

UK National Reference Laboratory (NRL) for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs, Cefas, Weymouth

Annual Technical Report for 2017/18

Final report version 1

19.9.18

7 pages

Contract Reference: FS616016 - Cefas ref C7417

Document approved by:	C7417 Project Sponsor – M. Algoet, 19.06.18	Review date:	N/A
Document checked by:	Craig Baker-Austin, Principle Investigator, 12.06.18 Myriam Algoet, 24.05.18 & 19.06.18 Michelle Price-Hayward, 17.05.18	Classification:	Official
Document prepared by:	Louise Stockley	Location:	C7417 cdp

SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2017 - MARCH 2018

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1. Introduction

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) Weymouth is designated as the UK National Reference Laboratory (NRL) for monitoring bacteriological and viral contamination of bivalve molluscs. This contract was renewed in 2017 as a 2-year Gov-Gov arrangement between Cefas and the Food Standards Agency (FS). This report summarises the activities carried out by the NRL for the financial year 2017-18 according to the requirements of Regulation (EC) No. 882/2004 as defined in the Service Level Agreement between the Food Standards Agency and Cefas. The description of activities included herein comprises co-ordination of UK Official Control Laboratories (OCL), provision of advice to the Competent Authority (CA) and collaboration with the European Union Reference Laboratory (EURL) through participation in comparative testing, research and development and representation at EURL workshops.

2. Co-ordination of the activities of the laboratories responsible for Official Control in the area of competence in the UK

The ninth meeting of OCLs undertaking microbiological testing of bivalve shellfish was held at the Cefas laboratory on the 3rd and 4th of October 2017. Eighteen delegates attended the two-day event. The first day (pm) consisted of a practical laboratory-based session focussing on the use of phage-based typing methods to detect faecal pollution in shellfish harvesting areas. A wide variety of topics were covered on the second day. These included OCL performance in proficiency testing (PT), a comparison of pulsifiers with other techniques for homogenisation, the legal ramifications of procedure in official control testing in England and Wales, progress at ISO on revisions to the *E. coli* and Salmonella standards, and the upcoming EU-wide survey of norovirus in oysters. There was also some discussion on upcoming changes to EU regulations (e.g. the replacement of EC Reg 882/2004 by EC Regulation 625/2017) and the future of the live bivalve network following Brexit.

3. Advice and representation within the UK and EU

3.1. Provision of advice to the OC Laboratory Network

The NRL provided advice throughout the year to OCLs. Advice provided at the OCL meeting on 3/4th Oct 2017 included practical aspects of LBS testing such as use of homogenisation methods. Advice on sourcing of media reagents was given to the OCL network following supply issues with Oxoid (who produce the vast majority of MMGB media used for *E. coli* testing). The NRL provided advice to one OCL regarding results from last PT round (PT66), where unexpected results were generated. Advice was also given to the Northern Ireland OCL on distinguishing human and non-human pollution sources.

Separate liaison meetings were held with Public Health England (PHE) and Public Health Wales (PHW) to maintain the use of NRL protocols and advice and to ensure a consistent approach to sample transport and

microbiological examination of shellfish samples as well as reporting of results and timely identification of outwith results. Some specific issues have been highlighted that cross between NRL and classification areas, such as the need to provide greater clarity on temperature recording protocols for sampling officers to follow. Information and documents were provided to PHE in support of its development of the general food microbiology NRL. The NRL has provided continued assistance in this area.

Ad hoc advice was given to individual laboratories on the following aspects of the microbiological examination of live bivalve shellfish:

- i. Appropriate methods for EPT purposes in LBS.
- ii. Norovirus testing method suitable for industry regarding LBS in the UK.
- iii. Risks associated with vibrios in seafood produce in the UK.
- iv. Temperature recording approaches for OCL's (e.g dataloggers).
- v. Adequate reporting of results, with impacts for classification purposes and possible impacts of new class A criteria on testing approaches.

3.2. Representation within the UK/EU

The NRL provided specific advice at the EURL workshop (Split, Croatia, May 2017) on a variety of issues, in particular *Vibrio* method standardisation and use of whole genome sequencing to investigate *Vibrio* outbreaks. The NRL also participated in MS-level discussions at the EURL meeting regarding shellfish-associated outbreaks as well as use of testing laboratories for norovirus analysis in shellfish. The NRL also contributed to a variety of these associated outputs and actions including developing a protocol for laboratories to use for the choice of norovirus-testing purposes.

The NRL participated in the BSI Food Microbiology Committee during 2017-2018 and provided oral and written comment on standards relevant to the area of shellfish microbiology (a short summary of changes to ISO documents was provided with the quarterly RAG report). The NRL also circulated key information on standards to the laboratory network and the competent authority, including changes to methods that impinged on current protocols. Future potential changes to relevant protocols were also discussed at the October OCL network meeting.

3.3 NRL website

The NRL [website](#) was maintained during the period by adding new material and removing obsolete material. Several additions to the NRL website took place in 2017-2018, including the following:

- I. Edits to 'Current activities' page.
- II. New *E. coli* testing protocol added June 2017.
- III. Changes of address and contact details of OCL's identified from the October 2017 network meeting.
- IV. Amendments to broken links etc.

4. Provision of assistance to the CA

4.1 Provision of technical advice and support to CA in development and management of the OCL network.

The NRL has provided regular reporting to FSA, particularly regarding progress of the EU NoV baseline survey work over the last year. Main activities in this area were as follows:

- i. Semi-annual catch up meetings were held in June 2017 at FSA Aviation house (02/06/17) and during the NRL network meeting in October 2017.
- ii. provided advice to FSA on methodological problems associated with *E. coli* testing approaches.
- iii. Provided advice and updates regarding outbreaks (e.g. norovirus outbreaks linked to UK domestic produce) during the winter of 2017. Provided further advice regarding norovirus testing in the UK to the CA (Winter 2017).
- iv. Contributed to a FSA-led stakeholder meeting, May 2018, that was instigated following the elevated results at Porton in July 2015. This contribution and follow up included provision of information and data to different stakeholder groups (Seafish, SAGB, EA, FSA, etc) on PT and the EQA scheme and how this is used to judge laboratory competence.
- v. Published an overview of *E. coli* testing methods in February 2018 based on work carried out by the NRL.
- vi. Significant assistance and technical advice and oversight was provided to the CA with regards to the UK-side of the EU norovirus baseline survey, which began in November 2016 and finishes in November 2018.

4.2 Dissemination to CA and OCLs of information provided by the EURL for monitoring bacteriological and viral contamination of bivalve molluscs.

Information disseminated by the EURL was provided to both the FSA and OCL via direct circulation of documentation, through the yearly network meeting or via the NRL website. Specific topics circulated are listed below:

- i. Report consisting of notes and resolutions from the 16th Workshop of NRLs for meeting provided at the NRL workshop meeting, October 2017.
- ii. Report of the EURL proficiency test distribution for norovirus and hepatitis A in bivalve molluscs.
- iii. Report on the whole animal PT distribution and EQA report.

- iv. Changes to EU legislation and how these pertain to NRLs and also OCLs (in particular the wider remit of EC No 625/2017).

4.3 Other advice

The NRL gave other advice to FSA in relation to the following:

- Current or upcoming amendments to ISO methods and the associated impact on shellfish-associated methods (provided with quarterly RAG reports).
- Appropriate testing methods for norovirus following outbreaks (Winter 2017).
- Human health risks associated with Vibrios for FSA-led “data hack” in October 2017.

5. Compliance assessment via audits and proficiency testing (PT)

5.1 Assessment of OCL performance in whole animal distributions

In November 2017, the NRL organised a PT distribution comprising of whole Common mussels (*Mytilus edulis*) and homogenised Pacific oysters (*Crassostrea gigas*) for enumeration of *E. coli* and the detection of *Salmonella* spp.. Whole matrix and shellfish homogenate samples were distributed to all 12 UK OCLs to test aspects of the methodology not covered by the standard shellfish EQA scheme i.e. opening of shellfish and preparation of initial dilutions. All samples were received within 24 hours of dispatch by the NRL and the arrival temperatures recorded by the OCLs showed that the internal temperature did not exceed recommended transport temperature of <10°C. Eleven laboratories analysed the samples on the day of arrival; one laboratory (243) analysed the samples the following day. Laboratory 532 did not examine either samples for *Salmonella* spp. as they do not undertake this test in their laboratory.

All laboratories returned replicate *E. coli* MPN/100g results falling between ± 3 SD of the participants' median for sample 1, with 11 laboratories receiving a maximum score of 12. Laboratory 9 had scores deducted as the tube combination selected was not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL. The eleven laboratories that returned results for *Salmonella* spp. all correctly reported the absence of *Salmonella* spp. in Sample 1 and received a score of 2.

For shellfish homogenates (Pacific oysters) 11 laboratories returned replicate *E. coli* MPN/100g results between ± 3 SD of the participants' median for Sample 2, with 10 received a maximum score of 12. Laboratory 67 reported both replicate results between ± 3 and ± 5 SD of the participants' median. Laboratory 9 had scores deducted as the tube combination selected was not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL. Twelve laboratories returned results for *Salmonella* spp. and reported the presence of *Salmonella* spp. in Sample 2 and received a score of 2.

Table 1. Summary statistics of OCLs results – *E. coli*

<i>E. coli</i>	Sample 1 - Mussels	Sample 2 - Homogenate
Participants reporting duplicate results for <i>E. coli</i> MPN	12	12
Participants reporting single MPN results only	0	0
Participants reporting MPN results within the expected range for both replicates ¹	12	11
Participants reporting MPN results outside the expected range for one replicate	0	0
Participants reporting MPN results outside the expected range for both replicates	0	1
Participants reporting MPN results as censored results for one or both replicates	0	0
Participants reporting tube combination and / or MPN results inconsistent with ISO 7218 ²	1	1

¹ expected range = participants' median \pm theoretical 3SD_T,

² points deducted from participants returning results inconsistent with ISO 7218

Table 2: OCLs results and allocated scores for sample 1 and 2

Lab ID	Sample 1 - Mussels					Sample 2 - Homogenate				
	<i>E. coli</i> MPN/100g			<i>Sal. spp.</i> in 25g		<i>E. coli</i> MPN/100g			<i>Sal. spp.</i> in 25g	
	Rep. 1	Rep. 2	Score	Result	Score	Rep. 1	Rep. 2	Score	Result	Score
7	780	450	12	Not detected	2	4900	13000	12	Present	2
9	1700	2400	8	Not detected	2	17000	17000	8	Present	2
67	1700	2200	12	Not detected	2	1400	1400	6	Present	2
97	1700	1300	12	Not detected	2	13000	35000	12	Present	2
145	3300	1400	12	Not detected	2	24000	17000	12	Present	2
166	2300	1700	12	Not detected	2	17000	17000	12	Present	2
243	330	490	12	Not detected	2	24000	3300	12	Present	2
271	490	310	12	Not detected	2	24000	7900	12	Present	2
532	780	780	12	NE	-	11000	11000	12	NE	-
578	1300	1700	12	Not detected	2	13000	13000	12	Present	2
1160	690	2300	12	Not detected	2	22000	35000	12	Present	2
1817	1400	780	12	Not detected	2	17000	7000	12	Present	2

NE – Not Examined

5.2 Assessment of OCL performance in external quality assessment (EQA)

The ongoing performance of UK OCLs was assessed for three distributions of the Cefas/PHE EQA shellfish scheme **between July 2016 and March 2017** (SF054, SF055 and SF056). Scores were allocated in accordance with the Cefas/PHE shellfish EQA scheme scoring system. Twelve OCLs analysed all 3 distributions during the reporting period for the enumeration of *E. coli* and the detection of *Salmonella* spp (see table 3 and 4, below). The performance of the participating OCLs is summarised in Tables 3 and 4. All OCLs achieved scores in excess of 70% over three distributions for the enumeration of *E. coli*, demonstrating satisfactory performance. All OCLs achieved 100% for the detection of *Salmonella* spp. Laboratory 14 informed the NRL of its closure in April 2017 and therefore it did not participate in the last distribution.

Table 3. Performance of UK OCLs in Cefas/PHE EQA distributions for *E. coli*

OCL ID	Distribution SF054		Distribution SF055		Distribution SF056		All distributions		
	SF0116	SF0117	SF0118	SF0119	SF0120	SF0121	Cumulative score	Max score	%
7	12	12	12	12	12	12	72	72	100
9	12	12	8	7	12	12	63	72	88
14	12	12	12	12	NE	NE	-	-	-
67	12	12	12	12	12	12	72	72	100
97	12	12	12	12	12	12	72	72	100
145	12	12	12	12	12	12	72	72	100
166	12	12	12	12	12	12	72	72	100
243	12	12	12	12	12	12	72	72	100
271	12	12	12	12	12	12	72	72	100
532	12	12	12	12	12	12	72	72	100
578	12	12	12	12	12	12	72	72	100
1160	12	12	12	12	12	12	72	72	100
1817	12	12	12	12	8	8	64	72	89

NE – Not examined.

Table 4. Performance of UK OCLs in Cefas/PHE EQA distributions for *Salmonella* spp.

OCL ID	Distribution SF054		Distribution SF055		Distribution SF056		All distributions		
	SF0116	SF0117	SF0118	SF0119	SF0120	SF0121	Cumulative score	Max score	%
7	2	2	2	2	2	2	12	12	100
9	2	2	2	2	2	2	12	12	100
14	2	2	2	2	NE	NE	-	-	-
67	2	2	2	2	2	2	12	12	100
97	2	2	2	2	2	2	12	12	100
145	2	2	2	2	2	2	12	12	100
166	2	2	2	2	2	2	12	12	100
243	2	2	2	2	2	2	12	12	100
271	2	2	2	2	2	2	12	12	100
532	NE	NE	NE	NE	NE	NE	-	-	-
578	2	2	2	2	2	2	12	12	100
1160	2	2	2	2	2	2	12	12	100
1817	2	2	2	2	2	2	12	12	100

NE – Not examined.

5.3 Participation in EURL/PHE EQA shellfish scheme for *E. coli* and *Salmonella* spp.

The NRL participated in the EURL/PHE EQA shellfish scheme comprising of LENTICULE discs for *E. coli* and *Salmonella* spp.. Six samples were analysed across three distributions which took place in June 2017 (SF0122, SF0123), October 2017 (SF0124, SF0125) and February 2018 (SF0126, SF0127). The results obtained by the UK NRL were assessed together with all other participants (<https://eurlcefass.org/media/14053/proficiency-testing-74-eqa-2017-eurl-pt-74-final-report.pdf>). The UK NRL achieved a rolling performance assessment of 100% for *E. coli* enumeration and *Salmonella* spp. detection.

5.4 Participation in the PHE EQA for pathogenic *Vibrio* spp. scheme.

The UK NRL participated in the PHE pathogenic *Vibrio* scheme. Six samples were analysed across three distributions in July 2017 (V0140, V0141), November 2017 (V0142, V0143) and February 2018 (V0144,

V0145). The NRL results for the detection of *V. parahaemolyticus* were satisfactory for all sample distributions. *V. cholerae* and *V. vulnificus* were incorrectly reported in 2 distributions respectively. Follow up investigations on this issue are ongoing.

5.5 Participation in EURL supplementary PT for E. coli and Salmonella spp.

The UK NRL participated in the EURL PT distribution (PT 73) for enumeration of *E. coli* and the detection of *Salmonella* spp. in shellfish matrices, comprising of 2 samples of Pacific oysters (*Crassostrea gigas*) in November 2017. The NRL achieved performance assessment of 100% for *E. coli* and *Salmonella* spp. for all samples.

5.6. Participation in EURL PT for norovirus and hepatitis A.

The UK NRL participated in one PT distribution organised by the EURL for norovirus and hepatitis A virus in September 2017 (PT 72). The distribution comprised of four separate shellfish matrices. The NRL received 100% scores for presence/absence data and quantification for all sample types.

5.7. Participation in PHE EQA for norovirus and hepatitis A.

The UK NRL participated in two PT distribution organised by the PHE in July 2017 and February 2018 Each distribution comprised of two laboratory-constructed (LENTICULE) samples. The NRL reported an unsatisfactory result for Norovirus Genogroup II. Follow-up action regarding this poor performance was undertaken by the NRL, including a review of the SOPs and root cause analysis of the erroneous test result and incorrect reporting of the sample result. Additional measures including a double check of SOP 1330 has been included to resolve this issue.

5.8 Meetings, workshops and task forces

The NRL director will be participating in the 17th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs to be held by the EURL in Southampton, May 2018. Minutes and an overview report detailing participation and major outcomes will be provided to the FSA and the laboratory network following the workshop.

The NRL has participated in TC and face-to-face meetings with the FSA, Seafish, EA and industry following the initiation of the shellfish working group.

Dr Craig Baker-Austin

Date...19/06/18.....

NRL Director

EURL for monitoring bacteriological and viral contamination of bivalve molluscs

WORK PROGRAMME of EURL for **MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS**

PERIOD: 2018

Version 1.2
(date 16/04/2018)

CONTACT DETAILS

EURL Director: Rachel Hartnell Rachel.hartnell@cefas.co.uk

EURL Coordinator: James Lowther james.lowther@cefas.co.uk

Centre for Environment, Fisheries and Aquaculture Science
Barrack Rd.,
Weymouth
DT4 8UB
Tel: +44 1305 206600

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SUMMARY

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ACTIVITIES

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EURL for monitoring bacteriological and viral contamination of bivalve molluscs

INTRODUCTION

The functions and duties of the European Union Reference Laboratories are specified in Article 32 of Regulation (EC) 882/2004, and from April 29th 2018, in Article 94 of Regulation (EU) 2017/625.

The responsibilities and tasks of the EURL for monitoring bacteriological and viral contamination of bivalve molluscs include both methodological and supervisory responsibilities for NRLs; relevant methods include those for both human pathogens and indicators of the sanitary quality of bivalve shellfish. In addition, the responsibilities and tasks of the EURL for this commodity include the application of those methods in bivalve shellfish production areas within the scope of classification and monitoring.

The current work programme detailed below is to cover the dates, 1st January 2018 – 31st December 2018. This work programme 2018 covers Transfer Activities (as defined below) which will ensure the transfer of knowledge, materials (including reference materials) and data obtained and produced by the laboratory in the framework of its function and duties as EURL towards the laboratories appointed to fulfil the duties of the EURL for monitoring bacteriological and viral contamination of bivalve molluscs as from 1 January 2019 in order to enable the newly appointed laboratories to fulfil their duties as EURLs as from that date.

Transfer Activities includes tasks to ensure the transfer of the activity to the new laboratories appointed as EURLs to fulfil the duties of the EURL for monitoring bacteriological and viral contamination of bivalve molluscs which shall be limited to the following specific tasks:

- a. Exchange of testing knowledge and key laboratory reagents to allow the new laboratories to undertake confirmatory testing from January 1st 2019;
- b. Transfer of protocols for the qualitative and quantitative determination of statutory and non-statutory determinants in bivalve molluscs, and best practice guidance for technical assessment of bivalve mollusc production areas.

([(a) and (b)] together, being, the “Transfer Activities”)

If further tasks, other than the Transfer Activities, are required to ensure the transfer of the activity to the new laboratories appointed to fulfil the duties of the EURL for monitoring bacteriological and viral contamination of bivalve molluscs as from 1st January 2019 then the scope and pricing of such further tasks shall be subject to a separate agreement with the Commission and will not form part of this work programme 2018.

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Regulation (EU) 625/2017 Art 94(2):

European Union reference laboratories designated in accordance with Article 93(1) shall be responsible for the following tasks insofar as they are included in the reference laboratories' annual or multiannual work programmes that have been established in conformity with the objectives and priorities of the relevant work programmes adopted by the Commission in accordance with Article 36 of Regulation (EU) No 652/2014:

(taking into account Art 147 of (EU) 625/2017)

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TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- **Art. 94.2.a** ***Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.***
- **Art. 94.2.b** ***Providing reference materials to national reference laboratories***
- **Art. 94.2.c** ***Coordinating the application by the national reference laboratories and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests.***
- **Art. 94.2.l** ***Where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.***

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Sub-activity 1.1 *Documentation of methods*

Objectives: Provision of high quality methods and associated guidance to NRLs and other relevant laboratories

Description: To develop, maintain and update standard operating procedures for the detection and/or quantification of statutory (*E. coli*, *Salmonella* spp.) and non-statutory determinands (*Vibrio* spp., norovirus, hepatitis A virus), in bivalve molluscs, in addition to guidance notes on method uncertainty, generation of method characteristics and method validation etc. as required.

To supply technical advice on bacteriological and viral methods to NRLs, Official Control testing laboratories, and third country laboratories.

To provide assistance and advice on implementation of methods, accreditation to IEC ISO17025 and quality control requirements

Expected Output: Current method documents on the EURL website will be maintained and updated, while additional documents will be developed and published as appropriate.

Duration: According to the annual cycle

Sub-activity 1.2 *Distribution of reference and other materials*

Objectives: Provision of reference materials of relevance to NRLs and other relevant laboratories

Description: To distribute reference strains, reference materials and control materials for statutory (*E. coli*, *Salmonella* spp.) and non-statutory determinands (*Vibrio* spp., norovirus, hepatitis A virus), on request of NRLs, third country laboratories etc.

Expected Output: n/a

Duration: Throughout 2018 as required

Sub-activity 1.3 *Organisation of proficiency testing*

Objectives: Provision of proficiency testing schemes for statutory and non-statutory determinands

Description: To organise comparative testing for EU MS NRLs, EFTA country NRLs (Norway, Iceland), for statutory determinands (*E. coli* and *Salmonella* spp.) in bivalve molluscs. To include organisation of one whole-animal distribution during 2018, and co-ordination of NRL (and relevant EURL) participation in one or more of the three non-matrix External Quality Assessment (EQA) schemes organised in collaboration with Public Health England (PHE) per calendar year (to include one participation funded by the EURL). For both EURL- and EURL/PHE-organised schemes, the EURL will analyse results and produce a report, plus additional advice, follow-up and recommendations. To improve quality assurance in test results for EU controls comparative tests organised by the EURL will be available to laboratories outside of the NRLs network on a cost recovery basis

To organise comparative testing for EU MS NRLs, EFTA country NRLs (Norway, Iceland), and non-NRL laboratories designated to undertake analysis in the EFSA baseline survey, for norovirus and hepatitis A virus in bivalve molluscs. To include organisation of one whole-animal distribution during 2018, and cooperation with PHE to deliver two non-matrix based schemes proficiency testing schemes for norovirus and hepatitis A virus during 2018 (to include one participation funded by the EURL). For both EURL- and EURL/PHE-organised schemes, the EURL will analyse results and produce a report plus

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additional advice, follow-up and recommendations. To improve quality assurance in test results for EU controls comparative tests organised by the EURL will be available to laboratories outside of the NRLs network on a cost recovery basis.

Expected Output: Reports on each matrix PT and each series of EURL/PHE-organised schemes

Duration: According to the annual cycle

Sub-activity 1.4 Development and standardisation of methods

Objectives: Improving methods and associated laboratory practise for statutory and non-statutory determinands in both regulatory and investigative contexts

Description: To act as convener for CEN/TC275/WG6/TAG4 'Viruses in food' to assist in the development of EN methods for the determination of viruses in foodstuffs. In 2018 including, but not restricted, to providing responses to official technical and editorial comments from voting members at ISO SC9 level for the publication of EN/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.

To lead and co-ordinate the activities of CEN/TC275/WG6/TAG15 'Vibrios' in the elaboration of methods for the quantification of pathogenic *V. parahaemolyticus* in bivalve shellfish

To act as sub-project leader for the revision of the ISO 7218 General rules for microbiology, covering revision of sections on MPN tables (with relevance to the EU reference method for *E. coli*) and molecular detection methods (viruses and vibrios)

To contribute to the project for revision of ISO 22117 on organisation of proficiency testing

To represent the EURL at the annual plenary meeting of the CEN WG6 and ISO SC9 microbiology working groups

To develop methods for inclusion in the ISO 21872 series (detection of vibrios), particularly to enable enumeration of total/pathogenic *V. parahaemolyticus* in bivalve shellfish

To continue to work with the NRLs network to identify means to improve the harmonisation of quantification between laboratories using ISO 15216-1, the standard method for detection of viruses in foods

To develop methods to improve determination of the potential infectivity of noroviruses detected in bivalve shellfish

To develop alternative (more rapid) testing methods for statutory and non-statutory determinands.

To investigate the application of testing methods to remote sensing platforms.

To develop methods for hepatitis E virus and/or other emerging viruses in shellfish

Expected Output: EN ISO standards and methodology publications

Duration: Throughout 2018

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TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- **Art. 94.2.d *Coordinating practical arrangements necessary to apply new methods of laboratory analysis, testing or diagnosis, and informing national reference laboratories of advances in this field.***
-
- **Art. 94.2.e *Conducting training courses for staff from national reference laboratories and, if needed, from other official laboratories, as well as of experts from third countries.***
-
- **Art. 94.2.g *Providing information on relevant national, Union and international research activities to national reference laboratories.***

Sub-activity 2.1 *Application of new methods*

Objectives: Facilitating the application of new methods

Description: To maintain EURL expertise in new methodologies through attendance at relevant international conferences, keeping up-to-date with the scientific literature, etc.

To provide guidance and review of procedures/data to laboratories wishing to undertake studies to validate alternative methods according to ISO 16140. To include formal assessment of validation data as required to support official approval (at SCFCAH) for alternative methods.

Expected Output: Dissemination of information on relevant new methodologies through the network in the form of informal communications, briefing notes, generic SOPs and presentations at the annual meeting of NRLs.

Duration: Throughout 2018

Sub-activity 2.2 *Training*

Objectives: Improving the harmonisation of method application across the NRLs network, and in third countries

Description: To provide specialist training to NRLs, third country laboratories and others in relation to official control analyses (E. coli, Salmonella spp.) and non-statutory analyses (Vibrio spp., norovirus, hepatitis A virus) and other aspects of bivalve shellfish hygiene as required.

Expected Output: Establishing competency in the relevant determinands

Duration: Throughout 2018

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Sub-activity 2.3 *Informing NRLs*

Objectives: To provide relevant information on research activities to the NRLS network (and others)

Description: To organise, host, and participate in the annual EURL workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs, and produce resolutions and other workshop outputs. In 2018, the workshop will be held in the United Kingdom, (16th-18th May 2018). Invitations to be extended to NRLS from EFTA countries (Norway, Iceland), the EURLs for E.coli, Salmonella, viruses in foods and marine biotoxins, and additional international experts where their participation is of benefit to the network. For those countries with split NRLs in this field (one each for Bacteriology and Virology), an additional invitee to allow for adequate coverage will be allowed.

Further to the above, to undertake EURL activities and commitments agreed in resolutions at the annual workshop.

To maintain a website for the EURL including all relevant information for NRLs and other stakeholders.

Expected Output: Meeting resolutions, actions list and meeting report. Up-to-date website maintained.

Duration: Meeting to be held over 3 days in May 2018. Website to be maintained as required throughout 2018.

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TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- **Art. 94.2.f** *Providing scientific and technical assistance to the Commission within the scope of their mission.*
- **Art. 94.2.h** *Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC).*
- **Art. 94.2.i** *Assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens.*

Sub-activity 3.1 Assistance to the commission

Objectives: To provide support to DG SANTE and other parts of the Commission in matters related to microbiological contamination of bivalve molluscs.

Description: To attend the meetings of the EU Member States restricted working group on bivalve molluscs.

To provide expert scientific and technical advice with regard to criteria for noroviruses and hepatitis A virus in live bivalve mollusc (LBM) production areas and/or products placed on the market.

To provide scientific and technical advice to DG SANTE in support of trade of LBM between EU and US, in particular with respect to any additional technical considerations or activities related to *Vibrio parahaemolyticus*.

To assist DG SANTE Directorate F with audits of MS and third countries as requested and as other EURL priorities allow (mission costs funded by Directorate F). To provide specialist technical advice and support to Directorate F in support of their remit as requested.

To participate in the EURL Director's co-ordination meeting and other EURL co-ordination meetings/workshops as appropriate.

To provide additional ad hoc assistance as required.

Expected Output: Scientific and technical advice to support EU regulations

Duration: Throughout 2018

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Sub-activity 3.2 *Guidance documents*

Objectives: To develop and maintain guidance for the use of the Commission, the Member State Competent Authorities and other stakeholders in classification and monitoring of bivalve shellfish production areas

Description: To lead the expert WG on microbiological best practice for monitoring of bivalve shellfish, to develop additional guidance on seasonal classification and classification of ponds, cleres etc. and to consider the elaboration of supporting guidance for monitoring of norovirus following the completion of the baseline survey.

To provide technical advice and any necessary revision to the **Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with regard to Regulation 854/2004**, with regard to mitigation of faecal contamination, virus (or other microbiological) contamination or outbreaks.

To provide technical advice and any necessary revision to the **Microbiological Monitoring of Bivalve Mollusc Harvesting Areas Guide to Good Practice: Technical Application**, with regard to mitigation of faecal contamination, virus (or other microbiological) contamination or outbreaks.

To develop a guidance document for competent authorities and industry stakeholders on assessment of virus testing laboratories.

Expected Output: Updated versions of the Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with regard to Regulation 854/2004 and the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas Guide to Good Practice: Technical Application to be published on the EURL website.

Guidance on assessment of virus testing laboratories to be launched on the EURL website.

Duration: Throughout 2018

Sub-activity 3.3 *Cooperation with international agencies*

Objectives: To work with other international agencies to ensure the best integration of relevant expertise in matters related to microbiological contamination of bivalve molluscs and related fields.

Description: To cooperate with EFSA in the analysis of data generated during the baseline survey for norovirus in oysters, and to provide further assistance through provision of control reagents to all designated laboratories.

To support the FAO in the development of the best practice guidance for the application of bivalve molluscan shellfish sanitation programmes.

In collaboration with ECDC, to develop a proposal to extend the existing epidemiological surveillance systems for *Vibrio cholerae* to include *Vibrio parahaemolyticus*.

To participate in additional relevant EU and International scientific committees as required (EFSA, WHO/FAO, ISSC etc).

To collaborate with colleagues at the EURL for marine biotoxins to disseminate information on the linkages between tetrodotoxins and *Vibrio* spp.

Expected Output: Contribution to interim reports on the EFSA baseline survey, and to the FAO guidance. Joint proposal with ECDC on *Vibrio* surveillance.

Duration: Throughout 2018

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Sub-activity 3.4 *Diagnosis of outbreaks*

Objectives: To support the Commission and the Member State Competent Authorities in the analysis of outbreaks related to microbiological contamination of bivalve molluscs.

Description: To maintain EURL laboratory competence and expertise in analytical methods for monitoring virological contaminants of bivalve molluscs (norovirus and hepatitis A virus). To include maintenance of requirements for ISO IEC 17025 accreditation for quantitative determination of norovirus in LBM, and extension of scope to include quantitative determination of HAV

To maintain EURL laboratory competence and expertise in analytical methods for monitoring bacteriological contaminants of bivalve molluscs (*E. coli*, *Salmonella* spp., marine vibrios) using reference methods. To include maintenance of ISO IEC 17025 accreditation of enumeration of *E. coli*, and the detection of *Salmonella* spp. and *Vibrio parahaemolyticus* and extension of accreditation to include PCR confirmation of *Vibrio parahaemolyticus* and detection of *Vibrio vulnificus* and *V. cholerae*. To perform the above-named tests on outbreak material or on occasion of disputed test results (on request).

Expected Output: Maintenance of methodology competence at the EURL to ISO17025. Provision of test sample reports on any outbreak samples tested.

Duration: Maintenance of accreditation according to the annual cycle. Investigation of outbreaks as required throughout 2018.

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REAGENTS AND REFERENCE COLLECTIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- **Art. 94.2.j** ***Coordinating or performing tests for the verification of the quality of reagents and lots of reagents used for the diagnosis of foodborne, zoonotic or animal diseases and pests of plants.***

- **Art. 94.2.k** ***Where relevant for their area of competence, establishing and maintaining:***
 - i. reference collections of pests of plants and/or reference strains of pathogenic agents;***
 - ii. reference collections of materials intended to come into contact with food used to calibrate analytical equipment and provide samples thereof to national reference laboratories;***
 - iii. up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents.***

Sub-activity 4.1 *Verification of reagent quality*

Objectives: To ensure the fitness-for-purpose of reagents used for the diagnosis of microbiological contamination of bivalve molluscs.

Description: To undertake quality assurance testing and where necessary fitness-for-purpose assessment of reagents as required (including those supplied ready-to-use from manufacturers) according to the EURL quality assurance procedures, to ensure the quality of the analysis carried out by the EURL, and to provide NRLs and other relevant labs with appropriate guidance on the use of reagents.

Expected Output: Up-to-date information on the suitability of reagents to NRLs and other relevant laboratories.

Duration: As required throughout 2018.

Sub-activity 4.2 *Reference collections and lists*

Objectives: To maintain reference collections of microbes, and lists of reference materials and reagents

Description: To maintain a strain bank of microbiological determinands (e.g. strains of *Vibrio* spp., *Salmonella*, *E.coli*) relevant to LBM, to provide NRLs and other relevant labs with control organisms in

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order to implement, verify and challenge microbiological methods. In addition, to provide selected strains for performance assessment of bacteriological media.

To produce and maintain stocks of reference materials (plasmids, EC RNA, process control material etc.) used in the detection and quantification of viruses.

To establish a list of available reference substances and reagents, and relevant manufacturers.

Expected Output: Provision of strains, reference materials etc. to NRLS and others to facilitate standardisation between laboratories and harmonisation of methodology. Provision of a list of reference materials on the EURL website.

Duration: Throughout 2018

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REQUIREMENTS RELATED TO OTHER LEGISLATION

Please specify applicable legislation:
(Number of Sub-activity boxes can be adjusted)

Sub-activity 5.1 *Advice on application of legislation in force*

Objectives: Provision of up-to-date information on regulations applying to LBM

Description: To provide support and technical assistance to DG SANTE and Member State Competent Authorities and 3rd countries on Commission Regulations (EC) 852/2004, 853/2004, 854/2004 and 2073/2005 and subsequent implementing or amending regulations where these apply to live bivalve molluscs to include as a minimum, maintenance of an up-to-date list of relevant regulation on the EURL website.

Expected Output: Dissemination of information on relevant regulations. Maintenance of the list of regulation on the website.

Duration: As required throughout 2018.

REMARKS

For 2018, our work programme and budgets assumes that provision of Commission-financed EURL services, will be extended beyond the network of EU MS NRLs (24 labs including split Virology and Bacteriology labs in Italy), to three EFTA country NRLs (Iceland and split Virology and Bacteriology labs in Norway). In addition, where appropriate these services will be extended to non-NRLs designated for analysis of samples in the EFTA baseline survey for norovirus in oysters (one lab each in Denmark and Spain).

To support method development and standardisation activities, it is proposed that a studentship is supported – this will be included as sub-contracting costs in the budget.