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

Annual Technical Report for 2013/14

Cefas Weymouth Laboratory, Barrack Road,
The Nothe, Weymouth, Dorset, DT4 8UB, UK
Telephone: +44 (0) 1305 206600 Fax: +44 (0) 1305 206601
E-mail: shellfishmicro.nrl@cefas.co.uk
Website: http://www.cefas.defra.gov.uk/nrl.aspx

Report Title	Annual technical report for the UK National Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs 2013/14
Project Name	The Provision of Services for the UK National Reference Laboratory for Bacteriological and Viral Contamination of Bivalve Molluscs
Client/Customer	Food Standards Agency (FSA)
Cefas Project Reference	C5937
Document Number	C5937_2014_01
Revision	Final V1.0
Date	06/05/2014

Revision History

Revision number	Date	Pages revised	Reason for revision
0.1	24/04/2013	All	Draft for review by customer
1.0	06/05/2014	1, 6	Final after customer comments

	Name	Position	Date
Authors	Ron Lee, Craig Baker-Austin, Louise Stockley	NRL delivery	15/04/2014
Checked	Craig Baker-Austin	NRL Deputy Director	15/04/2014
Approved	Ron Lee	NRL Director	06/05/2014

This report was produced by Cefas for its Customer, FSA, for the specific purpose of providing a summary of the activity undertaken by the NRL in accordance with the Memorandum of Understanding with the FSA. Cefas accepts no liability for any costs, liabilities or losses arising as a result of the use of or reliance upon the contents of this report by any person other than its Customer.

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

Tel 01305 206 600

www.cefas.defra.gov.uk

ANNUAL TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2013 - MARCH 2014

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 2013-14 according to the requirements of Regulation (EC) No. 882/2004 and as defined in the Memorandum of Understanding 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 Official Control Laboratories and other relevant laboratories undertaking microbiological examination of bivalve molluscs

The fifth meeting of laboratories undertaking microbiological testing of bivalve shellfish was held at the NRL on the 16th and 17th October 2013. Twenty-three delegates attended the two day event although a small number did not attend the first day. The first day (pm) comprised a visit to a sewage treatment works followed by a laboratory demonstration on the enumeration of norovirus in sewage. This was followed by presentations of the results from research projects looking at the reduction of norovirus through sewage treatment and the impact of norovirus in a shellfish harvesting area. Topics covered on the second day included OCL performance in proficiency testing, the effects of homogenisation method on *E. coli* results in cockles, the use of net bags versus plastic bags for the transport of samples, revision of NRL generic laboratory protocols, progress at ISO on revisions to standards relevant to the microbiological examination of bivalve shellfish and the interpretation of the results of vibrio testing of seafood.

3. Advice and representation within the UK and EU

3.1 Provision of advice to the NRL Laboratory Network

The NRL undertook statistical analyses of past proficiency testing results with respect to homogenisation methods that had been used by OCLs. This related to concerns relating to the efficiency of homogenisation of cockles. The analyses resulted in NRL advice that stomaching is not the preferred method of homogenisation for cockles. This advice has been incorporated into the NRL generic protocols.

The NRL provided advice to individual OCLs on the following matters:

The NRL held separate liaison meetings with the Public Health England and Public Health Wales to maintain the use of NRL protocols and advice and to ensure a consistent approach to sample transport and microbiological examination of shellfish samples.

The NRL Director attended the first annual meeting of the PHE OCL User Day.

3.2 Provision of advice to the FSA

Comment was provided to FSA on proposed amendments to Regulation (EC) No. 882/2004 as they potentially affected NRLs and the functioning of laboratory networks.

Advice was provided to FSA with respect to:

- proposed changes to Codex Alimentarius standards relating to bivalve molluscs (including scallops)
- a query from the Canadian authorities relating to methods used by a UK laboratory for examination of a batch of bivalve molluscs for *Vibrio* spp.
- revision of the list of official feed and food control laboratories on the FSA website. Liaison was undertaken with the FSA with respect to laboratories included in the NRL laboratory network.

Information was provided to FSA in Scotland regarding laboratories known to offer microbiological laboratory services to the shellfish industry

In addition, advice on sample transport and laboratory methods was given to Cefas colleagues providing statutory services to FSA.

3.2 Representation at EURL meetings

i. EURL Annual Workshop

The NRL director and deputy director participated in the 12th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held in Rome in May 2013. A report detailing participation and major outcomes was provided to the FSA and the laboratory network following the workshop.

ii. Participation in other EURL activities

Two members of staff attended a workshop on methods for pathogenic vibrios organised by the EURL in January 2014.

iii. Advice on best scientific practice

A formal response was submitted to the FSA regarding proposals for controls improving risk management in the EU with respect to human enteric viruses. Further advice was also provided to the FSA on the subject.

Copies of NRL protocols relating to the avoidance of conflicts of interest in acting as an EURL, NRL and OCL provided to PHE for information in its role as NRL for food microbiology.

iv. Maintenance of expertise

Staff attended internal Cefas refresher training on the application of ISO 17025. Laboratory activities were undertaken to ensure maintenance of expertise by laboratory staff in support of continuation of accreditation.

v. Participation in standardization activities

The NRL participated in the BSI Food Microbiology Committee and provided oral and written comment on standards relevant to the area of shellfish microbiology. The NRL also circulated key consultations on standards to the laboratory network and submitted comments on behalf of the network to BSI. In addition, the NRL identified to BSI problems with the Most Probable Number tables in ISO 7218: Amd1 (2013) and undertook associated correspondence with representatives on the ISO Statistics committee.

4. Production of standard operating procedures, codes of practice and guidance documents

The NRL produced a significant revision of the NRL generic protocol for the enumeration of *E. coli* in bivalve molluscs. This incorporated amendments to ISO 16649-3 and ISO 7218 where they affected the content of the protocol. Following consultation with the laboratory network, the final document was published on the NRL website in March 2014.

The NRL also produced a revised draft of the NRL generic protocol for the detection of *Salmonella* spp. in bivalve molluscs. This was circulated to the laboratory network for comment and the final version will be published in April/May 2014.

5. Compliance assessment via audits and ring trials

5.1 Assessment of laboratory performance

i. Provision of proficiency tests

In November 2013, the NRL organised a distribution comprising common mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*) for enumeration of *Escherichia coli* and the detection of *Salmonella* spp. Whole matrix samples were provided to laboratories to test aspects of the methodologies not covered by the standard shellfish EQA scheme i.e. opening of shellfish and preparation of initial dilutions. Material was distributed to 13 UK OCLs. Participation and performance assessments for *E. coli* and *Salmonella* spp. for each sample are given in Tables 1 and 2. For both samples (PT 51 – sample 1 and sample 2) (Table 2), all laboratories reported *E. coli* MPN/100g within the expected range and received a maximum score of 12. For *Salmonella* spp., twelve out of thirteen laboratories returned results as expected and received a score of 2: the other laboratory does not undertake microbiological examination of official control samples of bivalve molluscs for *Salmonella* spp.. A summary of the methods used by OCLs for the detection of Salmonella spp. will be produced prior to the 2014 laboratory network meeting.

Table 1. Summary of participation by OCLs – PT 51 Sample 1 and Sample 2

E. coli	**Sample 1 - Mussels**	**Sample 2 - Oysters**
Participants reporting duplicate results for *E. coli* MPN	13	13
Participants reporting MPN results within the expected range for both replicates[1]	13	13
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	0
Participants reporting MPN results as censored results for one or both replicates	4	5

***Salmonella* spp. summary statistics**		
Participants reporting results for *Salmonella* spp.	12	12
Participants reporting the presence of *Salmonella* spp.	0	0
Participants reporting the absence of *Salmonella* spp.	12	12

[1]expected range = participants' median ± theoretical 3SD for sample 1 and 2 ($<2.0 \times 10^1 – 2.3 \times 10^3$)

Table 2. Performance assessment of returned participants' results

Lab ID	Sample 1 - Mussels					Sample 2 - Oysters				
	E. coli MPN/100g			*Salmonella* spp. in 25g		*E. coli* MPN/100g			*Salmonella* spp. in 25g	
	Rep. 1	Rep. 2	Score	Result	Score	Rep. 1	Rep. 2	Score	Result	Score
7	20	20	12	ND	2	70	<20	12	ND	2
9	<20	20	12	ND	2	20	<20	12	ND	2
14	20	<20	12	ND	2	<20	20	12	ND	2
67	45	45	12	ND	2	140	45	12	ND	2
97	<20	20	12	ND	2	20	<20	12	ND	2
145	80	20	12	ND	2	50	90	12	ND	2
166	50	50	12	ND	2	20	50	12	ND	2
243	50	50	12	ND	2	80	80	12	ND	2
271	<20	<20	12	ND	2	20	20	12	ND	2
532 [a]	20	70	12	NT	-	110	50	12	NT	-
578	20	20	12	ND	2	20	50	12	ND	2
1160	20	20	12	ND	2	50	80	12	ND	2
1817	20	80	12	ND	2	<20	<20	12	ND	2

[a] This laboratory does not undertake *Salmonella* testing of official control samples of bivalve molluscs.

5.2 Support for OCLs

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

- The availability and use of instruments for the opening of large numbers of bivalve shellfish.
- Homogenisation methods for bivalve shellfish.
- Investigation and application of remedial measures with respect to an unusually high proportion of void MPN tube combinations.
- Methods suitable for the detection and enumeration of pathogenic *Vibrio* spp. in bivalve molluscs, together with the interpretation of results.
- Retesting following problems in an EURL PT distribution. The NRL arranged for the laboratory to receive additional material for testing.

5.3 OCL performance in the shellfish external quality assessment (EQA) scheme

The performance of UK OCLs was subject to ongoing review according to the agreed scoring system. Formal performance assessments were undertaken for three distributions of the Cefas/PHE EQA shellfish scheme (SF044, SF045 and SF046). Thirteen laboratories completed three distributions during this reporting period for *E. coli* enumeration and twelve completed three distributions for the detection of *Salmonella* spp. Laboratory performance is summarised in Tables 3 and 4. All laboratories achieved scores in excess of 70% over three distributions, the measure of performance considered by the scheme organisers to demonstrate satisfactory performance. One laboratory was given a score of 2 out of 12 for one sample of distribution SF046: the NRL subsequently contacted the laboratory with respect to the investigative and corrective actions.

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

Lab no.	Distribution SF044		Distribution SF045		Distribution SF046		All distributions		
	SF0096	SF0097	SF0098	SF0099	SF0100	SF0101	Cumulative score	Max score	%
7	12	12	12	12	12	12	72	72	100
9	12	12	12	12	12	2 [a]	62	72	86
14	12	12	12	12	12	12	72	72	100
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	12	12	72	72	100

[a] Both replicate results outside 4SD of expected range.

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

Lab no.	Distribution SF044		Distribution SF045		Distribution SF046		All distributions		
	SF0096	SF0097	SF0098	SF0099	SF0100	SF0101	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 of bivalve molluscs.
NE – Not examined.

5.4 Participation in EURL practical initiatives

No proposals for practical work by the EU NRLs were received.

5.5 NRL participation in EURL proficiency tests

i. 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 analyzed across three distributions in February 2013 (SF0096, SF0097). July 2013 (SF0098, SF0099), November 2013 (SF0100, SF0101). 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.

ii. Participation in PHE EQA for pathogenic *Vibrio* spp.

The NRL participated in the PHE FEPTU *Vibrio* scheme. Six samples were analyzed across three distributions in July 2013 (V0110, V0111), November 2013 (V0114, V0115) and February 2014 (V0118, V0119). The NRL results for detection *V. cholerae* were satisfactory on all occasions for both samples but the presence of *V. parahaemolyticus* was not detected in one sample from the July 2013 distribution. An investigation into the occurrence was undertaken and *V. parahaemolyticus* was isolated from a repeat sample.

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

The NRL participated in the EURL PT distribution for *E. coli* enumeration and detection of *Salmonella* spp. in whole animal distribution comprising Pacific oysters (*Crassostrea gigas*) and Common mussels (*Mytilus edulis*) in November 2013. The NRL achieved performance assessment of 100% for *E. coli* in both samples and for *Salmonella* spp. in Sample 1. The *Salmonella* results for Sample 2 were not scored by the EURL due to a quality control problem with the sample.

iv. Participation in EURL PT for norovirus and hepatitis A

The NRL participated in one proficiency testing distribution organised by the EURL for norovirus and hepatitis A virus in December 2013 (PT 50). The distribution comprised four shellfish matrix and two laboratory constructed (LENTICULE) samples. The NRL scored 100% for all performance measures (relative accuracy, specificity and sensitivity).

5.6 Provision of laboratory-based training

No training needs were identified at the NRL laboratory network meeting.

6. Co-ordination within the UK of EURL initiatives

Advice from the EURL with respect to virus testing of bivalve molluscs was passed on to the NRL laboratory network.

Responses were provided to the EURL with respect to the UK aspects of draft summary documents prepared following the EURL Workshop held in May 2013.

Anonymised *E. coli* data was provided to the EURL on behalf of the UK. The data was to be used in analyses to support revision of one section of the *EURL Good Practice Guide for the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas*.

7. Communication

7.1 NRL website

The NRL website (http://www.cefas.defra.gov.uk/nrl.aspx**)** was maintained during the period by adding new material and removing obsolete material. In particular, the following changes were made:

- New style laboratory network page .
- Content of Current Activities page updated.
- Substructure created for the Laboratory Network Meetings section of the Information Centre (will allow addition of presentations as well as the meeting minutes).
- Homogenisation method comparison report added to Information Centre.
- Issue 10 of the NRL generic *E. coli* protocol added to the Information Centre.

7.2 Information from the EURL

Information disseminated by the EURL was provided to both the FSA and OCL via direct circulation of documentation or via the NRL website. Specific papers circulated are listed below:

- Resolutions of the 12th Workshop of NRLs for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs.

- Report of an EURL proficiency test distribution for norovirus and hepatitis A in bivalve molluscs.

A query received from NRL France via the EURL, relating to a norovirus outbreak potentially connected with UK clams, was passed to FSA for consideration.