



SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR FOODBORNE VIRUSES AND BACTERIOLOGICAL CONTAMINATION OF BIVALVE MOLLUSCS AND SHELLFISH CLASSIFICATION: APRIL 2020 - MARCH 2021

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Date: April 2021



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Cefas Document Control

Submitted to:	FSA Valerie Mcfarlane
Date submitted:	28/04/21
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Version:	Final V1
Recommended citation for this report:	NRL technical report. (2021). Report title. Cefas NRL Project Report for FSA (C7871), 19 pp.

Version control history

Version	Author	Date	Comment	
V1	Craig Baker-Austin, James Lowther and Louise Stockley	26/04/21	Draft report collated	
V2	Craig Baker-Austin	26/04/21	Sent to MPH for review	
Final V1	Final V1 Sharron Ganther		Final to FSA	

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1. Introduction

During the reporting period the Centre for Environment, Fisheries and Aquaculture Science (Cefas) Weymouth was designated as the UK National Reference Laboratory (NRL) for foodborne viruses and bacteriological contamination of bivalve shellfish. Previously the laboratory was designated as UK NRL for monitoring bacteriological and viral contamination of bivalve molluscs; this contract was renewed in 2017 as a Government-to-Government agreement between Cefas and the Food Standards Agency (FSA). In 2018, the laboratory was separately designated as UK NRL in the newly formed network of NRLs for foodborne viruses (initially covering non-shellfish matrices only). Following the UK's decision to leave the EU, the European Commission redistributed the responsibilities of the former EURL for monitoring bacteriological and viral contamination of bivalve molluscs (based at Cefas) between four different EURLs:

- EURL for foodborne viruses (all matrices including bivalve shellfish)
- EURL for Escherichia coli (E. coli)
- EURL for Salmonella
- EURL for marine biotoxins and classification and monitoring of bivalve mollusc production areas

From 1st April 2019 to 31st March 2021 the two shellfish-related NRL designations at Cefas were combined under a single designation (although the NRL maintained different directors for foodborne viruses and bacteriological contamination of shellfish, respectively). As a result of restructuring at the EURLlevel, prior to the end of transition arrangements on December 31st 2020, the NRL at Cefas was one of multiple UK NRLs appointed within three of the four NRL networks described above. The other NRLs appointed by FSA were:

- The Agri-Food & Biosciences Institute (AFBI) for marine biotoxins
- Public Health England (PHE) for Salmonella and E. coli (in non-shellfish matrices)

The description of activities included herein comprises co-ordination of UK Official Laboratories (OLs), provision of advice to the Competent Authority (CA) and collaboration with the EURLs through participation in comparative testing, research and development and representation at EURL workshops (where relevant), support during outbreaks, and provision of reference materials among others. The roles and responsibilities of the NRLs have changed significantly in recent years. The services required to be delivered under the scope of the current Cefas/FSA agreement include the following basic duties of the National Reference Laboratory, based on Articles 100-101 of Regulation (EU) 2017/625 (retained from January 1st 2021 in UK law subject to amendments through Statutory Instrument 2019 No. 665; The Official Controls for Feed, Food and Animal Health and Welfare

(Amendment etc.) (EU Exit) Regulations 2019):

- (a) cooperate internationally in their area of competence (and where possible, with the relevant EURL);
- (b) collaborate with international laboratories (where possible with the relevant EURL) and participate in training courses and inter-laboratory comparative tests organised by these laboratories:
- (c) coordinate the activities of OCLs responsible for the analysis of samples to ensure the verification of compliance with feed and food law;
- (d) where appropriate, organise comparative tests between OCLs and ensure an appropriate follow-up of such comparative testing;
- (e) ensure the dissemination of any information required by the competent authority;
- (f) provide scientific and technical assistance to the Competent Authority for the implementation of MANCPs (multi annual national control plans) in accordance with Article 109 and of coordinated control plans adopted in accordance with Article 112 of (retained) Regulation (EU) 2017/625;
- (g) where necessary, conduct training courses for OCL staff;
- (h) upon request by the appropriate authority, actively assist in relevant emergency situations and in cases of non-compliance of consignments, carry out confirmatory analysis;
- (i) be responsible for carrying out other specific duties as required by the competent authority, where appropriate and by prior agreement.

This technical report summarises the activities carried out by the NRL for the financial year 2020-21 according to the requirements of (retained) Regulation (EC) No. 882/2004 and (retained) Regulation (EU) 2017/625 as defined in the Service Level Agreement (SLA) between the FSA and Cefas over the time period April 2020-March 2021. This report constitutes the last report of the contract ending 31st March 2021.

It should be noted that because of the impacts of the current Covid-19 pandemic, some objectives associated with this contract were altered over the last year (with agreement of the FSA). These included changing the network meeting to a pre-recorded virtual event rather than a physical face-to-face workshop, a short delay in the delivery of certain laboratory tasks to meet Cefas Covid-19 secure requirements and attendance at meetings by virtual means only.

Furthermore, attendance at EU meetings and opportunities for collaboration with EURLs were restricted in year (in line with Government advice) as to not interfere with the EU Transition negotiations.

Delivery of the responsibilities of the NRL has been brigaded into the following objectives in accordance with the SLA agreed between Cefas and the FSA. These include the following key responsibilities:

- 1. Provision of secretariat services (Objective 1)
- 2. Advice and representation within the UK and internationally (Objective 2)
- 3. Production of standard operating procedures, codes of practice and guidance documents (Objective 3)
- 4. Compliance assessment via audits and Proficiency Testing (PT) (Objective 4)
- 5. Co-ordination within the UK of international initiatives (Objective 5)
- 6. Provision of additional services where requested by FSA (Objective 6)
- 7. Other meetings, workshops and task forces (Objective 7)

2. Provision of secretariat services (Objective 1):

2.1. Dissemination of information via NRL website

The NRL website was completely redesigned to provide an interface with the viruses in food NRL and other NRLs since the disbandment of the LBM EURL (Spring 2020). This included clearer signposting, and separation of key areas of work between the two NRL functions. Clear demarcation of the navigation bar to include sections such as methods, current activities, PT, laboratory network information, and an information centre page with reports, publications and other relevant documents relating to bivalve shellfish sanitation were also prepared. Numerous updates were also amalgamated into the website in the last year. A new combined NRL website was designed in consultation with FSA, content agreed and a beta version of the website launched in the Spring of 2020. This website can be accessed here. https://www.cefas.co.uk/nrl/

2.2. Reporting to FSA

The NRL has provided regular reporting to the FSA, through the means of email, telephone (where appropriate), and via quarterly RAG reports. Because of the current Covid-19 pandemic no face-to-face meeting were conducted in the last year. Regular updates on the impact of Covid-19 on the delivery of the programme were provided to FSA during the first period of lockdown when laboratory delivery was placed on hold but remote delivery maintained through home-working. Laboratory delivery resumed when lockdown restrictions were lifted in June 2020, albeit with adjustments to working practices and capacity to meet Cefas Covid-19 secure processes.

Notes from contractual update meetings were provided to the FSA on the 23rd August 2020 and subsequently sent and approved by FSA. The NRL teams held a teleconference with the FSA project officer and other staff to discuss progress on 23rd July 2020. The NRLs provided updates to FSA on expected interactions with the EURLs during the remainder of the transition period on 18th August 2020. A report on the October NRL meeting (8th October 2020), and the minutes from the NRL meeting of the OCL network were sent to FSA on 28th October 2020. The NRL teams also held a teleconference with the FSA project officer and other staff to discuss progress on 27th January 2021, a final copy of the minutes was sent to FSA on 31/03/2021.

Various ad hoc advice was given to the FSA on:

- i. Alternative methods such as impedance for testing of shellfish for official control purposes (Spring 2021).
- ii. Appropriate methods for end product testing (EPT) purposes in LBM, including *E. coli* and norovirus (NoV) testing.
- iii. Consultation regarding changes to EU legislation (e.g. 2017/625), and associated impacts on OL testing.
- iv. Input regarding outbreak investigations in particular, risks associated with vibrios in seafood produce in the UK, including testing approaches to identify and quantify these pathogens in shellfish matrices.
- v. Echinoderm testing and current EU regulations.
- vi. Ad hoc advice to FSA on Hepatitis A virus testing (HAV) in fruit and vegetables.
- vii. During May 2020 the NRL teams responded to questions from FSA on possible risks to activities arising from the end of EU exit transition arrangements at the end of 2020.

2.3. Dissemination of information/advice from engagement with international organisations reporting to FSA

The NRL requested clarification regarding the classification of echinoderms from the bacteriological contamination of bivalve molluscs EURL (Vigo, Spain) in the Spring and Summer of 2021. We are still awaiting a response despite repeated attempts to obtain information and clarity on this issue.

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

The bacteriological NRL alongside the classification team at Cefas held a liaison meeting with Public Health Wales (PHW) on 3rd December 2020. Issues covered during this meeting included impacts of Covid on OCL testing, staffing considerations and issues, an update on classification changes (e.g. in Wales) as well as reporting of results and timely identification of outwith results against agreed

KPI's (key performance indicators). Draft notes of this meeting were sent to PHW laboratories on the 3rd December 2020.

3. Advice and representation within the UK and internationally (Objective 2).

3.1. Provision of advice to the OCL Network

The NRL provided various advice throughout the year to OCLs (summarised in the quarterly RAG reports sent to FSA). In particular, during the summer of 2020, advice was given to various OCLs (Belfast and Colindale/Porton Down) regarding risk assessment work carried out by Cefas associated with Covid-19 and routine testing of shellfish. Much of the advice provided to the OCL network is summarised during the network meeting each year. The twelfth meeting of OCLs undertaking bacteriological testing of bivalve shellfish was held on the 8th October 2020. Twenty-one delegates attended the event. Because of the current Covid-19 pandemic, the workshop was hosted remotely, using pre-recorded presentations (sent to the delegates on 6th October 2020), with a followup questions and answer session on 8th October 2020. A wide variety of topics were covered during this workshop. These included OCL performance in PT, an assessment of Covid-19 risks related to processing shellfish (James Lowther, Cefas), new data on reducing NoV contamination from oysters under depuration conditions (Andrew Younger, Cefas), assessing Vibrio risks in a UK context (Alexandra Hughes, Cefas), and new methods for the rapid quantification of E. coli in bivalve molluscan shellfish (David Walker, Cefas). Information on progress at ISO on revisions to relevant methods and standards was also presented, as well as a discussion on recent changes to EU regulations pertaining to OCL testing. There was also some discussion on the future of the live bivalve network following EU Transition, and media availability as well as impacts of the current Covid-19 pandemic on OCL delivery.

3.2. Representation at relevant international meetings

Following the disbandment of the EURL in this area, there was no annual EURL meeting in the Spring of 2020. In accordance with instructions received from FSA and UK government re: non-attendance at EU meetings in the lead up to EU Transition, Cefas did not attend any EU meetings during this period.

Nationally, the NRL participated in the BSI Food Microbiology Committee during 2020-21 and provided various oral and written comment on standards relevant to the area of shellfish microbiology. Craig Baker-Austin (Director of the UK NRL for bacteriological contaminants of shellfish) provided a presentation on method standardisation at the CEN ISO meeting, July 2020.

Craig Baker-Austin participated in ISO WG standardisation efforts (vibrios) during Autumn of 2020, including reviewing FDIS documents and providing comments to the ISO secretariat. The NRL was also recently involved in reviewing relevant documentation from BSI on methods impinging on our laboratory methods (e.g. Salmonella, seafoods, sample preparation etc). The NRL also circulated key information on standards to the laboratory network and the CA (during the October network meeting), including changes to methods that impinged on current protocols. Again, where significant changes in testing methods were identified through the year, these were communicated to the OCL network during the yearly network meeting (typically October each year).

James Lowther (Director of the UK NRL for foodborne viruses) attended the 3rd workshop of NRLs (including a special session on Next Generation Sequencing) hosted remotely by the EURL in Uppsala, Sweden, on the 1st - 3rd September, 2020. Due to central government advice, permission to attend this meeting was granted in the capacity of an invited expert representing the FAO Reference Centre for Bivalve Mollusc Sanitation. James Lowther attended (remotely) a meeting on the application of digital PCR to quantification of viruses in bivalves, jointly hosted by the EURL and the Netherlands Food and Consumer Product Safety Authority, 23rd March, 2021.

3.3. Participation in other international activities

Craig Baker-Austin helped lead revisions to ISO 21872-1 (*Vibrio* in seafood ISO method) throughout this period. He also led the WG27 (ISO *Vibrio* working group), including providing draft change text to amendments and clarification to the ISO secretariat edits to this microbiological standard.

3.4. Advice on best practice

The NRL provided advice on best practice over the last year, in a variety of different areas of competence.

The Foodborne Viruses NRL provided *ad hoc* advice to FSA on HAV (testing in fruit and vegetables during the late Spring of 2021. In particular, the Foodborne Viruses NRL provided further *ad hoc* advice to FSA on HAV testing in vegetables and fruit (particularly dates), and the issues underlying the difficulty of obtaining viral genome sequences from contaminated foods.

The bacteriological contamination NRL provided miscellaneous advice to FSS (Foods Standard Scotland) on a number of matters during the summer and autumn of 2020, including an NRL perspective on laboratory performance (e.g. commercial testing laboratories and their testing methods for *E. coli* determination in shellfish). This involved a review of both testing protocols used (OCL versus commercial method), SOPs and results generated from both the commercial laboratory against the reference method (MPN). The NRL attended a meeting to discuss these issues with FSS

3.5. Maintenance of expertise

Laboratory activities have been undertaken towards maintenance of UKAS accreditation for a variety of bacteriological and viral methods in bivalve shellfish. Special provisions were taken in order to maintain adherence to ISO 17025 for shellfish virus testing despite the restrictions due to the Covid-19 pandemic. James Lowther attended a day-long seminar on the application of Illumina NGS technology for microbiology on 28th April 2020. An audit of the NoV and HAV tests was conducted remotely by UKAS between 9-13th Nov 2020. Upon clearance of a single finding, accreditation of these methods to ISO 17025 was renewed. An audit of the *Vibrio parahaemolyticus* test was conducted remotely by UKAS between 9-13th Nov 2020. We are still waiting clearance of a single finding regarding this method from UKAS. There is no update on this as of April 2021.

A placement student at Cefas (Dawn Lau) has been engaged in a project using whole genome sequencing data to assess risks associated with vibrios. This project is looking at the types of pathogenic vibrios circulating globally and comparing these strains with those currently identified in the UK (from Public Health England) from a variety of foodborne sources. This data analysis will build upon and help maintain a UK-specific database for analysis of these foodborne pathogens. Dawn's work has contributed to a review paper that the NRL will publish in a special edition of "Pathogens" in early 2021 (contribution commissioned by journal).

3.6. Involvement in standardisation activities relevant to work area

The NRL has been actively involved in method standardisation activities linked to its area of competence.

Craig Baker-Austin participated in ISO WG standardisation efforts (vibrios) throughout 2020, including reviewing FDIS documents and providing comments to the secretariat. James Lowther represented BSI as the UK National Standards Body at the plenary meeting of the ISO Food Microbiology Committee ISO/TC34/SC9 held on 3rd-5th June 2020.

James Lowther as leader of the ad hoc Group on ISO 15216-1 AMD-1 has contributed the following:-

- Led the response to comments received during the vote on the Committee Draft of the amendment
- Prepared and submitted to the ISO secretariat the draft for the second vote.
- Led the response to comments received during the second vote on the DIS of the amendment

 Helped to prepare the final draft and publishers' proofs of the amendment (the final vote was dispensed with as no technical comments were received during the second vote).

Publication of the amendment is anticipated in early FY 2021-22 at which point the *ad hoc* group will be disbanded. James Lowther attended meetings of CEN/TC463/WG1 on general requirements for PCR methods for food microbiology on 18th May 2020 and 9th March 2021 as a UK expert. James Lowther attended meetings of the non-culturable organisms (viruses and parasites) sub-group of ISO/TC34/SC9/WG3 on method validation for food microbiology on 10th August 2020, 10th February 2021 and 24th February 2021 as a UK expert. In collaboration with other members of the BSI Food Microbiology standardisation mirror group AW/009, the NRL identified and informed BSI of issues with the BSI publication BS EN ISO 6887-3:2017 + A1:2020 (consolidated version of the standard for preparation of fish and fishery products for microbiological examination, including amendments). A new corrected version was published in February 2021.

3.7. Support with emergency situations

The NRL provided advice and feedback on two separate outbreaks of apparent foodborne (seafood) illness including an outbreak of mussel-associated gastroenteritis in Devon (September 2020).

3.8. Scientific and technical assistance with the implementation of MANCPs and coordinated control plans

The NRL contributed by providing information on sampling priorities for FSA for National Monitoring Plan (NMP) sampling priorities for 2020-21 (December 2020). Specifically, the NRL received a request on the 30th November from the FSA regarding the annual review of the UK's National Monitoring Plan (NMP) sampling priorities for the import of products of animal origin (POAO) for the period April 2021 to March 2022. This included a revision of previous microbial targets for consideration. A consolidated response to the FSA National Monitoring Plan sampling priorities was subsequently submitted to the FSA for microbiological and virus contaminants in bivalve shellfish for 2021-22 on the 15th December 2020.

4. Production of standard operating procedures, codes of practice and guidance documents (Objective 3).

4.1. Contribution to the development of standardised protocols and advisory documents

The bacteriological NRL provided amendments to advisory documents pertaining to end product testing (EPT) of shellfish for *E. coli*. The document was published on Cefas NRL website following

further internal edits on the 18th January 2021.

4.2. Validation of reagents

During the NRL meeting on the 8th October 2020, it was noted that there have been issues identified in both the validation, sourcing media and reagents necessary for OCL testing. These include both *E. coli* and *Salmonella* spp. testing. The NRL outlined some alternatives as well as advice to OCLs regarding this matter.

4.3. Dissemination to CA and OCLs of information provided by the EURL for monitoring bacteriological contamination of bivalve molluscs and shellfish classification.

None received over the last reporting period (April 2020-March 2021).

5. Compliance assessment via audits and PT (Objective 4).

5.1. Assessment of OCL performance in whole animal distributions

With agreement from FSA, the whole animal PT distribution organised by the NRL, planned for November 2020, was delayed until the following year due to restrictions associated with the Covid-19 pandemic. This PT was distributed in April 2021.

5.2. Support for OCLs

Various support was provided to OCLs over this last reporting period. For example, during the recent Cefas/PHE EQA scheme (2019/2020), poor performance from one OCL was identified and rectified. Briefly, OCL 243, achieved a score of only 50% over 2 distributions (a score of >70% is deemed satisfactory, see below section 5.3). The NRL recommended OCL 243 complete an investigation into their procedures and referred the OCL to the trouble shooting guidance available on the NRL website. An investigation was requested on the 21st April 2020. A root cause investigation by the laboratory identified that sample tubes had not been labelled until after inoculation transfer (BPW to selective broths). This appears to have led to samples becoming mixed up during the testing process. Changes to the SOP and training of staff were instigated following this incident. In November 2020 the NRL received the investigation report from OCL 243 relating to poor performance in the EQA scheme, which was deemed satisfactory, so no further assistance was required.

5.3. Assessment of OCL performance in Cefas/PHE external quality assessment (EQA) shellfish scheme for *E. coli* and *Salmonella* spp.

The results from the most recent EQA scheme (SF0136 and SF0138) were sent to OCLs in April 2020. The ongoing performance of UK OCLs was assessed for two distributions of the Cefas/PHE EQA shellfish scheme comprising of four Lenticule® discs for *E. coli* and *Salmonella* spp. between June 2020 and February 2021 (SF066 and SF067). Scores were allocated in accordance with the Cefas/PHE shellfish EQA scheme scoring system. Eleven OCLs analysed two 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 distributions for the enumeration of *E. coli*, the measure of performance considered by the scheme organisers to demonstrate satisfactory performance. All OCLs achieved scores of 100% over 2 distributions for the detection of *Salmonella* spp.

Table 1 Performance assessment for E. coli MPNs for distributions, SF066 - SF067

	Distribution SF066		Distribution SF067		All distributions		
Lab no.	SF0140	SF0141	SF0142	SF0143	Cumulative score	Max score	%
7	12	12	12	12	48	48	100
9	12	12	12	12	48	48	100
67	12	12	12	12	48	48	100
97	12	12	12	12	48	48	100
145	7	12	12	12	43	48	90
166	12	12	12	12	48	48	100
243	12	12	12	4	40	48	83
271	12	7	12	12	43	48	90
532	12	12	12	12	48	48	100
578	12	12	12	12	48	48	100
1817	12	12	12	12	48	48	100

Table 2 Performance assessment for Salmonella spp. for distributions, SF066 - SF067

	Distribution SF066		Distribution SF067		All distributions		
Lab no.	SF0140	SF0141	SF0142	SF0143	Cumulative score	Max score	%
7	2	2	2	2	8	8	100
9	2	2	2	2	8	8	100
67	2	2	2	2	8	8	100
97	2	2	2	2	8	8	100
145	2	2	2	2	8	8	100
166	2	2	2	2	8	8	100
243	2	2	2	2	8	8	100
271	2	2	2	2	8	8	100
532 ^a	NE	NE	NE	NE	-	-	-

578	2	2	2	2	8	8	100
1817	2	2	2	2	8	8	100

^a This laboratory does not undertake *Salmonella* spp. testing of official control samples.

5.4. Participation in international practical initiatives

No international practical activities were organised in the last reporting period (April 2020-March 2021) because of the current Covid-19 pandemic.

5.5. Provision of laboratory-based training

Neither the foodborne virus nor bacteriological contamination of molluscs NRL conducted practicalbased training this year due to restrictions related to the Covid-19 pandemic.

5.6. NRL participation in EURL PT for Salmonella spp.

The UK NRL participated in one PT distribution organised by the EURL for *Salmonella spp.* during August 2020. The distribution comprised of four samples of spiked mussels. The UK NRL correctly reported the presence / absence of *Salmonella* spp. in all four samples.

5.7. NRL participation in FAO Reference Centre PT for E. coli and Salmonella spp.

The FAO Reference Centre for Bivalve Mollusc Sanitation did not organise a PT distribution during the Covid-19 pandemic.

5.8. NRL participation in EQA shellfish scheme for *E. coli* and *Salmonella* spp.

The UK NRL participated in 2 distributions of the Cefas/PHE EQA shellfish scheme which took place in September 2020 (SF0140, SF0141) and March 2021 (SF0142, SF0143). The results reported by the UK NRL were assessed and a cumulative performance assessment of 100% for the enumeration *E. coli* and detection of *Salmonella* spp. was recorded.

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

The UK NRL participated in one distribution of the PHE pathogenic *Vibrio* scheme comprising two samples in January 2021 (V0158, V0159). The UK NRL reported satisfactory results for the detection of *V. parahaemolyticus* for both samples. *V. vulnificus* was incorrectly reported for 1 sample received.

5.10. NRL participation in EURL PT for norovirus and hepatitis A

The UK NRL participated in one PT distribution organised by the EURL for NoV and HAV during June 2019 (PT 79). The distribution comprised 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.

5.11. NRL participation in FAO reference centre PT for norovirus and hepatitis A

The FAO Reference Centre for Bivalve Mollusc Sanitation did not organise a PT distribution during the last reporting period (April 2020-March 2021) because of the ongoing Covid-19 pandemic.

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

The foodborne virus NRL participated in two PT schemes organised by the EURL for Foodborne Viruses, scheme EFV 04 for NoV and HAV in leafy greens (October 2020) and scheme EFV 05 for NoV and HAV in oysters (November 2020). Based on the EURL expected results the NRL correctly identified the presence or absence of the target viruses in all samples. The NRL participated in a PT schemes organised by PHE, scheme NHV008 for NoV and HAV in lenticules (February 2021). Based on the PHE expected results the NRL correctly identified the presence or absence of the target viruses in all samples. Full PT Scheme reports on schemes organised in FY 20-21, including analysis of quantification, are anticipated from the EURL and PHE in the early part of FY 21-22. These will be forwarded to the FSA once we have received them.

6. Co-ordination within the UK of International initiatives (Objective 5).

No recommendations were received by the NRL during this reporting period.

7. Provision of additional services where requested by FSA (Objective 6).

None requested in this reporting period.

8. Other meetings, workshops and task forces (Objective 7)

The bacteriological 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 (SSWG). The bacteriological NRL attended and contributed to the SSWG meeting on 4th June 2020, 17th September 2020 and 22nd January 2021. The group set up following an incident of anomalously high *E. coli* results in July 2015. The NRL provided a review of a report provided by Seafish regarding differences in the

implementation of OC testing for shellfish classification across Europe, which was submitted to Seafish on March 2^{nd} 2021.





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