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

Annual Technical Report for 2015/16

Draft report version 2

16.05.16

9 pages

Contract Reference: Cefas ref (C5937)

Document approved by:	**C5937 Project Manager – Craig Baker-Austin**	**Review date:**	**N/A**
Document checked by:	**Craig Baker-Austin**	**Classification:**	**Official**
Document prepared by:	**Louise Stockley**	**Location**	**NRL drive**

SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2015 - MARCH 2016

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 report summarises the activities carried out by the NRL for the financial year 2015-16 according to the requirements of Regulation (EC) No. 882/2004 and as defined in the Service Level Agreement between the Food Standards Agency (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 seventh meeting of OCLs undertaking microbiological testing of bivalve shellfish was held by the NRL on the 28th and 29th of October 2015. Eighteen delegates attended the two day event. The first day (pm) consisted of a practical laboratory-based session focussing on two different molecular approaches used for detecting foodborne pathogens in shellfish (pathogenic vibrios and norovirus), detected using conventional PCR and real-time PCR, respectively. Numerous topics were covered on the second day including OCL performance in proficiency testing (PT), amendments to the current MPN calculator, background, use and variability of shellfish testing methods for *E. coli,* progress at ISO on revisions to the *Vibrio* standard and the use of pulsifier versus stomacher for homogenisation purposes for *E. coli* recovery from shellfish matrices, and the upcoming EU-wide survey of norovirus in oysters.

3. Advice and representation within the UK and EU

3.1. Provision of advice to the OCL Laboratory Network

The NRL produced a revision of the NRL generic protocol for the enumeration of *E. coli* in bivalve molluscs and incorporated amendments relating to the MPN calculator which were discussed and presented during the October 2015 laboratory network meeting. A new version of the *E. coli* generic protocol with amended MPN tables was uploaded to the NRL website on the 26th February 2016.

The NRL provided advice to OCL laboratory (Porton FW&E) following elevated *E. coli* results in July 2015. This included provision of advice on protocols/methods, best practice and follow-on recommendations during two separate laboratory visits in August and September 2015. Miscellaneous advice was provided to various OCLs regarding the use of MPN calculator, time and temperature and also the reporting of results for OCL purposes (Autumn and Winter 2015).

The NRL participated in the BSI Food Microbiology Committee during 2015-2016 and provided oral and written comments on standards relevant to the area of shellfish microbiology. The NRL also circulated key consultations on standards to the laboratory network and the CA, including changes to methods that impinge on current protocols. Future potential changes to relevant protocols were also discussed at the October OCL

network meeting. A list of recent edits (and anticipated future changes) to NRL protocols are highlighted in Annex I (page 7).

The NRL held separate liaison meetings 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.

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

i. Homogenisation methods for bivalve shellfish.
ii. The application and use of new MPN calculator.
iii. Adequate reporting of results and timely identification of outwith results (e.g. from 'rare' Class A sites).
iv. Advice on sample transport criteria and laboratory testing methods.

3.2 NRL website

The NRL website (http://www.cefas.defra.gov.uk/nrl/information-centre/reports.aspx) was maintained during the period by adding new material and removing obsolete material. Several additions to the NRL website took place in 2015-2016, including the following:

- Issue 12 of the *E. coli* generic protocol (February 2016).
- Edits to 'current activities' page.
- PT 62 results report
- Update to OCL address and contact details of OCL's identified from the October 2015 network meeting.
- Addition of network meeting notes from the 2015 EU network meeting.

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.

Significant technical advice was given to the FSA following elevated *E. coli* results from the Porton FW&E laboratory in July 2015. The NRL produced, finalised and submitted the NRL investigation and report on this incident in December 2015 to FSA. Significant additional input included often weekly Telephone conference (TC) contact between July 2015 until Autumn 2015 following this incident. The NRL provided additional information and data on request regarding the *E. coli* MPN method in general. The NRL presented the results of the investigation at a stakeholder event organised by the FSA in January 2016. The NRL subsequently drafted a review document to the FSA regarding the background, use, limitations and variability of the MPN method in March 2016. Advice was given to the FSA in relation to an upcoming EU-wide surveillance of norovirus in Member State harvesting areas. The information provided to the CA included advice on several aspects of the anticipated project including the timeframe of the survey (the NRL suggested a longer time

frame given the potential of an anomalously cold or warm year as well as variations in norovirus in the community), and the pathogens to be assessed (e.g. norovirus, hepatitis A).

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 OCLs via direct circulation of documentation or via the NRL website. Specific papers circulated are listed below:

- Resolutions of the 14th Workshop of NRLs for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs, IFREMER, France, May 2015.
- Report of the EURL proficiency test distribution for norovirus and hepatitis A in bivalve molluscs.
- Report on the whole animal PT distribution and EQA report.

4.3 Other advice

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

- Proposals for the amendment of Regulation (EC) No. 882/2004, with regards to aspects relating to the responsibilities of Reference Laboratories.
- Reference and alternative methods for *E. coli* testing of shellfish, in particular following the episode of elevated results in July 2015.
- Proposals for amending the EU standards for class A areas.
- Proposals for controls to improve risk management in the EU with respect to human enteric viruses, including the planned EU harmonised study for viruses in EU bivalve shellfish produce.

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

5.1 Assessment of OCL performance in whole animal distribution

In October 2015, the NRL organised a distribution comprising of common mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*) for the enumeration of *E. coli*. Whole matrix samples were provided to 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. Material was distributed to all 13 UK OCLs. The arrival temperatures recorded by OCLs showed the temperatures recorded did not exceed recommended transport temperatures of <10°C. Participation and performance assessments for *E. coli* for each sample are given in Tables 1, 2 and 3. All OCLs returned results for this distribution. For sample 1, 9 OCLs returned replicate *E. coli* MPN/100g results between ±3 SD of the participants' median and received a maximum score of 12. OCLs 166, 578 and 1160 reported one replicate result between ±3 and ±5 SD of the participants' median. For sample 2, 12 OCLs reported the absence of *E. coli* in this sample and received a maximum score of 12. OCL 166 reported the presence of *E. coli* in this sample and scored 2. For sample 3, 10 OCLs returned replicate *E. coli* MPN/100g results between ±3 SD of the participants' median, with 9 OCLs receiving the maximum score of 12. OCLs 14 and 145 reported one replicate result between ±3 and ±5 SD of the participants' median. OCL 166 reported

both replicates outside ±5 SD of the participants' median. Two points were deducted from OCL 97 as the *E. coli* MPN/100g value was not correctly reported for the tube combination provided and OCL 9 did not examine sample 1 as insufficient shellfish numbers were available.

Table 1. Summary of participation by OCLs – PT 62 Sample 1, Sample 2 and Sample 3

E. coli	Sample 1 - Mussels	Sample 2 - Oysters	Sample 3 - Oysters
OCLs reporting duplicate results for *E. coli* MPN	12	13	13
OCLs reporting single MPN results only	0	0	0
OCLs reporting MPN results within the expected range for both replicates [1]	9	12	10
OCLs reporting MPN results outside the expected range for one replicate	3	1	2
OCLs reporting MPN results outside the expected range for both replicates	0	0	0
OCLs reporting MPN results as censored results for one or both replicates	0	12	1
OCLs reporting single MPN result within the expected range	0	0	0
OCLs reporting single MPN result outside the expected range	0	0	0

[1] expected range = participants' median ± theoretical $3SD_T$

Table 2. Performance assessment of returned participants' results

OCL ID	Sample 1 - Mussels			Sample 2 - Oysters			Sample 3 - Oysters		
	E. coli MPN/100g			*E. coli* MPN/100g			*E. coli* MPN/100g		
	Rep. 1	Rep. 2	Score	Rep. 1	Rep. 2	Score	Rep. 1	Rep. 2	Score
7	78	130	12	<18	<18	12	230	130	12
9	NE	NE	-	<18	<18	12	45	45	12
14	45	68	12	<18	<18	12	2100	490	9
67	130	230	12	<18	<18	12	230	78	12
97	130	78	12	<18	<18	12	450	330	10
145	140	220	12	<18	<18	12	20	170	9
166	20	68	9	490	330	2	<18	<18	2
243	230	270	12	<18	<18	12	130	110	12
271	130	78	12	<18	<18	12	170	220	12
532	130	110	12	<18	<18	12	140	170	12
578	220	790	9	<18	<18	12	130	220	12
1160	78	20	9	<18	<18	12	170	220	12
1817	78	45	12	<18	<18	12	220	130	12

NE – Not Examined

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

Formal performance assessments were undertaken for three distributions of the Cefas/PHE EQA shellfish scheme (SF051, SF052 and SF053). Scores were allocated in accordance with the Cefas/PHE EQA shellfish scheme scoring system. Thirteen OCLs analysed all 3 distributions during the reporting period for the enumeration of *E. coli* and the detection of *Salmonella* spp. OCLs performances are summarised in Tables 3 and 4. Eleven OCLs achieved scores in excess of 70% over three distributions for the enumeration of *E. coli*, the measure of performance considered by the scheme organisers to demonstrate satisfactory performance. All OCLs achieved 100% for the detection of *Salmonella* spp. OCLs 9 and 243 achieved an overall score 67%

and 61% respectively. The NRL contacted each OCL requesting follow-up action to be taken due to underperformance in distribution SF051. Follow-up action and non-conformances were received from both OCLs. OCL 9 identified the root cause for the deduction in score was due to an error when rehydrating and preparing the samples (the MPN results reported for each sample fell into the expected range of the opposite sample). It was also noted that this error would not be possible with real shellfish samples. The response received from OCL 243 stated that the cause of the deduction in score was because staff were not properly trained on how to use the MPN calculator. It was noted that the results reported were incorrectly reported. Further training would be given to address this issue.

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

OCL	Distribution SF051		Distribution SF052		Distribution SF053		All distributions		
ID	SF0110	SF0111	SF0112	SF0113	SF0114	SF0115	Cumulative score	Max score	%
7	12	12	12	12	12	12	72	72	100
9	0	0	12	12	12	12	48	72	67
14	12	12	12	12	12	12	72	72	100
67	12	12	12	10	12	12	70	72	97
97	12	12	12	12	12	12	72	72	100
145	8	8	12	12	12	9	61	72	85
166	12	12	12	12	12	9	69	72	96
243	2	2	12	12	8	8	44	72	61
271	12	12	12	12	12	12	72	72	100
532	8	8	12	12	10	12	62	72	86
578	12	12	12	12	12	12	72	72	100
1160	12	12	12	9	12	12	69	72	96
1817	12	12	12	12	12	12	72	72	100

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

OCL	Distribution SF051		Distribution SF052		Distribution SF053		All distributions		
ID	SF0110	SF0111	SF0112	SF0113	SF0114	SF0115	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	2	2	12	12	100
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 [a]	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

[a] This laboratory does not undertake *Salmonella* testing of official control samples.
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 for *E. coli* and *Salmonella* spp. Six samples were analysed across three distributions in July 2015 (SF0110, SF0111), November 2015 (SF0112, SF0113) and February 2016 (SF0114, SF0115). The results obtained by the UK NRL were assessed together with all

other participants. The UK NRL achieved a rolling performance assessment of >70% for *E. coli* enumeration and *Salmonella* spp. detection.

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

The NRL participated in the PHE pathogenic *Vibrio* scheme. Six samples were analysed across three distributions in July 2015 (V0128, V0129), November 2015 (V0130, V0131) and February 2016 (V0132, V0133). The NRL results for the detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* were satisfactory for 4 samples but *V. cholerae* was not detected in sample V0129 and *V. vulnificus* was not detected in sample V0133. To date, the NRL is not accredited for the detection of *V. cholerae* and *V. vulnificus*. Follow up action regarding this poor performance was undertaken by the NRL including a review of the laboratory SOP and staff training. The detection of *V. cholerae* and *V. vulnificus* have been incorporated into the laboratory SOP and is being bench tested prior to the obtaining UKAS accreditation. All staff trained in the *Vibrio* method re-examined repeat PHE samples and successfully detected the presence of *V. cholerae* and *V. vulnificus.*

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

The NRL participated in the EURL PT distribution (PT 60) for *E. coli* enumeration in the whole animal distribution comprising of Common mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*) in November 2015. The NRL achieved performance assessment of 100% for *E. coli* in both samples.

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

The NRL participated in one PT distribution organised by the EURL for norovirus and hepatitis A virus in June 2015 (PT 59). The distribution comprised of two laboratory constructed (LENTICULE) samples. The NRL scored 100% for all performance measures (relative accuracy, specificity and sensitivity).

5.7 Meetings, workshops and task forces

The NRL director participated in the 14th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held at the IFREMER, France, in May 2015. Minutes and an overview report detailing participation and major outcomes was provided to the FSA and the laboratory network following the workshop. The NRL has participated in numerous TC, Video conference (VC) and face-to-face meetings with the FSA, OCL's and industry following the July 2015 *E. coli* incident. A full version of the NRL report based on the incident is available on the FSA website (http://www.food.gov.uk/sites/default/files/nrl-investigation.pdf). One member of staff (Dr C. Baker-Austin) attended and ran a workshop on molecular methods for pathogenic vibrios at the Croatian NRL in April 2015 under the auspices of the EURL.

Dr Craig Baker-Austin Date…27/04/16…….

NRL Director

Annex I

A number of standard microbiological ISO methods were amended between 2015-2016. A short summary below highlights where amendments to relevant standard methods impinged or altered NRL methods. The *E. coli* NRL generic protocol was subsequently issued (issue 12) and distributed to all OCLs, reflecting these changes during the winter of 2016. An updated version of this generic protocol was also published on the NRL website. The Salmonella protocol is not being issued until the ISO has been published (anticipated to be later this year).

***E. coli* ISO 16649-3** – This ISO has recently been published (Autumn 2015). Some notable edits to the NRL protocol include:

- Reword note on how the MPN tables were generated.
- The addition of Peptone salt solution (PSS).
- Amendments to reference strains to be consistent with ISO 16649-3, and the inclusion of links to identify WDCM numbers.
- The addition of notes stating all dilutions tested should be included when obtaining the MPN value.
- TBGA/TBX plates incubation time changed from 22±2 hrs to 21±3 hrs. Rewording of text regarding the streaking and stacking (<6 high) TBGA/TBX plates.
- Rewording to address issues when calculating the MPN value. The inclusion of the Excel spreadsheet MPN calculator for 3 and 4 dilutions.

Salmonella ISO 6579 – This ISO is currently in the process of being published (May 2016). These are some intended edits once this is complete:

- Incubation of BPW range is being changed to 34 ˚C to 38 ˚C. Range stated in generic protocol is less than this at 37±1 ˚C.
- Amendment to selective plates. No specification to be given for second incubation medium.

About us

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

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

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

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

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

.

Customer focus

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

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

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

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

Head office
Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK

Tel +44 (0) 1502 56 2244
Fax +44 (0) 1502 51 3865
Web www.cefas.defra.gov.uk

Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601

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