISO 16649-3 with the approach used in ISO 7218 Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations and reduces laboratory error in result interpretation. A link to the new calculator was included in the latest version of the EURL generic *E. coli* protocol on the EURL website. The EURL also published guidance on determining uncertainty of measurement for the enumeration of *E. coli* in bivalve molluscs by ISO 16649-3.

The EURL produced a guidance document giving guidance on *Salmonella* detection kits for detection and identification of *Salmonella* spp. in bivalve molluscs, to provide additional information for laboratories requiring verification data for alternative methods for detection of *Salmonella* spp. in bivalve molluscs.

Training

VIRUS METHOD TRAINING FOR BASELINE SURVEY LABS June - July 2016

The EURL provided 3-day training workshops in consecutive weeks (28th - 30th June & 5th - 7th July 2016) for laboratories designated for analysis in the EFSA European baseline survey of norovirus in oysters covering all elements of the method including analysis of data and calculation of quantities and production of associated control materials. The workshops were attended by representatives of designated labs from Ireland, Sweden, Denmark, the Netherlands, Spain, Portugal, Italy, Croatia and Greece.

VIBRIO METHOD TRAINING WORKSHOP

The EURL provided a 5-day training workshop on bacteriological and molecular methods for the detection of pathogenic vibrios for 5 delegates from the Saudi Arabia Food and Drug Authority on 24th - 28th April 2017.

Additional ad hoc training and study visits

The EURL hosted a visitor from the Institute of Public Hygiene and Veterinary Health in Romania in November 2016 and a visitor from JRC in March 2017 for training in practical methods for detection of viruses

Other technical assistance

Reference and/or ready-to-use control materials for the virus detection method were provided to laboratories designated for analysis in the EFSA European baseline survey of norovirus in oysters in 10

European countries. Control materials for the method were also separately provided to JRC, and laboratories in France, Hungary, Greece the United Kingdom and Switzerland.

Provision of advice to NRLs and others

In 2016 and 2017 the EURL provided advice (briefing notes, technical reports, etc) to laboratories within the network of National Reference Laboratories and others. Advisory activities requiring input of half a person day or more, or those with written outputs are included here:

- Advice on the implementation of methods for detection of viruses in shellfish (ISO 15216) and determination of method characteristics (LOD, LOQ etc.) to labs in China, Canada, France, Germany, Denmark, Greece, the Netherlands and Peru.
- Presentation of EURL activities to annual French Live Bivalve Mollusc Stakeholders meeting 'Health, Environment and Microbiology' September 2016, Nantes.
- Advice to NRL Croatia on the length of review periods for interpretation of monitoring data for the purposes of classification of LBM production areas.
- Advice to NRL Croatia on application of *E. coli* MPN/100g hygiene standards to LBM placed on the market.
- Advice to the Competent Authority of the UK devolved administration (Scotland) on the classification review frequency following implementation of Commission Regulation (EU) 2015/2285.
- Advice to the UK Competent Authority on the rationale for and calculation of 99.9%tile value for rainfall events and the relationship between one in five-year rainfall event when considering anomalous results from LBM classification programmes.
- Advice to the UK Competent Authority on the technical hygiene requirements associated with the export of live bivalves to the USA.

Additionally, *ad hoc* technical advice was provided to laboratories via e-mail or telephone.

Other Scientific activities

EURL staff have produced a number of peer-review papers in scientific journals, book chapters, etc.:

Baker-Austin C. and J. D. Oliver. Vibrio vulnificus: new insights into a deadly opportunistic pathogen. *Environmental Microbiology*, published ahead of print, Oct 2017.

Baker-Austin, C., J. A. Trinanes, N. Gonzalez-Escalona, and J. Martinez-Urtaza. Non-cholera vibrios – the microbial barometer of climate change. *Trends in Microbiology,* **25**(1):76-84, 2017.

Baker-Austin, C., J. A. Trinanes, S. Salmenlinna, M. Löfdahl, A. Siitonen and J. Martinez-Urtaza. Heatwave-associated vibriosis in Sweden and Finland, 2014. *Emerging Infectious Diseases*, **22**(7):1216-1220, 2016.

Boxman, I.L., L. Verhoef, H. Vennema, S. Ngui, I.H. Friesema, C. Whiteside, **D. Lees** and M. Koopmans. International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012. *Eurosurveillance* **21**:1-9, 2016.

Bresnan, E., **C. Baker-Austin, C. J. A. Campos,** K. Davidson, M. Edwards, A. Hall, **D. Lees,** A. McKinney, S. Milligan and J. Silke. Impacts of climate change on human health. *Marine Climate Change Science Review,* 2017.

Campos, C. J. A., G. Goblick, **R. Lee,** K. Wittamore and **D. N. Lees.** Determining the zone of impact of norovirus contamination in shellfish production areas through microbiological monitoring and hydrographic analysis. *Water Research* **124**: 556–565, 2017.

Campos, C. J. A., O. C. Morgan, S. Kershaw and D. N. Lees. Risk factors for norovirus contamination of shellfish water catchments in England and Wales. *International Journal of Food Microbiology* **241**: 318–324. 2017.

Campos, C.J.A., J. Avant, J. Lowther, D. Till and **D.N. Lees**. Human norovirus in untreated sewage and effluents from primary, secondary and tertiary treatment processes. *Water Research* **103**: 224–232, 2016.

Church, S., **C. Baker-Austin** and S. Michell. *Vibrio vulnificus* Type VI Secretion System 1 contains anti-bacterial properties. *PLOS One,* **11**(10):e0165500, 2016.

De Souza, R. V., **C. Campos, D. Lees,** L. Garbossa and W. Seiffert. Compliance of brown mussel (*Perna perna*) production areas in the South of Brazil with the bacteriological criteria of the shellfish hygiene systems in the European Union and United States of America: assessing the impacts on consumer safety. *Journal of Water and Health* **15**(5): 834–838, 2017.

De Souza, R. V., **C. J. A**. **Campos,** L. H. P. Garbossa, L. F. N. Vianna, A. Vanz, G. Rupp and W. Q. Seiffert, A critical analysis of the international legal framework regulating the microbiological classification of bivalve shellfish production areas. *Reviews in Aquaculture,* in press. 2017.

European Food Safety Authority (Working group and sub-group members including **D. Lees** and **J. Lowther**). Technical specifications for a European baseline survey of norovirus in oysters. *EFSA Journal* 2016;14(3):4414 [62 pp.], 2016.

Garbossa, L., R. de Souza, C. **Campos,** A. Vanz, L Vianna and G. Rupp. Thermotolerant coliform loadings to coastal areas of Santa Catarina (Brazil) evidence the effect of growing urbanisation and insufficient provision of sewerage infrastructure. *Environmental Monitoring and Assessment* **189**(1): 27, 2017.

Jennings, S., **C. Baker-Austin** *et al.* Aquatic food security: insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. *Fish and Fisheries*, 2016. DOI: 10.1111/faf.12152

Lowther J. A., A. Bosch, S. Butot, J. Ollivier, D. Mäde, S. A. Rutjes, G. Hardouin, B. Lombard P. In't Veld and A. Leclercq. Validation of ISO method 15216 part 1 - Quantification of hepatitis A virus and norovirus in food matrices. *International Journal of Food Microbiology*, published ahead of print, Nov 2017.

Martinez-Urtaza J., **R. van Aerle,** M. Abanto, J. Haendiges, R. A. Myers, J. Trinanes, **C. Baker-Austin** and N. Gonzalez-Escalona. Genomic Variation and Evolution of Vibrio parahaemolyticus ST36 over the Course of a Transcontinental Epidemic Expansion. *MBio.* **8**(6), 2017.

Olalemi, A., **C. Baker-Austin**, J. Ebdon and H. Taylor. Bioaccumulation and faecal bacterial and viral indicators in *Mytilus edulis* and *Crassostrea gigas. International Journal of Hygiene and Environmental Health,* **219**: 592–598, 2016.

Taiwo M., C. Baker-Austin, A. Powell, E. Hodgson, O. Natas and **D. Walker**. Comparison of toxR and tlh based PCR assays for *Vibrio parahaemolyticus*. *Food Control* **77**: 116-120. 2017.

Turner A. D., M. Dhanji-Rapkova, L. Coates, L. Bickerstaff, S. Milligan, A. O'Neil, D. Faulkner, H. McEneny, **C. Baker-Austin**, **D. N. Lees** and M. Algoet. Detection of Tetrodotoxin Shellfish Poisoning (TSP) Toxins and Causative Factors in Bivalve Molluscs from the UK. *Marine Drugs*. 15(9), 2017.

Wagley S., R. Borne, J. Harrison, **C. Baker-Austin**, D. Ottaviani, F. Leoni, V. Vuddhakul and R. W. Titball. Galleria mellonella as an infection model to investigate virulence of Vibrio parahaemolyticus. *Virulence*. published ahead of print, Sept 2017.

Walker, D. I., A. Younger, L. Stockley and C. Baker-Austin. *Escherichia coli* testing and enumeration in live bivalve shellfish – present methods and future directions. *Food Microbiology*, in press. 2017.

Walker, D. I., J. McQuillan, **M. Taiwo,** R. Parks, C. Stenton, H. Morgan, M. Mowlem and **Lees, D. N.** A highly specific Escherichia coli qPCR and its comparison with existing methods for environmental waters. *Water Research*, **126**: 101–110. 2017.

Winterbourn J.B., K. Clements, **J.A. Lowther**, S.K. Malham, J.E. McDonald and D.L. Jones. Use of *Mytilus edulis* biosentinels to investigate spatial patterns of norovirus and faecal indicator organism contamination around coastal sewage discharges. *Water Research* **105**:241-250, 2016.

Scientific presentations (international conferences)

Avant, J. The contribution of proficiency testing to standardisation, implementation and quality assurance of virus testing in shellfish**,** 11th International Conference on Molluscan Shellfish Safety, Galway, Ireland, May 2017.

Baker-Austin, C. Pathogenic vibrios – the microbial barometer of climate change. University of North Carolina, Institute for Marine Sciences, Morehead city, NC, USA, October 2016.

Baker-Austin, C. *Vibrio* wound infections in Sub-Arctic waters. *Vibrio*, Roscoff, France, March 2016.

Campos, C. Identifying norovirus exclusion zones in shellfish production areas through microbiological monitoring, drogue tracking and sewage effluent tracing: studies in a shallow estuary and a deep coastal embayment**,** 11th International Conference on Molluscan Shellfish Safety, Galway, Ireland, May 2017.

Campos, C. Microbiological pollution in shellfish production areas: environmental risk factors and management options. Marine BioIsle Seminar, Universidade dos Açores, Ponta Delgada, Portugal, October 2016.

Campos, C. Satellite tracking of algal blooms and water quality. Association of Scottish Shellfish Growers Annual Conference, Oban, Scotland, October 2017.

Campos, C. Satellite-based HAB and water quality monitoring for shellfish farms to support management decisions. 58th Marine Measurement Forum, Plymouth Marine Laboratory, Plymouth, United Kingdom, September 2016.

Lees, D. Improving health controls for viruses in bivalve molluscs. Keynote. Water Microbiology Conference, Chapel Hill, North Carolina, USA, May 2016.

Lowther, J. A one year survey of norovirus in UK oysters collected at the point of sale, 11th International Conference on Molluscan Shellfish Safety, Galway, Ireland, May 2017.

Lowther, J. A One Year Survey of Norovirus in UK Oysters Collected at the Point of Sale, 5th Food and Environmental Virology conference, Kusatsu, Japan, September 2016.

Lowther, J. Methodology for detection of norovirus and hepatitis A virus in foods; current status and future challenges, FSA-EFSA International Workshop on Foodborne Viruses, London, England, February 2016.

Powell, A. Emerging Vibrio risk in Northern Europe, the Crucible of Climate Change. American Society for Microbiology (ASM) Conference on Vibrio: The Biology of Vibrios, Chicago, United States, November 2017.

Younger, A. Evaluation of the protection against norovirus afforded by Official Control monitoring of shellfish production areas under EU Regulations**,** 11th International Conference on Molluscan Shellfish Safety, Galway, Ireland, May 2017.

4. Confirmatory testing and quality assurance

EURL standard operating procedures (SOPs) for ISO/IEC 17025 accredited methods have been reviewed and revised according to the annual cycle. All SOPs are available as generic protocols for statutory (and some non-statutory) methods through the EURL website and on request from the EURL co-ordinator.

Accreditation to ISO 17025 was retained for the following methods and associated procedures:

- Detection of *Salmonella* spp in bivalve molluscan shellfish (ISO 6579).
- Enumeration of *E. coli* in bivalve molluscan shellfish (ISO 16649-3).
- Detection of *V. parahaemolyticus* in bivalve molluscan shellfish (ISO 21872-1).
- Quantification of norovirus in bivalve molluscan shellfish (ISO 15216-1).

In addition, accreditation to ISO 17025 was obtained for the following method

- Quantification of hepatitis A virus in bivalve molluscan shellfish (ISO 15216-1).

The EURL is accredited for these analyses by the United Kingdom Accreditation Service (UKAS) schedule number UKAS 2293. UKAS is a member of the European co-operation for accreditation (EA). No confirmatory testing for third parties as a result of disputed analysis was undertaken in 2016 or 2017.

5. Comparative testing and ring trials

Participation of Member State NRLs, EFTA and third countries in EURL organised proficiency testing (PT) in 2016 - 2017 is tabulated in Table 2 and summarised in this section. In most cases, PT distributions are open to non-NRL participants on a cost recovery basis. Samples were provided free of charge to NRLs under the agreed annual work programme of the EURL. Full PT reports are available on the EURL website https://eurlcefas.org. Reference samples produced by the EURL were assessed for homogeneity in accordance with ISO 22117.

Proficiency testing for statutory determinands

E. coli and Salmonella spp. proficiency testing – PT 64, PT 67, PT 73 and PT 74.

NRLs agreed at the annual workshop of NRLs in 2012 that participation in two annual PT distributions is mandatory, at a minimum, with the EURL matrix PT (comprising of bivalve mollusc samples) to be compulsory and at least one PHE EQA scheme distribution to be examined per year. All designated NRLs completed the EURL matrix PT in both years (currently there are no NRLs designated in Malta, Cyprus, the Czech Republic or Estonia). NRLs in Bulgaria, Finland and Norway did not take part in the PHE EQA scheme during 2016. The reasons for lack of participation were reviewed at the 2017 annual workshop. To assist NRLs in participating in the mandatory PT distributions, the EURL agreed to pay NRLs participation in a

single PHE distribution each year from 2017 onwards (Resolution 8, 2016). All NRLs participated in the required number of PT schemes during 2017. Table 2 shows the level of NRL's participation during 2016 – 2017 for all organised PT distributions. The minimum requirement for satisfactory performance during a calendar year is a cumulative score of greater than 70%. Tables 3 (PT 64 and PT 67) and Tables 4 (PT 73 and PT 74) show abstracted performance scores for NRLs for 2016 and 2017 respectively.

PT 64 - Matrix distribution 2016

Matrix distributions enable participants to examine all aspects of the methodology. In November 2016, the EURL distributed three samples of bivalve molluscan shellfish (Pacific oysters - *Crassostrea gigas*) to participants. Thirty-nine laboratories participated, including all 25 nominated NRLs for bacteriology within the network. Participants' duplicate *E. coli* MPN values for each sample were compared to the median of all participants' results. Upper and lower acceptability limits were calculated as the participants' median ±3 theoretical standard deviations (SD) and ±5 SD (≈99% and 99.9% confidence intervals respectively). Performance assessment was determined according to the EURL/PHE EQA scheme for a single distribution, with modifications to reflect replicate analyses of a single sample (Table 3). NRLs Bulgaria, Finland and Poland had points deducted as one or both MPN values reported for sample 1 or sample 3 were outside ±3 SD of the participants' median. Further points were deducted from NRLs Germany, Finland and Latvia due to the reporting of tube combinations inconsistent with the guidance given in ISO7218:2007/Amd 1:2013 for interpretation of MPN tables and/or the EURL generic protocol for enumeration of *E. coli* in bivalve molluscs. For *Salmonella* spp. analyses, NRL Norway failed to detect the presence of *Salmonella* spp. in sample 3; all other NRLs correctly detected its presence (Table 3). Due to *Salmonella* spp. being detected naturally in 4 out of 20 replicates reference samples for sample 2 (un-spiked sample), scores were not allocated to participants.

PT 67 – Non-matrix distributions 2016

NRLs were offered distributions (SF053, SF054, SF055) in February, June and October 2016 for examination of *E. coli* and *Salmonella* spp. in simulated bivalve mollusc matrices. Uptake to the PHE/EQA scheme was not universal (Table 3); with NRLs in Bulgaria, Finland and Norway not registering for any distributions.

PT 73 - Matrix distribution

In November 2017, the EURL distributed two samples of bivalve molluscan shellfish (Common mussels – *Mytilus edulis* and Pacific oysters - *Crassostrea gigas*) to participants. Thirty-three laboratories participated, including all 25 nominated NRLs for bacteriology within the network. Participants' duplicate *E. coli* MPN values for each sample were compared to the median of all participants' results. Upper and lower acceptability limits were calculated as the participants' median ±3 theoretical standard deviations (SD) and ±5 SD (≈99% and 99.9% confidence intervals respectively). Performance assessment was determined according to the EURL/PHE EQA scheme for a single distribution, with modifications to reflect replicate analyses of a single sample (Table 4 and 5). NRLs Iceland, Netherlands and Sweden had points deducted as one or both MPN values reported for sample 2 were outside ±3 SD of the participants' median. Further points were deducted from NRLs Bulgaria, Croatia, Finland and Latvia due to the reporting of tube combinations inconsistent with the guidance given in ISO7218:2007/Amd 1:2013 for interpretation of MPN tables and/or the EURL generic protocol for enumeration of *E. coli* in bivalve molluscs, or for the reporting of incorrect MPN values for the reported tube combination. For *Salmonella* spp. analyses, NRLs Ireland and Latvia incorrectly reported the presence of *Salmonella* spp. in sample 1 and scored 0. All NRLs correctly detected the presence of *Salmonella* spp. in sample 2 (Table 4 and 5).

Table 2 - Summary of participation amongst NRLs and others in EURL organised proficiency testing for calendar years 2016-2017

EURL PT reference number	Proficiency testing description	Year	EU MS National Reference Laboratories																											EFTA		Third countries									
			Austria	Belgium and Luxembourg [1 2 3 5 6]	Bulgaria	Croatia	Cyprus [3]	Czech Republic	Denmark [1 2 3 5 6]	Estonia	Finland [5]	France [1 3 4 6]	Germany [1 2 3 4 5]	Greece [2 3 6]	Hungary	Ireland	Italy [2 3 5]	Latvia	Lithuania	Malta	Netherlands [1]	Poland [3]	Portugal [1 3]	Romania [2 3]	Slovakia	Slovenia	Spain [1 2 3 5]	Sweden	United Kingdom [2 3 5]	Iceland	Norway	Australia	Canada [3 5]	Chile	New Zealand	Peru [1]	Saudi Arabia	Singapore	Switzerland	South Korea
PT 61	Norovirus and Hepatitis A virus in LENTICULES	2016	✓	✓	✓	✓	X	X	✓	X	X	✓	✓	✓	X	✓	✓	X	X	X	✓	✓	✓	X	✓	✓	✓	✓	✓	X	✓	X	X	X	✓	✓	X	✓	✓	✓
PT 64	*E. coli* and *Salmonella* spp. in shellfish matrix	2016	✓	✓	✓	✓	X	X	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X	X	X
PT 65	Norovirus and Hepatitis A virus shellfish matrix and LENTICULES	2016	✓	✓	✓	✓	X	X	✓	X	✓	✓	X	X	X	✓	✓	X	X	X	✓	✓	✓	X	X	✓	✓	✓	✓	X	X	X	✓	X	X	✓	X	✓	X	✓
PT 67	*E. coli* and *Salmonella* spp. - EQA	2016	✓	✓	X	✓	X	X	✓	X	X	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X	X	X	X
PT 69	Norovirus and Hepatitis A virus in LENTICULES	2017	✓	✓	X	X	X	X	✓	X	✓	✓	✓	✓	X	✓	✓	X	X	X	✓	✓	✓	X	✓	✓	X	✓	✓	X	✓	X	X	X	X	X	X	X	X	X
PT 71	Development of Real-Time PCR for the detection of *Vibrio* spp.	2017	✓	✓	✓	X	X	X	X	X	X	✓	✓	X	X	X	✓	X	X	X	X	✓	X	X	X	✓	X	X	✓	X	✓	X	X	X	X	X	X	X	✓	X
PT 72	Norovirus and Hepatitis A virus shellfish matrix	2017	✓	✓	✓	✓	X	X	✓	X	X	✓	✓	✓	✓	✓	✓	X	X	X	✓	✓	✓	X	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PT 73	*E. coli* and *Salmonella* spp. in shellfish matrix	2017	✓	✓	✓	✓	X	X	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X	✓	X
PT 74	*E. coli* and *Salmonella* spp. - EQA	2017	✓	✓	✓	✓	X	X	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X	X	X

No designated NRL

Labs that received samples but did not return results are marked as not participating

[1] more than one laboratory participated in PT 61.

[2] more than one laboratory participated in PT 64.

[3] more than one laboratory participated in PT 66.

[4] more than one laboratory participated in PT 71.

[5] more than one laboratory participated in PT 72.

[6] more than one laboratory participated in PT 73.

Table 3. Performance assessment scores for NRLs in *E. coli* and *Salmonella* spp. PT in 2016

EU/EFTA Member State	*E. coli* scores											*Salmonella* spp. scores										
	PT 64		Distribution SF053		Distribution SF054		Distribution SF055		All distributions			PT 64		Distribution SF053		Distribution SF054		Distribution SF055		All distributions		
	S - 1	S - 3	SF0114	SF0115	SF0116	SF0117	SF0118	SF0119	Cumulative score	Max score	%	S - 1	S - 3	SF0114	SF0115	SF0116	SF0117	SF0118	SF0119	Cumulative score	Max score	%
Austria	12	12	12	12	12	12	-	-	72	72	100	-	2	2	2	2	2	-	-	10	10	100
Belgium	12	12	12	12	12	12	12	12	96	96	100	-	2	2	2	2	2	2	2	14	14	100
Bulgaria	9	12							21	-	-	-	2							2	-	-
Croatia	12	12	12	12	12	12	-	-	72	72	100	-	2	2	2	2	2	-	-	10	10	100
Denmark	12	12	-	-	12	12	-	-	48	48	100	-	2	-	-	2	2	-	-	6	6	100
Finland	0	2							2	-	-	-	2							2	-	-
France [1]	12	12	9	12	12	12	0	0	69	96	72	-	2	2	2	2	2	0	0	10	14	71
Germany	8	12	12	12	-	-	12	12	68	72	94	-	2	2	2	-	-	2	2	10	10	100
Greece	12	12	-	-	-	-	12	12	48	48	100	-	2	-	-	-	-	2	2	6	6	100
Hungary	12	12	4	4	-	-	12	12	56	72	78	-	2	2	2	-	-	2	2	10	10	100
Iceland	12	12	12	12	-	-	12	12	72	72	100	-	2	2	2	-	-	2	2	10	10	100
Ireland	12	12	12	12	12	12	12	12	96	96	100	-	2	-	-	-	-	2	2	6	6	100
Italy	12	12	12	12	-	-	12	9	69	72	96	-	2	2	2	-	-	2	2	10	10	100
Latvia	8	8	-	-	8	8	-	-	32	48	67	-	2	-	-	2	2	-	-	6	6	100
Lithuania	12	12	-		-	-	12	12	48	48	100	-	2	-	-	-	-	2	2	6	6	100
Netherlands [1]	12	12	9	12	12	12	12	12	93	96	97	-	2	2	2	-	-	-	-	6	6	100
Norway	12	12							24	-	-	-	0							0	-	-
Poland	9	12	12	12	12	12	12	12	93	96	97	-	2	2	2	2	2	2	2	14	14	100
Portugal	12	12	12	12	12	12	12	12	96	96	100	-	2	2	2	2	2	2	2	14	14	100
Romania	12	12	12	12	12	9	12	12	93	96	97	-	2	2	2	2	2	2	2	14	14	100
Slovakia	12	12	12	12	-	-	12	12	72	72	100	-	2	2	2	-	-	2	2	10	10	100
Slovenia	12	12	12	12	12	12	12	12	96	96	100	-	2	2	2	2	2	2	2	14	14	100
Spain	12	12	-	-	12	9	-	-	45	48	94	-	2	-	-	2	2	-	-	6	6	100
Sweden	12	12	12	12	-	-	12	12	72	72	100	-	2	2	2	-	-	2	2	10	10	100
UK	12	12	12	12	12	12	12	12	96	96	100	-	2	2	2	2	2	2	2	14	14	100

[1] laboratories submitted results from reference and alternative methods

Table 4. Performance assessment scores for NRLs in *E. coli* PT in 2017

EU/EFTA Member State	Performance assessment [1]							Information only [4]						
	PT 73		Distribution SF056		Cumulative score	Max Score	%	Distribution SF057		Distribution SF058		Cumulative score	Max Score	%
	S - 1	S - 2	SF0120	SF0121				SF0122	SF0123	SF0124	SF0125			
Austria	12	12	12	12	48	48	100	12	12	12	12	96	96	100
Belgium	12	12	12	12	48	48	100	12	12	12	12	96	96	100
Bulgaria	12	10	8	8	38	48	79	-	-	-	-	38	48	79
Croatia	12	10	12	12	46	48	96	-	-	-	-	46	48	96
Denmark	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Finland	8	8	8	8	32	48	67	-	-	-	-	32	48	67
France [2]	12	12	12	12	48	48	100	-	-	-	-	48	48	100
France [3]	8	8	-	-	-	-	-	-	-	-	-	-	-	-
Germany	12	12	12	12	48	48	100	12	12	-	-	72	72	100
Greece	12	12	8	8	40	48	83	-	-	-	-	40	48	83
Hungary	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Iceland	12	9	12	12	45	48	94	-	-	-	-	45	48	94
Ireland	12	12	12	9	45	48	94	12	12	12	12	93	96	97
Italy	12	12	12	12	48	48	100	-	-	12	12	72	72	100
Latvia	8	8	8	8	32	48	67	-	-	-	-	32	48	67
Lithuania	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Netherlands [2]	12	12	12	12	48	48	100	0	0	0	0	48	96	50
Netherlands [3]	8	4	8	8	28	32	88	8	8	8	8	60	64	94
Norway	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Poland	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Portugal	12	12	12	12	48	48	100	12	12	12	12	96	96	100
Romania	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Slovakia	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Slovenia	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Spain	12	12	12	12	48	48	100	-	-	-	-	48	48	100
Sweden	12	9	8	8	37	48	77	-	-	12	12	61	72	85
UK	12	12	8	8	40	48	83	12	12	12	12	88	96	92

[1] Cumulative score calculated using results from PT 73 and distribution SF056 only

[2] Laboratory results submitted using the reference method

[3] Laboratory results submitted using the alternative method

[4] Results provided for information only

Table 5. Performance assessment scores for NRLs in *Salmonella* spp. PT in 2017

EU/EFTA Member State	Performance assessment [1]							Information only [2]						
	PT 73		Distribution SF056		Cumulative score	Max Score	%	Distribution SF057		Distribution SF058		Cumulative score	Max Score	%
	S - 1	S - 2	SF0120	SF0121				SF0122	SF0123	SF0124	SF0125			
Austria	2	2	2	2	8	8	100	2	2	2	2	16	16	100
Belgium	2	2	2	2	8	8	100	2	0	2	2	14	16	88
Bulgaria	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Croatia	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Denmark	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Finland	2	2	2	2	8	8	100	-	-	-	-	8	8	100
France	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Germany	2	2	2	2	8	8	100	2	2	-	-	12	12	100
Greece	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Hungary	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Iceland	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Ireland	0	2	2	2	6	8	75	2	2	-	-	10	12	83
Italy	-	2	2	2	6	6	100	-	-	2	2	10	12	83
Latvia	0	2	2	2	6	8	75	-	-	-	-	6	8	75
Lithuania	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Netherlands	2	2	2	2	8	8	100	0	0	0	0	8	16	50
Norway	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Poland	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Portugal	2	2	2	2	8	8	100	2	2	2	2	16	16	100
Romania	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Slovakia	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Slovenia	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Spain	2	2	2	2	8	8	100	-	-	-	-	8	8	100
Sweden	2	2	2	2	8	8	100	-	-	2	2	12	12	100
UK	2	2	2	2	8	8	100	2	2	2	2	16	16	100

[1] Cumulative score calculated using results from PT 73 and distribution SF056 only

[2] Results provided for information only

PT 74 – Non-matrix distributions 2017

NRLs were offered distributions (SF056, SF057, SF058) in February, June and October 2017 for examination of *E. coli* and *Salmonella* spp. in simulated bivalve mollusc matrices. All NRLs participated in at least one distribution (Table 4 and 5).

Cumulative performance of NRLs in proficiency testing for statutory determinands

Cumulative performance assessments for participating NRLs indicated generally satisfactory performance for both 2016 and 2017. In 2016, performance assessment was carried out on all statutory distributions (EURL and PHE/EQA). NRLs Bulgaria, Finland and Norway did not participate in the minimum number of PT distributions required and were not subjected to a cumulative assessment for the year. Of those labs that were assessed, NRL Latvia achieved a cumulative score of <70% for *E. coli.* In 2017, performance assessment was assessed using only the EURL whole animal distribution (PT 73) and 1 PHE/EQA distribution (SF056); NRLs Finland and Latvia achieved a cumulative score of <70% for *E. coli.* In both years all assessed labs achieved a cumulative score of >70% for *Salmonella* spp.

Proficiency testing for non-statutory determinands

Norovirus, Hepatitis A virus and Vibrio spp. – PT 61, PT 65, PT 69, PT 72 and PT 71.

The EURL also offers PT for non-statutory determinands, and in 2016 and 2017 PT distributions were provided for norovirus (genogroups I and II) and hepatitis A virus and for *Vibrio* spp. Non-statutory PT helps laboratories in the implementation and accreditation of new methods, and can demonstrate continuous improvements. This is particularly important for virus methods for which there is a need for capacity building across the network of NRLs to support potential future food hygiene legislation. For *Vibrio* spp. the PT was designed to investigate different methodologies in order to support the development of a new PCR-based ISO method for quantification of potentially enteropathogenic *Vibrio* spp.

PT 61 - Norovirus and hepatitis A virus in matrix material and LENTICULES

In September 2016 the EURL distributed 6 samples comprising naturally contaminated and bioaccumulated Pacific oysters (*Crassostrea gigas*) and laboratory constructed LENTICULE discs. Double-stranded DNA control material for each target was also included with the sample material to assist laboratories with quantification. Material was distributed to 47 laboratories including 19 NRLs. All participating NRLs returned results, with 13 obtaining the intended presence/absence result (as determined by EURL reference designations) for all determinands tested. Sixteen NRLs reported quantitative data for at least one sample/target virus combination with 7 reporting results within an acceptable range defined as the participants' median ±2δ median absolute deviation (MAD) for all sample/target virus combinations tested.

PT 65 - Noroviruses and hepatitis A virus in LENTICULES

In July 2016 the EURL distributed 2 laboratory constructed LENTICULE discs containing a combination of norovirus GI, GII and HAV. Material was distributed to 43 laboratories including 17 NRLs, of whom 16 returned results. Fourteen NRLs correctly reported presence/absence results (as determined by the EURL reference samples) for all determinands tested. Twelve NRLs reported quantitative data for at least one sample/target virus combination with 6 reporting results within an acceptable range defined as the participants' median ±2δ median absolute deviation (MAD) for all sample/target virus combinations tested.

PT 69 - Virus LENTICULE proficiency testing schemes in 2017

In collaboration with the UK PHE, the EURL organised two separate distributions each comprising of 2 laboratory constructed LENTICULE discs containing a combination of norovirus GI, GII and HAV. NHV001 was distributed in February 2017 and NHV002 was distributed in October 2017. Seventeen NRLs participated in NHV001 and five participated in NHV002. Fifteen NRLs correctly reported presence/absence results (as determined by the EURL reference samples) for all samples and determinands tested. NRLs overall accuracy for presence / absence across the two schemes was 96.9%. Thirteen NRLs reported quantitative data, of whom 3 reported quantities in the unsatisfactory range for one or more sample/target virus. All other quantitative results reported by NRLs were in the satisfactory range.

PT 72 - Norovirus and hepatitis A virus in matrix material

In September 2017 the EURL distributed 4 samples comprising of naturally contaminated and bioaccumulated Pacific oysters (*Crassostrea gigas*). Double-stranded DNA control material for each target was also included with the sample material to assist laboratories with quantification. Material was distributed to 43 laboratories including 21 NRLs, of whom 20 returned results. Seventeen NRLs correctly reported presence/absence results (as determined by the EURL reference samples) for all determinands tested. Sixteen NRLs reported quantitative data for at least one sample/target virus combination with 10 reporting results within an acceptable range defined as the participants' median ±2δ median absolute deviation (MAD) for all sample/target virus combinations tested.

PT 71 – *Vibrio* spp. development of PCR (conventional or real-time) for detection

The EURL organised an Interlaboratory study (ILS) to support methodological improvements for the identification of *Vibrio* spp. using Polymerase chain reaction (PCR) and real-time PCR (RT-PCR) formats. The aim of this ILS was to facilitate method development rather than laboratory performance, in particular for PCR methodologies focussed on the pathogen *V. parahaemolyticus*. Thirty individual samples were distributed to 13 participants including 10 NRLs. Following the analysis of the reported data several notable issues and conclusions were identified, as detailed in the scheme report available on the EURL website.

6. Development of analytical methods

A meeting of CEN/TC275/WG6/TAG4 (led by EURL staff) was convened in Brussels on 2nd - 3rd February 2016. Based on the results of the votes (and associated comments) on ISO/DIS 15216-1, the revision of ISO/TS 15216-1:2013, Microbiology of food and animal feed: determination of norovirus and hepatitis A virus in foodstuffs by PCR, Part 1: Quantitative determination, a series of revisions was agreed and a new final draft of the standard ISO/FDIS 15216-1 was prepared. Following the final positive vote on this document, the new standard including validation data was published in March 2017.

Subsequently, TAG4 has begun work to harmonise part 2 of ISO 15216 (detection) with the newly published part 1.

Following successful conclusion of a research collaboration with the EU Joint Research Council, Geel, to elaborate control materials to assist in the standardisation of ISO methods for noroviruses, a teleconference between the EURL and representatives of JRC was held on 28th October 2016, to identify further opportunities for collaboration in this area.

Further research into methods for the determination of the infectivity of noroviruses detected in bivalve shellfish by PCR was carried out, and progress with the use of FRNA bacteriophage as an indicator of infectivity was made.

The initial technical enquiry on ISO/DIS 21872, the draft document amalgamating and revising ISO/TS 21872-1:2007, Microbiology of food and animal feeding stuffs: Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp., Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* and ISO/TS 21872-2:2007, Part 2: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*, was carried out from 17th March to 17th June 2016. The DIS was approved with 23 positive votes and no negative votes. Following this the EURL convened a meeting of CEN/TC275/WG6/TAG15 to discuss the vote and the comments received and a final draft revision ISO/FDIS 21872 was prepared and forwarded to the CEN/ISO secretariat in preparation for the final vote. Following the approval of the method, the new standard was published in July 2017.

A teleconference meeting of CEN/TC275/WG6/TAG15 was convened on 19th May 2016 to discuss the first steps necessary for development of methods to enable enumeration of total / pathogenic *V. parahaemolyticus*. Further meetings are scheduled for the early part of 2017. Subsequently a physical meeting was convened in London on 9th February 2017. An ILS (PT 71) was designed to support method development in this area.

The EURL worked with statistical experts from the Bundesanstalt für Materialforschung und -prüfung, Berlin, Germany (Prof. Cordula Wilrich), to develop a bivalve-specific *E. coli* MPN calculator, to assist NRLs and OCLs and to standardise the approach to MPN calculations derived from positive and negative tube combinations.

The EURL worked with the relevant expert from the US FDA (Dr Greg Goblick) to work up data resulting from practical dye tracing studies carried out in 2015. A joint peer reviewed paper was published in 2017. This work includes establishing experience with dye dilution studies used by the US FDA to establish buffer zones in complex situations. The EURL is in a good position to advise MS, in support of the trade annexes in the EU guidance, should MS need to establish buffer zones in production areas targeted for exports to the US.

About us

Cefas is a multi-disciplinary scientific research and consultancy centre providing a comprehensive range of services in fisheries management, environmental monitoring and assessment, and aquaculture to a large number of clients worldwide.

We have more than 500 staff based in 2 laboratories, our own ocean-going research vessel, and over 100 years of fisheries experience.

We have a long and successful track record in delivering high-quality services to clients in a confidential and impartial manner.
(www.cefas.defra.gov.uk)

Cefas Technology Limited (CTL) is a wholly owned subsidiary of Cefas specialising in the application of Cefas technology to specific customer needs in a cost-effective and focussed manner.

CTL systems and services are developed by teams that are experienced in fisheries, environmental management and aquaculture, and in working closely with clients to ensure that their needs are fully met.
(www.cefastechnology.co.uk)

.

Customer focus

With our unique facilities and our breadth of expertise in environmental and fisheries management, we can rapidly put together a multi-disciplinary team of experienced specialists, fully supported by our comprehensive in-house resources.

Our existing customers are drawn from a broad spectrum with wide ranging interests. Clients include:

- international and UK government departments
- the European Commission
- the World Bank
- Food and Agriculture Organisation of the United Nations (FAO)
- oil, water, chemical, pharmaceutical, agro-chemical, aggregate and marine industries
- non-governmental and environmental organisations
- regulators and enforcement agencies
- local authorities and other public bodies

We also work successfully in partnership with other organisations, operate in international consortia and have several joint ventures commercialising our intellectual property

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.defra.gov.uk

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

printed on paper made from a minimum 75% de-inked post-consumer waste