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

Annual Technical Report for 2014/15

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SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2014 - MARCH 2015

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 2014-15 according to the requirements of Regulation (EC) No. 882/2004 and as defined in the Service Level Agreement between the Food Standards Agency and Cefas. The description of activities included herein comprises co-ordination of UK Official Control Laboratories (OCL), provision of advice to the Competent Authority (CA) and collaboration with the European Union Reference Laboratory (EURL) through participation in comparative testing, research and development and representation at EURL workshops.

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

The sixth meeting of laboratories undertaking microbiological testing of bivalve shellfish was held at the NRL on the 7th and 8th of October 2014. Eighteen delegates attended the two day event. The first day (pm) consisted of a visit to a local shellfish depuration facility followed by a theoretical overview of shellfish purification approaches used commercially in the UK and across Europe. Numerous topics were covered in the second day. These included OCL performance in comparative (proficiency) testing, amendments to sample transporting time/temperature controls, 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.

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. This incorporated amendments to specifically relating to time/temperature controls where they affected the content of the protocol, and were agreed during the October 2014 network meeting. During the meeting, it was agreed that a standardised approach to sample receipting criteria with respect to time and temperature criteria would be preferable to OCL's. It was proposed and accepted by the OCL network that based upon the data generated by the NRL the following criteria could be justified by the data:

- Initiation of testing within 24 hours of collection where possible but with an absolute upper limit of 48 hours
- Sample receipting temperature between 1 and 10°C

It was noted that changes to existing protocols would require formal approval from the relevant Competent Authorities prior to amendment of procedures, which were subsequently approved. Following consultation with the laboratory network, the final document was distributed to the OCL network (January 2015).

The NRL participated in the BSI Food Microbiology Committee and provided oral and written comment on standards relevant to the area of shellfish microbiology (November 2014). The NRL also circulated key consultations on standards to the laboratory network.

The NRL held separate liaison meetings with the 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. Information and documents were provided to the PHE in support of its development of the general food microbiology NRL.

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

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 use of 4 tube dilutions for *E. coli* and the need to use the appropriate MPN calculator
iii. Problems with an unusually high proportion of void MPN tube combinations.
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 2014-2015, including the following:

- Addition of the NRL generic protocol to the methods section, with amended time/temperature controls (January 2015).
- Changes of address and contact details of OCL's identified from the October 2014 network meeting.
- Addition of network meeting notes from the 2014 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.

Advice was given to FSA in relation to an upcoming EU-wide surveillance of norovirus in Member State harvesting areas. The detailed response to the CA included advice on several aspects of the anticipated project including the following:

- Species of bivalve molluscs to be studied across the EU in the harmonised study
- Pathogens to be assessed
- Scope of the study approach, including type of sampling methodology employed and study duration

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 or via the NRL website. Specific papers circulated are listed below:

- Resolutions of the 13th Workshop of NRLs for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs
- 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 FSA in relation to the following:

- Proposals for the amendment of Regulation (EC) No. 882/2004, with regard to aspects relating to the responsibilities of reference laboratories
- Proposals for amending the EU standards for class A areas
- Proposals for controls improving 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 laboratory performance in whole animal distribution

In December 2014, the NRL organised a distribution comprising common mussels (*Mytilus edulis*) and Pacific oysters *(Crassostrea gigas*) for enumeration of *Escherichia coli* and detection of *Salmonella* spp. Whole matrix samples were provided to laboratories to test aspects of the methodologies not covered in the standard shellfish EQA scheme i.e. opening of shellfish and preparation of initial dilutions. Material was distributed to 13 UK OCLs. Performance assessment and participation are given in Tables 1 and 2. All samples, with the exception laboratory 578, were received within the time/temperature limits as recommended by the NRL. All laboratories analysed the samples on the day of arrival. Information provided by laboratories on the temperature of the samples on arrival showed the maximum temperature recorded by participants did not exceed the recommended transport temperature of <10°C. Ten laboratories returned duplicate *E. coli* MPN/100g results falling between ±3 SD of the participants' median for sample 1. Laboratories 166, 243 and 1160 reported one replicate result within ±3 SD of the participants' median. Twelve laboratories returned replicate *E. coli* MPN/100g results between ±3 SD of the participants' median for sample 2. Laboratories 243 reported one replicate result within ±3 SD of the participants' median. Eleven laboratories reported the absence of *Salmonella* spp. in sample 2. However laboratory 166 reported the presence of *Salmonella* spp.

Table 1: Summary of participation by OCLs – PT 56 Sample 1 and Sample 2

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

***Salmonella* spp. summary statistics**	**Sample 1 - Mussels**	**Sample 2 - Oysters**
Participants reporting results for *Salmonella* spp.	12	12
Participants reporting the presence of *Salmonella* spp.	0	1
Participants reporting the absence of *Salmonella* spp.	12	11

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	130	170	12	ND	2	170	110	12	ND	2
9	78	78	12	ND	2	220	78	12	ND	2
14	230	130	12	ND	2	220	170	12	ND	2
67	260	310	12	ND	2	110	170	12	ND	2
97	330	690	12	ND	2	330	330	12	ND	2
145	490	490	12	ND	2	330	330	12	ND	2
166	790	2400	9	ND	2	330	330	12	Present	0
243	3300	NE	4	ND	2	230	NE	7	ND	2
271	140	130	12	ND	2	78	130	12	ND	2
532[a]	78	210	12	NE	-	45	130	12	NE	-
578	330	230	12	ND	2	220	220	12	ND	2
1160	2300	330	9	ND	2	330	68	12	ND	2
1817	330	270	12	ND	2	78	130	12	ND	2

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

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

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 (SF048, SF049 and SF050). Twelve laboratories completed all three distributions during this reporting period for *E. coli* enumeration and eleven 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. Laboratory 271 did not examine distribution SF049. Laboratories undertaking microbiological examination of official control samples of bivalve molluscs are reminded that participation in the EQA scheme (as well as the NRL whole animal PT) is a requirement.

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

Lab no.	Distribution SF048		Distribution SF049		Distribution SF050		All distributions		
	SF0104	SF0105	SF0106	SF0107	SF0108	SF0109	Cumulative score	Max score	%
7	12	10	12	12	12	12	70	72	97
9	12	12	12	12	9	12	69	72	86
14	12	12	12	12	12	12	72	72	100
67	12	12	12	12	12	12	72	72	100
97	10	12	12	12	12	12	70	72	97
145	12	12	12	12	12	12	72	72	100
166	12	12	9	12	12	12	69	72	100
243	12	12	12	12	10	12	70	72	97
271	12	12	NE	NE	9	12	45	48	94
532	12	12	12	12	12	12	72	72	100
578	12	12	12	12	8	12	68	72	94
1160	12	10	12	12	12	12	70	72	97
1817	12	12	12	12	12	12	72	72	100

NE – Not examined.

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

Lab no.	Distribution SF048		Distribution SF049		Distribution SF050		All distributions		
	SF0104	SF0105	SF0106	SF0107	SF0108	SF0109	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	NE	NE	2	2	8	8	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 2014 (SF0104, SF0105), November 2014 (SF0106, SF0107) and February 2015 (SF0108, SF0109). 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 *scheme.*

The NRL participated in the PHE FEPTU *Vibrio* scheme. Six samples were analysed across three distributions in July 2014 (V0122, V0123), November 2014 (V0124, V0125) and February 2015 (V0126, V0127). The NRL results for detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* were satisfactory on two occasions for all samples but *V. cholerae* was detected in one sample from the last distribution. Follow up action regarding this poor performance was undertaken by the NRL, including a review of approach and a re-test of sample.

5.5 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 of Manila clams (*Tapes phillipinarum*) and Common mussels (*Mytilus edulis*) in October 2014 (PT 54). The NRL achieved a performance assessment of 100% for both *E. coli* and *Salmonella* spp. in sample 1. A performance assessment was not performed on sample 2 (Mussels) as reference results showed this sample not to be homogenous.

5.5 Participation in EURL PT for norovirus and hepatitis A.

The NRL participated in two PT distribution organised by the EURL for norovirus and hepatitis A virus in July 2014 (PT 53) and December 2014 (PT 55). PT 53 distribution comprised of two laboratory constructed (LENTICULE) samples. The NRL's performance score was 'A' for both norovirus (GI and GII) and HAV. PT 55 distribution comprised of four shellfish matrix and two laboratory constructed (LENTICULE) samples. The NRL's performance score was 'C' for norovirus (GI and GII) and 'A' for HAV. Performance scoring; A = satisfactory (100% accuracy), B = questionable (one incorrect result), C = unsatisfactory (two or more incorrect results). Norovirus (NoV) and HAV are scored separately. Follow up action regarding this poor performance was undertaken by the NRL, including an investigation alongside the EURL to identify the possible cause of poor performance.

5.6 Meetings, workshops and task forces

The NRL director participated in the 13th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held at the EURL, Weymouth, in May 2014. A report detailing participation and major outcomes was provided to the FSA and the laboratory network following the workshop. 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.

Dr Craig Baker-Austin Date…06/05/15……
NRL Director

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