

Cefas contract report C7417

FSA Reference: FS616026

Summary technical report for the UK National Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs – April 18 to March 19

May 2019



**Summary Technical Report for the UK National
Reference Laboratory for Monitoring Bacteriological
and Viral Contamination of Bivalve Molluscs**

April 18 to March 19

Final report v1

17 pages

Not to be quoted without prior reference to the authors

Authors: Craig Baker-Austin, Cefas Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB

Cefas Document Control

Report submitted to:	FSA
Date submitted:	16/01/2020
Project reference:	C7417
Project manager:	Myriam Algoet
Report compiled by:	C. Baker-Austin & L. Stockley
Quality controlled by:	M. Price-Hayward, 30/04/19
Approved by and date:	Myriam Algoet, 16/01/2020
Version:	Final V1
Classification:	Not restricted
Review date:	N/A

Version Control History

Author	Date	Comment	Version
C. Baker-Austin	29/04/19		Draft V1 for internal review
L. Stockley	30/04/19		Draft V2 updated with PT information
C. Baker-Austin	01/05/19		Draft V3 for internal approval
M. Algoet	01/05/19		Draft report (Draft V4) for customer review
M. Algoet	16/01/2020		Final report issued

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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 Government to Government agreement between Cefas and the Food Standards Agency (FSA). This report summarises the activities carried out by the NRL for the financial year 2018-19 according to the requirements of Regulation (EC) No. 882/2004 and Regulation 625/2017 as defined in the Service Level Agreement between the FSA 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 tenth meeting of OCLs undertaking microbiological testing of bivalve shellfish was held at the Cefas laboratory on the 2nd and 3rd of October 2018. Seventeen delegates attended the two-day event. The first day (pm) consisted of a practical laboratory-based session outlining the use of root-cause analysis for interpreting and investigating laboratory incidents. A wide variety of topics were covered on the second day. These included OCL performance in proficiency testing (PT), risks associated with hepatitis E in shellfish, new methods for *E. coli* detection in shellfish matrices, progress at ISO on revisions to the *E. coli* and Salmonella standards, a discussion on recent changes to EU regulations pertaining to OCL testing and the progress of the UK contribution to the EU-wide survey of norovirus in oysters. There was also some discussion on the future of the live bivalve network following Brexit, and the upcoming role of Cefas as an FAO (Food and Agriculture Organisation of the United Nations) reference centre in seafood safety.

3. Advice and representation within the UK and EU

3.1. Provision of advice to the OCL Network

The NRL provided advice throughout the year to OCLs (summarised in the quarterly RAG reports sent to FSA). Briefly, this has included advice to the NI Public Health Laboratory OCL regarding interpretation of *E. coli* results from various testing laboratories (Spring 2019). Further practical advice included changes to EU legislation (e.g. 625/2017), loop sizes for microbiological testing, *E. coli* and norovirus guidance for end product testing (EPT), updates on method uncertainty and assessing method uncertainty to different OCLs (Winter 2018). Further advice was given to various OCLs on implementation of ISO 17025 pertaining to reporting results of shellfish testing (Guernsey OCL), as well as provision of feedback and advice regarding EPT to Porton OCL during Autumn 2018. Advice to OCLs on methods, in particular the MPN method (and use of calculator) was provided throughout the year. Some miscellaneous advice was also provided at the OCL meeting on 2nd and 3rd October 2018 on practical aspects of live bivalve mollusc (LBS) testing.

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 better means of traceability through the microbial testing process.

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, including *E. coli* and norovirus testing.
- ii. Changes to EU legislation (e.g. 625/2017), and associated impacts on OCL testing.
- iii. Risks associated with vibrios in seafood produce in the UK.

- iv. Updates on method uncertainty and assessing method uncertainty for microbiological testing.
- v. Traceability of samples through an OCL testing procedure.

3.2. Representation within the UK/EU

The NRL provided specific advice at the EURL workshop (Southampton, May 2018) on a variety of issues, in particular *Vibrio* method standardisation, likely EU-wide *Vibrio* controls and use of whole genome sequencing to investigate *Vibrio* outbreaks. The NRL also participated in Member State-level discussions at the EURL meeting regarding shellfish-associated outbreaks and closure/reopening criteria used in the UK. The NRL participated in the BSI Food Microbiology Committee during 2018-2019 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 to the FSA. The NRL also circulated key information on standards to the laboratory network and the CA, 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 2018-2019, including the following:

- i. Maintenance and updating of various links on the NRL website carried out.
- ii. New *E. coli* testing protocol added May 2018.
- iii. New contact details and map added to Cefas NRL website to reflect changes to laboratory network (see <https://www.cefas.co.uk/nrl/laboratory-network/>).
- iv. The current NRL website is being completely redesigned to provide an interface with the viruses in food NRL and other NRLs since the disbandment of the LBS EURL (Spring 2019).

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.* Catch up meetings were held on 6th June 2018 at Cefas and at FSA (Clive House, London) on 22nd November 2018.
- ii.* Provided various advice to FSA on variability of the E. coli testing methods currently used for shellfish and submitted advice to FSA regarding anomalous/unusual results obtained from an offshore producer (January 2019).
- iii.* Contributed regular updates to FSA and FSS on progress of EU-wide baseline survey (various).
- iv.* Provided various advice regarding norovirus testing in the UK to the CA.
- v.* Contributed to an 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.
- vi.* Provided FSA with documentation regarding norovirus and E. coli testing (EPT testing documents) on 27th November 2018.
- vii.* Provision of opinion to FSA on EU Exit - APH EURLs Project Board Actions: Risks and workshops in July 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 17th Workshop of NRLs for meeting provided at the NRL workshop meeting, October 2018.

- 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. Updates on method uncertainty and assessing method uncertainty for microbiological testing.
- v. Changes to EU legislation and how these pertain to NRLs and also OCLs (in particular the wider remit of EC No 625/2017, discussed at OCL meeting in Oct 2018).

4.3 Other advice

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

- i. Current or upcoming amendments to ISO methods and the associated impact on shellfish-associated methods (generally provided with quarterly RAG reports).
- ii. Specific provision of information to FSA on virological testing in commercial labs and provision of guidance on this to FSA on 7th June 2018.
- iii. Participation in industry and FSA-led groups e.g. shellfish stakeholder group meeting during summer of 2018. Provided FSA presentation of EU baseline survey work to Jill Wilson on 19th July 2018.

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

5.1 Assessment of OCL performance in whole animal distributions

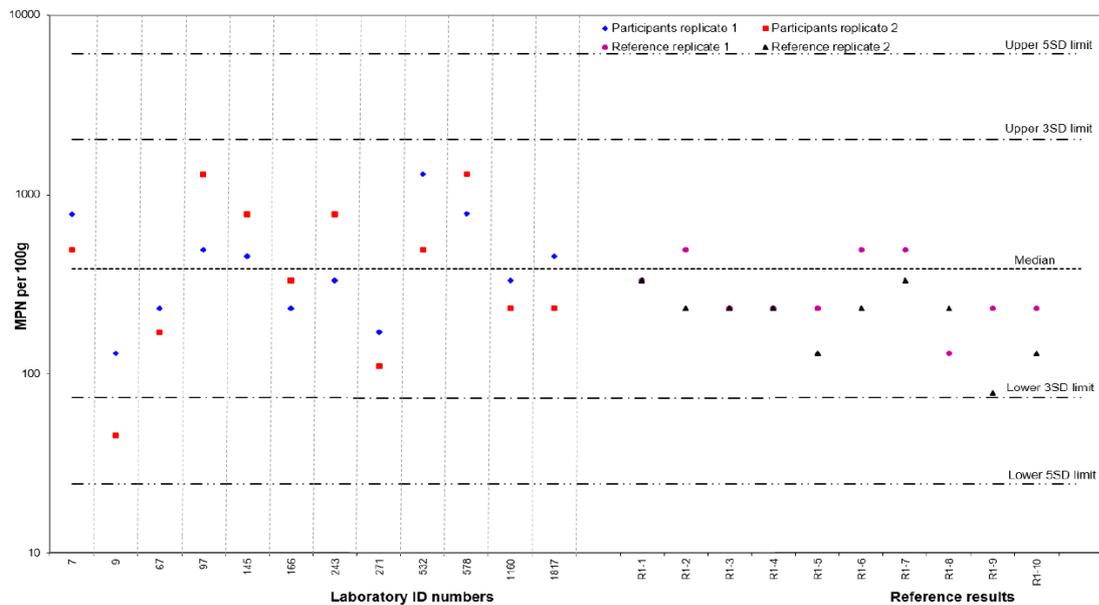
In November 2018, the NRL organised a PT distribution to the OCL network comprising of whole Pacific oysters (*Crassostrea gigas*), encompassing three separate samples (PT 76). Briefly, Sample 1: A single batch of 2000 Pacific oysters was collected from a UK commercial harvesting area on the 26th November 2018. Prior to packing, the shellfish were placed in a large disinfected container and thoroughly mixed. Sample 1 comprised of approximately 24 randomly selected oysters from this bulk material. Samples 2 and 3 were prepared from a single batch of approximately 700 Pacific oysters collected from a UK commercial harvesting area on the 21st

November 2018. On arrival, the oysters were shucked and homogenised before being pooled together to form one homogenate. The pooled homogenate was aliquoted in 100 ml volumes on the 24th November and stored at 3 ± 2 °C. Prior to distribution, the homogenate aliquots were labelled as either Sample 2 or Sample 3. Sample 2 was spiked with *E. coli* (2.2×10^4 cfu/sample) and *Salmonella* spp. (*S. Nottingham* - 1.5×10^2 cfu/sample); Sample 3 was spiked with *E. coli* (9.6×10^3 cfu/sample) and *Salmonella* spp. (*S. Nottingham* - 2.9×10^2 cfu/sample).

Whole matrix and shellfish homogenate samples were distributed to all 12 UK OCLs to test aspects of the methodology not covered by the Cefas/PHE EQA shellfish scheme i.e. opening of shellfish and preparation of initial dilutions. All samples were received within 24 hours of dispatch by the NRL with the exception of Laboratory 578, which was unable to analyse samples 2 and 3 as they had been removed at airport security. Information provided by laboratories on the samples' arrival temperature showed the maximum temperature recorded by participants did not exceed the recommended transport temperature of <10 °C set out in the NRL generic protocol. Eleven laboratories analysed the samples on the day of arrival, laboratory 1160 analysed the samples the following day. Laboratory 532 did not examine the sample material for *Salmonella* spp. as they do not undertake this test in their laboratory.

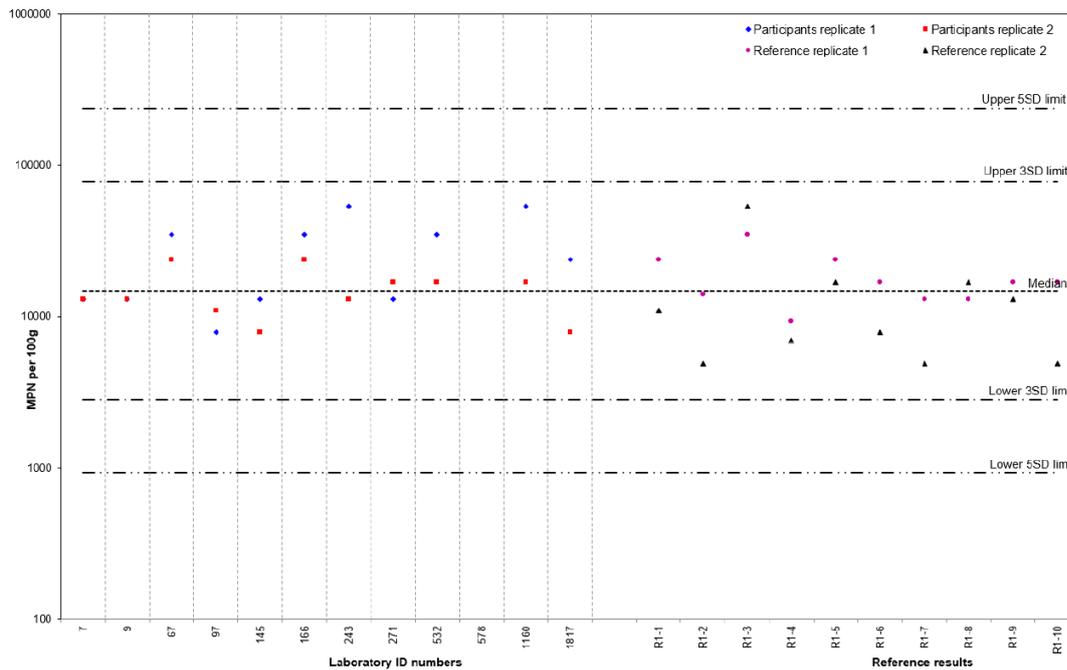
For sample 1, (*E. coli*) – Eleven laboratories returned replicate *E. coli* MPN/100g results falling between ± 3 SD of the participants' median (Figure 1) with all 11 laboratories receiving a maximum score of 12. Laboratory 9 had scores deducted as 1 replicate result reported fell between ± 3 and ± 5 SD of the participants' median and both tube combinations selected were not consistent with the rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL, receiving a score of 5. For *Salmonella* spp. eleven laboratories returned results for *Salmonella* spp. with all correctly reporting the absence of *Salmonella* spp. and received a score of 2.

Figure 1. Sample 1 – Pacific oysters - Participants' and NRL reference *E. coli* MPN results plotted against the participants' median



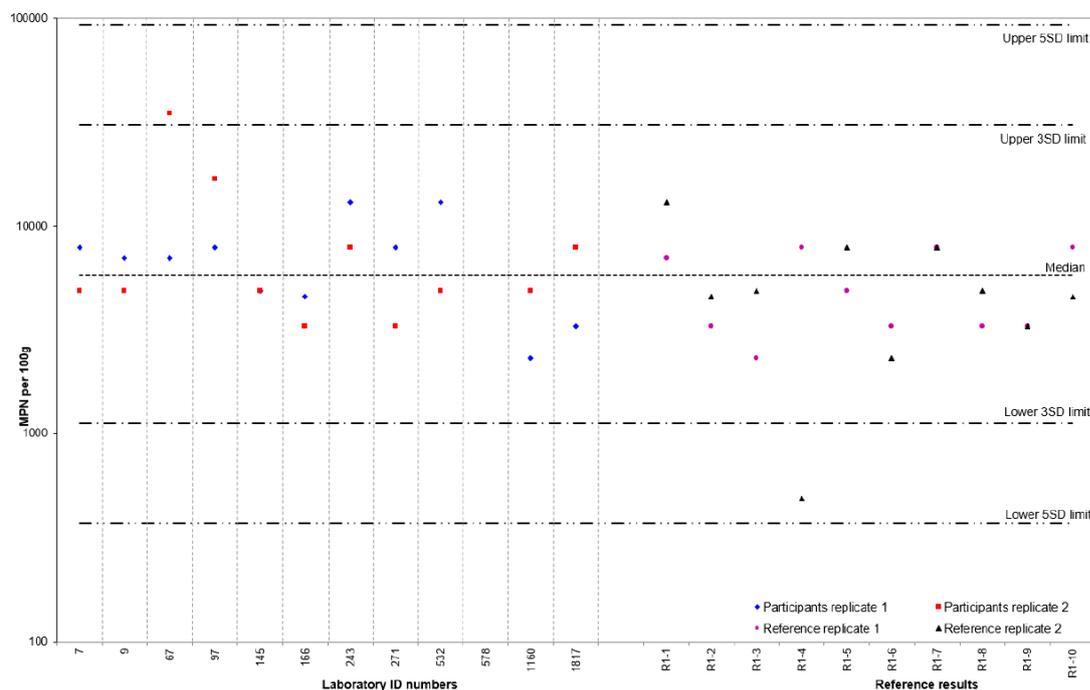
For sample 2, (*E. coli*) - Eleven laboratories returned replicate *E. coli* MPN/100g results between ± 3 SD of the participants' median (Figure 2), with 10 received a maximum score of 12. Laboratory 9 had scores deducted as the tube combination selected was not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL, receiving a score of 8. For *Salmonella* spp. – Ten laboratories returned results for *Salmonella* spp. and reported the presence of *Salmonella* spp. and received a score of 2.

Figure 2. Sample 2 – Pacific oysters - Participants' and NRL reference *E. coli* MPN results plotted against the participants' median



For sample 3, (*E. coli*) - Ten laboratories returned replicate *E. coli* MPN/100g results between ± 3 SD of the participants' median (Figure 3), with 9 received a maximum score of 12. Laboratory 67 had scores deducted as 1 replicate result reported fell between ± 3 and ± 5 SD of the participants' median and 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, receiving a score of 8. For *Salmonella* spp. – Ten laboratories returned results for *Salmonella* spp. and reported the presence of *Salmonella* spp. and received a score of 2.

Figure 3. Sample 3 – Pacific oysters - Participants' and NRL reference *E. coli* MPN results plotted against the participants' median



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 comprising of six lenticule discs for *E. coli* and *Salmonella* spp. between June 2018 and February 2019 (SF060, SF061 and SF062). Scores were allocated in accordance with the Cefas/PHE shellfish EQA scheme scoring system. Twelve OCLs analysed 2 or more distributions during the reporting period for the enumeration of *E. coli* and the detection of *Salmonella* spp. as agreed at the NRL laboratory network meeting in 2018. OCL performances are summarised in Tables 1 and 2. All OCLs achieved scores in excess of 70% over 2 or more distributions for the enumeration of *E. coli* and the detection of *Salmonella* spp., the measure of performance considered by the scheme organisers to demonstrate satisfactory performance.

Table 1. Performance of UK OCLs in Cefas/PHE EQA distributions for *E. coli*

OCL ID	Distribution SF060		Distribution SF061		Distribution SF062		All distributions		
	SF0128	SF0129	SF0130	SF0131	SF0132	SF0133	Cumulative score	Max score	%
7	12	12	12	12	12	2	62	72	86
9	12	12	12	12	12	12	72	72	100
67	DNR	DNR	12	12	12	12	48	48	100
97	12	12	9	12	DNR	DNR	45	48	94
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	DNR	DNR	12	12	12	12	48	48	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	7	12	67	72	93
1817	12	12	12	12	12	12	72	72	100

DNR – Did not register for this distribution.

Table 2. Performance of UK OCLs in Cefas/PHE EQA distributions for *Salmonella* spp.

OCL ID	Distribution SF060		Distribution SF061		Distribution SF062		All distributions		
	SF0128	SF0129	SF0130	SF0125	SF0128	SF0129	Cumulative score	Max score	%
7	2	2	2	2	2	2	12	12	100
9	2	2	2	2	2	2	12	12	100
67	DNR	DNR	2	2	2	2	8	8	100
97	2	2	2	2	DNR	DNR	8	8	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	DNR	DNR	2	2	2	2	8	8	100
532 ^a	2	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

^a Laboratory does not undertake *Salmonella* testing of OC samples. DNR – Did not register for this distribution.

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

The UK NRL participated in 3 distributions of the EURL/PHE EQA shellfish scheme which took place in February (SF0126, SF0127), June (SF0128, SF0129) and October 2018 (SF0130, SF0131). A cumulative performance assessment was undertaken using results obtained from the EURL matrix distribution (PT 76) and 1 EQA

distribution (SF059). The results obtained by the UK NRL were assessed together with all other participants (<https://eur.cef.eu/media/14118/proficiency-testing-78-eqa-final-report-v1.pdf>). The UK NRL achieved a cumulative performance assessment of 100% for *E. coli* enumeration and *Salmonella* spp. detection. Scores for SF060 and SF061 were also included and assigned cumulative scores for information only.

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

The UK NRL participated in the PHE pathogenic *Vibrio* scheme. Six samples were analysed across three distributions in June 2018 (V0146, V0147), October 2018 (V0148, V0149) and February 2019 (V0150, V0145). The UK NRL results for the detection of *V. parahaemolyticus* were satisfactory for 2 distributions. *V. vulnificus* were incorrectly reported for 1 sample received. Due to contamination issues, results were not reported for the October 2018 distribution. An investigation into the origin of the contamination concluded that high levels of *Serratia marcescens* was used prior to the testing of the EQA material. New measures are being introduced to limit further contamination episodes occurring again.

5.5 NRL participation in EURL supplementary PT for *E. coli* and *Salmonella* spp.

The UK NRL participated in the EURL PT distribution (PT 76) for enumeration of *E. coli* and the detection of *Salmonella* spp. in shellfish matrices in November 2018, comprising of 3 samples (1 x whole animal sample of Pacific oysters (*Crassostrea gigas*) and 2 x homogenised shellfish samples). The NRL achieved performance assessment of 100% for *E. coli* and *Salmonella* spp. for all samples.

5.6 NRL 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 during June 2018 (PT 75). The distribution comprised of four separate shellfish matrices (1 whole animal matrix and 3 x shellfish blends). The UK NRL received 100% scores for presence/absence data and quantification for all sample types.

5.7 NRL participation in PHE EQA scheme for norovirus and hepatitis A.

The UK NRL participated in two PT distributions organised by the Cefas/PHE in October 2018 (NHV004) and February 2019 (NHV005). Each distribution comprised of two Lenticule disc samples. The UK NRL reported satisfactory results for Norovirus Genogroup I and II and hepatitis A virus.

5.8 Meetings, workshops and task forces

The NRL director participated in the 17th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held by the EURL in Southampton, May 2018. Minutes and an overview report detailing participation and major outcomes were 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.

6. Planned activities for 2019-2020

The following activities are planned for the forthcoming year:

- Provision and publication of industry friendly guidance documents for norovirus and *E. coli* testing.
- Further evaluation of sample provenance and traceability for OCL testing across laboratory testing network, and current best practice.
- Hosting annual OCL network meeting and associated practical session (October 2019).
- Provision of advice and responding to enquiries as required e.g. from the competent authority, OCLs etc.
- Participation in EURL proficiency testing (*E. coli* EURL, Italy) for whole animal testing purposes, July 2019.
- Participation, where relevant, in activities outlined by the current EURLs for *E. coli*, Salmonella, and shellfish classification.
- Provide whole animal *E. coli* and Salmonella spp proficiency testing round for UK OCLs, November 2019.

- Participation in UK microbiological method standardisation activities through the British Standards Institution (group AW9).
- A review of peer reviewed literature published during 2019 regarding *E. coli*, *vibrio*, Salmonella testing and shellfish classification.
- Update and overhaul of UK NRL website for bacteriological contamination of shellfish.