

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

Annual technical report for calendar year 2018

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

Technical Report for Calendar Year 2018

Note: the work programme and all EURL reports mentioned in this technical report are available on the EURL website at <https://eurlcefaf.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, and from April 29th 2018, in Article 94 of Regulation (EU) 2017/625.

Introduction

The work programme for the EURL for 2018 was approved by the European Union in April 2018. 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 17th workshop of microbiological NRLs held in Southampton, United Kingdom, and other responsibilities outlined in Council Regulation (EC) 882/2004 and Regulation (EU) 2017/625.

During 2018, the EURL headed a network comprising 27 National Reference Laboratories (NRLs) representing 24 EU Member States (MS) and 2 European Free Trade Association (EFTA) MS (Table 1).

Table 1. Designated NRLs in Member States, EFTA and Accession states in calendar year 2018.

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 Veterinary 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 l'Île de'Yeu, BP 21105, 44311 Nantes Cedex 3
Germany	Bundesinstitut für Risikobewertung (BfR), Federal Institute for Risk Assessment, Diederdsdorfer Weg, D-12277, Berlin

Member State	Laboratory
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	Matis 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) Istituto Superiore di Sanità, Dipartimento 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), Lejupe 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
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 EFTA MS

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

Outputs of the EURL are listed below against duties as detailed in the Work Programme.

1. To ensure availability and use of high-quality methods and to ensure high quality performance by NRLs

Sub-activity 1.1 Documentation of methods

The EURL updated its generic protocol for detection of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in bivalve molluscan shellfish to reflect the publication of ISO 21872 in 2017 and produced new summary and comprehensive guidance notes on norovirus testing. Generic protocols for detection of *E. coli*, *Salmonella* spp., norovirus and hepatitis A virus and F-RNA bacteriophage, guidance notes on use of the MPN for enumeration of *E. coli* and determination of method characteristics for virus testing, and calculators for quantification of viruses in shellfish were maintained and made available to the network of NRLs via the EURL website:- <https://eurlcefafas.org/public-documents/methods.aspx>

Ad hoc advice on norovirus testing methods was provided to NRLs Greece and Bulgaria, and additional stakeholders in the UK and the Netherlands.

Ad hoc advice on vibrio detection methods was provided to NRLs Italy and Germany.

Sub-activity 1.2 Distribution of reference and other materials

In addition to regular provision of dsDNA and EC RNA control materials for norovirus to those NRLs involved in the baseline survey for norovirus in oysters, control materials for the virus method (plasmids, HAV, mengo virus etc.) were provided to NRLs Sweden, Spain and Greece.

Vibrio strains for positive control purposes were provided to NRL Italy.

Sub-activity 1.3 Organisation of proficiency testing

Participation of Member State NRLs, EFTA and third countries in EURL organised proficiency testing (PT) in 2018 is tabulated in Table 2 and summarised in this section. In most cases, PT distributions were 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://eurlcefafas.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 76 and PT 78.

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 EURL/PHE EQA scheme distribution to be examined per year. Twenty-four designated NRLs completed the EURL matrix PT in 2018 (currently there are no NRLs designated in Malta, Cyprus, the Czech Republic or Estonia). NRL Norway was unable to analyse the EURL matrix PT due to transport issues outside the courier's control. Table 2 shows the level of NRL's participation 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 (*E. coli*) and 4 (*Salmonella* spp.) show abstracted performance scores for NRLs for 2018.

PT 76 - Matrix distribution

In November 2018, the EURL distributed three samples of bivalve molluscan shellfish (Pacific oysters - *Crassostrea gigas*) to participants. Forty-one 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 ($\approx 99\%$ 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

Belgium, Bulgaria, France, Germany, Iceland, Ireland, Latvia, Slovakia, Sweden and Spain had points deducted as one MPN value reported for sample 1, sample 2 and/or sample 3 was outside ± 3 SD of the participants' median. NRL Finland had points deducted as one MPN value was reported for sample 1 outside ± 5 SD of the participants' median. Further points were deducted from NRLs Denmark, France 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 Lithuania detected the presence of *Salmonella* spp. in the naturally contaminated sample (sample 1). All other NRLs recorded its absence in this sample. All NRLs correctly detected the presence of *Salmonella* spp. in samples 2 and 3 (Table 4).

PT 78 – Non-matrix distributions

NRLs were offered distributions (SF059, SF060, SF061) in February, June and October 2018 for examination of *E. coli* and *Salmonella* spp. in simulated bivalve mollusc matrices. Uptake to the EURL/PHE EQA scheme was variable (Table 3 and 4) with all NRLs participating in the mandatory single EURL/PHE EQA scheme distribution (SF059).

Cumulative performance of NRLs in PT for statutory determinands

Cumulative performance assessments for participating NRLs indicated generally satisfactory performance for 2018. Performance assessment was assessed using only the EURL whole animal distribution (PT 76) and 1 EURL/PHE EQA distribution (SF059); NRL Latvia achieved a cumulative score of <70% for *E. coli*. All NRLs achieved a cumulative score of >70% for *Salmonella* spp.

Table 2 - Summary of participation amongst NRLs and others in EURL organised proficiency testing for calendar year 2018

EURL PT reference number	Proficiency testing description	EU MS National Reference Laboratories																							EFTA		Third countries											
		Austria	Belgium and Luxembourg ^{1,2}	Bulgaria	Croatia	Cyprus	Czech Republic	Denmark ^{1,2}	Estonia	Finland	France ¹	Germany ¹	Greece ²	Hungary	Ireland ²	Italy ^{1,2}	Latvia	Lithuania	Malta	Netherlands ¹	Poland	Portugal	Romania	Slovakia	Slovenia	Spain ^{1,2}	Sweden	United Kingdom	Iceland	Norway ³	Canada	Chile	New Zealand	Peru	Switzerland	South Korea		
PT 75	Norovirus and Hepatitis A virus shellfish matrix	✓	✓	✓	✓	X	X	✓	X	✓	✓	✓	✓	X	✓	✓	X	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	
PT 76	<i>E. coli</i> and <i>Salmonella</i> spp. in shellfish matrix	✓	✓	✓	✓	X	X	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X	X
PT 77	Norovirus and Hepatitis A virus in LENTICULES - EQA	✓	✓	X	X	X	X	X	X	✓	✓	✓	✓	X	✓	✓	X	X	X	✓	✓	✓	X	✓	✓	X	X	✓	X	X	X	X	X	X	X	X	X	
PT 78	<i>E. coli</i> and <i>Salmonella</i> spp. - EQA	✓	✓	✓	✓	X	X	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	X

 No designated NRL

¹ more than one laboratory participated in PT 75.

² more than one laboratory participated in PT 76.

³ Due to transport issues PT 76 sample material did not arrive in a fit condition to be analysed.

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

Table 3. Performance assessment scores for NRLs in *E. coli* PT in 2018

EU/EFTA Member State	Performance assessment ¹								Information only ⁴						
	PT 76			Distribution SF059		Cumulative score	Max Score	%	Distribution SF060		Distribution SF061		Cumulative score	Max Score	%
	S - 1	S - 2	S - 3	SF0126	SF0127				SF0128	SF0129	SF0130	SF0131			
Austria	12	12	12	12	12	60	60	100	12	12	12	12	108	108	100
Belgium	9	12	12	12	12	57	60	95	12	12	8	8	97	108	90
Bulgaria	9	12	12	12	12	57	60	95	-	-	-	-	57	60	95
Croatia	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Denmark	8	8	8	12	12	48	60	80	12	12	-	-	72	84	86
Finland	7	12	12	-	12	43	48	90	-	-	-	-	43	48	90
France ²	8	8	8	12	12	48	60	80	-	-	-	-	48	60	80
France ³	8	8	6	-	-	-	-	-	-	-	-	-	-	-	-
Germany	12	9	12	12	12	57	60	95	-	-	12	12	81	84	96
Greece	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Hungary	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Iceland	12	9	9	12	12	54	60	90	-	-	12	12	78	84	93
Ireland	9	12	12	9	12	54	60	90	12	12	12	12	102	108	94
Italy	12	12	12	12	12	60	60	100	-	-	12	12	84	84	100
Latvia	8	8	2	8	8	34	60	57	-	-	-	-	34	60	57
Lithuania	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Netherlands ²	12	12	12	12	12	60	60	100	NE	NE	NE	NE	60	60	100
Netherlands ³	8	8	8	8	8	40	40	100	8	8	8	8	72	72	100
Norway	-	-	-	12	12	-	-	-	-	-	12	12	48	48	100
Poland	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Portugal	12	12	12	12	12	60	60	100	-	-	12	12	84	84	100
Romania	12	12	6	12	12	54	60	90	-	-	12	12	78	84	93
Slovakia	12	9	12	12	12	57	60	95	-	-	-	-	57	60	95
Slovenia	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Spain	12	12	12	12	12	60	60	100	-	-	-	-	60	60	100
Sweden	12	9	9	12	12	54	60	90	-	-	12	9	75	84	89
UK	12	12	12	12	12	60	60	100	12	12	12	12	108	108	100

¹ Cumulative score calculated using results from PT 76 and distribution SF059 only

² Laboratory results submitted using the reference method

³ Laboratory results submitted using the alternative method

⁴ Results provided for information only

NE = Not examined

'-' = NRL did not participate in this distribution

Table 4. Performance assessment scores for NRLs in *Salmonella* spp. PT in 2018

EU/EFTA Member State	Performance assessment ¹								Information only ⁴						
	PT 76			Distribution SF059		Cumulative score	Max Score	%	Distribution SF060		Distribution SF061		Cumulative score	Max Score	%
	S - 1	S - 2	S - 3	SF0126	SF0127				SF0128	SF0129	SF0130	SF0131			
Austria	2	2	2	2	2	10	10	100	2	2	2	2	18	18	100
Belgium	2	2	2	2	2	10	10	100	2	0	2	2	16	18	89
Bulgaria	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Croatia	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Denmark	2	2	2	2	2	10	10	100	2	2	-	-	14	14	100
Finland	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
France	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Germany	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
Greece	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Hungary	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Iceland	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
Ireland	2	2	2	-	-	-	-	-	2	0	2	2	12	14	86
Italy	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
Latvia	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Lithuania	0	2	2	2	2	8	10	80	-	-	-	-	8	10	80
Netherlands	2	2	2	2	2	10	10	100	NE	NE	NE	NE	10	10	100
Norway	-	-	-	2	0	-	-	-	-	-	2	2	6	8	75
Poland	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Portugal	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
Romania	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
Slovakia	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Slovenia	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Spain	2	2	2	2	2	10	10	100	-	-	-	-	10	10	100
Sweden	2	2	2	2	2	10	10	100	-	-	2	2	14	14	100
UK	2	2	2	2	2	10	10	100	2	2	2	2	18	18	100

¹ Cumulative score calculated using results from PT 76 and distribution SF059 only

² Results provided for information only

NE = Not examined

‘-’ = NRL did not participate in this distribution

Proficiency testing for non-statutory determinands

Norovirus and Hepatitis A virus –PT 75 and PT 77.

The EURL also offers PT for non-statutory determinands, and in 2018 PT distributions were provided for norovirus (genogroups I and II) and hepatitis A virus. 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. NRL participation in PT for non-statutory determinands in 2018 is shown in Table 2.

PT 75 - Norovirus and hepatitis A virus in matrix material

In June 2018 the EURL distributed 4 samples comprising bioaccumulated and spiked 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 44 laboratories including 21 NRLs, of whom 20 returned results. Eleven 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 8 reporting results within an acceptable range defined as the participants' median $\pm 2\delta$ median absolute deviation (MAD) for all sample/target virus combinations tested.

PT 77 - Virus LENTICULE proficiency testing schemes in 2018

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. NHV003 was distributed in February 2018 and NHV004 was distributed in October 2018. Thirteen NRLs participated in NHV003 and seven participated in NHV004 (14 NRLs participated in at least one of the two schemes). Thirteen 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 99.2%. Ten NRLs reported quantitative data, of whom 1 reported a quantity in the questionable range for one sample/target virus combination. All other quantitative results reported by NRLs were in the satisfactory range.

Sub-activity 1.4 Development and standardisation of methods

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

- Completion of ISO/DIS 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. This draft international standard was subjected to a formal vote opening in April 2018. Following the successful vote, TAG4 responded to the comments received and a final draft ISO was completed in December 2018, ready for a final vote during 2019.
- Attendance at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6, 18th – 22nd June 2018, Lausanne, Switzerland

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

- Chairing a meeting of TAG15 in March 2018. At this meeting the results of PT 71 conducted in 2017 were discussed as were real-time PCR methods for *Vibrio parahaemolyticus*.

- Preparation of an amendment to ISO 21872-1:2017 including performance testing data for ASPW and SNA media.

At the plenary meetings of CEN/TC275/WG6 and ISO/TC34/SC9 in Lausanne, it was agreed to incorporate the projects within TAG15 into the work programme of ISO/TC34/SC9/WG27 “*Vibrio parahaemolyticus*” with the EURL remaining as project leader on the works that had previously fallen under TAG15. A long term plan for Dr Craig Baker-Austin of the EURL to assume convenorship of WG27 was formulated.

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.

2. To provide scientific and technical assistance to NRLs

Sub-activity 2.1 Application of new methods

At the annual workshop of microbiological NRLs held in Southampton, the EURL update the network of NRLs with presentations on the use of whole genome sequencing to investigate vibrio outbreaks and shellfish water quality monitoring using chemical tracers, environmental microbiology and satellite remote sensing.

EURL delegates attended conferences including the 6th conference of the International Society for Food and Environmental Virology, Tempe, Arizona, October 2018, the research conference on Vibrios and human health, Beaufort, North Carolina, April 2018 and the “Blue Planet” meeting on waterborne and seafood-borne pathogens in Abingdon, United Kingdom, August 2018.

Sub-activity 2.2 Training

The EURL hosted a visitor from NRL Greece in May 2018 for training in practical methods for detection of viruses

Sub-activity 2.3 Informing NRLs

EURL website

The EURL website <https://eurlcefafas.org> continued to provide a very useful repository for information for NRLs and other stakeholders.

Annual workshops of NRLs

The 17th workshop of NRLs was hosted at the Grand Harbour Hotel, Southampton, United Kingdom from 16th – 18th May 2018. Forty-six delegates representing 22 EU Member States, 2 EFTA countries and the EURL alongside invited experts from EFSA, the Blue Marine Foundation, Seafood Safety Assessment, the University of Strathclyde and the Centro de Control da Calidade do Medio Marino of Galicia, Spain and observers from DG SANTE and the EURLs for marine biotoxins and *Escherichia coli*. The workshop covered the future of the network and EURL provision for bivalve shellfish safety, official controls, marine vibrios, *E. coli* and *Salmonella*, viruses and outbreaks. Twenty-one resolutions were agreed by the workshop. Reports of the workshops of NRLs are available to download from the EURL website (<https://eurlcefafas.org>). Anonymised feedback from delegates attending the workshop showed exclusively good to very good performance, feedback breakdown is provided within the relevant annual workshop report.

3. To provide scientific and technical assistance to the European Commission and other organisations

Sub-activity 3.1 Assistance to the commission

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 2018 this has comprised:

- Provision of advice to the Commission and Member States in the restricted working group on bivalve molluscs. Two working groups attended in 2018 (January 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 Articles 18 and 19 of COM Implementing Regulation concerning official controls on food of animal origin, presentation of the revision of the Good Practice Guide for microbiological monitoring of bivalve mollusc production areas with regard to seasonal classification.
- Attendance at a meeting of EURLs in June 2018 to agree the new rearrangements and transition 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.
- Provision of expert technical opinion to Sante F with respect to review of example sanitary survey reports of bivalve production areas submitted to the Commission as partial evidence of fulfilment of the requirements of Commission Regulation (EC) 854/2004.
- Preparation for the two-week audit mission to South Korea (4th March – 15th March) 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.
- Attendance at the EURLs Directors meeting December 2018 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.

Sub-activity 3.2 Guidance documents

Following a meeting of the Good Practice Guide Working Group in November 2017, the EURL Guide to Good Practice: Technical Application and the EU Community Guide to the Principles of Good Practice were revised during the early part of 2018 to include revised recommendations on seasonal classifications for harvesting areas. The proposed changes were presented by the EURL at the meeting of NRLs in Southampton in May. They were further presented by the EURL and discussed at the Restricted Working Group on Bivalves in Brussels in December 2018. No changes to the proposed revised text were put forward by attendees of this meeting and so the Guides (EURL Guide to Good Practice: Technical Application issue 7 and EU Community Guide to the Principles of Good Practice issue 4) were issued in December.

Sub-activity 3.3 Cooperation with international agencies

Throughout 2018 the EURL took an active part in the expert Working Group on Norovirus in Oysters to plan analysis of the data generated in the EFSA baseline survey for norovirus in oysters. EURL staff attended meetings of the WG, held at EFSA in Parma, Italy on 26-27th September and 5-6th December, with additional contributions by correspondence.

The EURL further supported the baseline survey through the provision of ready-to-use control materials (dsDNA, EC RNA and mengo virus process control as required) to laboratories designated to analyse survey samples in 11 different European countries and through provision of ad hoc advice to those laboratories in addition.

The FAO Technical Guidance for the development of growing area aspects of bivalve mollusc sanitation programmes was published in October 2018. The EURL has contributed to the working group that has developed this guide since its inception in 2009.

Sub-activity 3.4 Diagnosis of outbreaks

EURL standard operating procedures (SOPs) for ISO/IEC 17025 accredited methods were reviewed and revised during 2018 according to the annual cycle. All SOPs were 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).
- 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).

On behalf of the Competent Authority in Iceland, the EURL tested a sample of oysters (produced in Iceland) that had been associated with an outbreak of norovirus in that country, as there was no domestic laboratory with that capability.

4. Reagents and reference collections

Sub-activity 4.1 Verification of reagent quality

Throughout 2018 the EURL undertook quality assurance testing of reagents as required according to the relevant quality assurance procedures. Through the maintenance of generic protocols and other guidance, the EURL continued to provide NRLs and other relevant labs with appropriate guidance on the use of reagents.

Sub-activity 4.2 Reference collections and lists

The EURL maintained stocks of reference materials (plasmids, EC RNA, process control material etc.) used in the detection and quantification of viruses.

The EURL *Vibrio* strain bank was augmented with several hundred UK strains as a result of environmental studies carried out in 2018. Discussions with the University of North Carolina (USA) and the University of Kyoto (Japan) were held in order to further augment the EURL strain bank with pathogenic *Vibrio* strains held in their collections.

5. Requirements related to other legislation

Sub-activity 5.1 Advice on application of legislation in force

The EURL maintained an up-to-date list of relevant regulation on the EURL website throughout 2018.

6. Other scientific outputs

Peer-reviewed papers

During 2018, EURL staff produced a number of peer-review papers in scientific journals:

Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor MA, Qadri F, **Martinez-Urtaza J** (2018). *Vibrio* spp. infections. *Nature Dis Primers*. 4:8.

de Souza RV, **Campos CJA**, Garbossa LHP, Seiffert WQ (2018) Developing, cross-validating and applying regression models to predict the concentrations of faecal indicator organisms in coastal waters under different environmental scenarios. *Sci Total Environ*. 630:20-31.

de Souza RV, **de Campos CJA**, Garbossa LHP, Vianna LFN, Seiffert WQ (2018) Optimising statistical models to predict faecal pollution in coastal areas based on geographic and meteorological parameters. *Mar Pollut Bull*. 129:284-292.

Hardstaff JL, Clough HE, Harris JP, **Lowther JA**, **Lees DN**, O'Brien SJ (2018). The use of capture-recapture methods to provide better estimates of the burden of norovirus outbreaks from seafood in England, 2004-2011. *Epidemiol Infect*. [Epub ahead of print]

Hartnell RE, **Stockley L**, Keay W, Rosec JP, Hervio-Heath D, Van den Berg H, Leoni F, Ottaviani D, Henigman U, Denayer S, Serbruyns B, Georgsson F, Krumova-Valcheva G, Gyurova E, Blanco C, Copin S, Strauch E, Wiczorek K, Lopatek M, Britova A, Hardouin G, Lombard B, In't Veld P, Leclercq A, **Baker-Austin C** (2018). A pan-European ring trial to validate an International Standard for detection of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* in seafoods. *Int J Food Microbiol*. [Epub ahead of print]

Lowther JA, **Gustar NE**, **Powell AL**, O'Brien S, **Lees DN** (2018). A One Year Survey of Norovirus in UK Oysters Collected at the Point of Sale. *Food Environmental Virol*. 10:278-287.

Martinez-Urtaza J, Trinanés J, Abanto M, Lozano-Leon A, Llovo-Taboada J, Garcia-Campello M, Pousa A, **Powell A**, **Baker-Austin C**, Gonzalez-Escalona N (2018). Epidemic dynamics of *Vibrio parahaemolyticus* illness in a hotspot of disease emergence. *Emerging Infect Dis*. 24:5.

McMenemy P, Kleczkowski A, **Lees D**, **Lowther J**, Taylor N (2018). A Model for Estimating Pathogen Variability in Shellfish and Predicting Minimum Depuration Times. *PLOS One*. 13: e0193865

Persson S, Eriksson R, **Lowther J**, Ellström P, Simonsson M (2018) Comparison between RT droplet digital PCR and RT real-time PCR for quantification of noroviruses in oysters. *Int J Food Microbiol*. 284:73-83.

Roig FJ, González-Candelas F, Sanjuán E, Fouz B, Feil EJ, Llorens C, **Baker-Austin C**, Oliver JD, Danin-Poleg Y, Gibas CJ, Kashi Y, Gulig PA, Morrison SS, Amaro C (2018) Phylogeny of *Vibrio vulnificus* from the analysis of the core-genome: implications for intra-species taxonomy. *Frontiers Microbiol*. 8:2613.

Schmidt W, Raymond D, Parish D, Ashton IGC, Miller PI, **Campos CJA**, Shutler JD (2018) Design and operation of a low-cost and compact autonomous buoy system for use in coastal aquaculture and water quality monitoring. *Aquacultural Engineering*. 80:28-36.

Schmidt W, Evers-King HL, **Campos CJA**, Jones DB, Miller PI, Davidson K, Shutler JD (2018) A generic approach for the development of short-term predictions of *Escherichia coli* and biotoxins in shellfish. *Aquac Environ Interact*. 10:173-185.

Turner AD, Fenwick D, **Powell A**, Dhanji-Rapkova M, Ford C, Hatfield RG, Santos A, **Martinez-Urtaza J**, Bean TP, **Baker-Austin C**, Stebbing P (2018) New Invasive Nemertean Species (*Cephalothrix Simula*) in

England with high levels of tetrodotoxin and a microbiome linked to toxin metabolism. *Marine Drugs*. 16:11.

Walker DI, Younger A, Stockley L, Baker-Austin C (2018). *Escherichia coli* testing and enumeration in live bivalve shellfish – Present methods and future directions. *Food Microbiol.* 73:29–38.

Younger AD, Teixeira Alves M, Taylor NGH, Lowther J, Baker-Austin C, Campos CJA, Price-Hayward M, Lees D (2018) Evaluation of the protection against norovirus afforded by *E. coli* monitoring of shellfish production areas under EU regulations. *Water Sci Technol.* 78:1010-1022.

Presentations at international conferences

During 2018, EURL staff gave presentations at a number of international scientific conferences:

Walker, D. Towards routine analysis of infectious norovirus in bivalve shellfish, 6th conference of the International Society for Food and Environmental Virology, Tempe, Arizona. 7th -10th October 2018.

Walker, D. Developing buffer zones for norovirus in shellfish production areas using chemical tracers, environmental microbiology and satellite remote sensing, 6th conference of the International Society for Food and Environmental Virology, Tempe, Arizona. 7th -10th October 2018.

Hartnell, R. E. Keeping shellfish consumers safe – managing and mitigating risk of foodborne illness from bivalve shellfish, 48th Conference of Western European Fish Technologists, Lisbon, Portugal, 15th – 18th October 2018.

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