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

Annual Technical Report for 2016/17

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SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2016 - MARCH 2017

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 2016-17 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 eighth meeting of OCLs undertaking microbiological testing of bivalve shellfish was held at the Cefas laboratory on the 20th and 21st of September 2016. Fifteen delegates attended the two-day event. The first day (pm) consisted of a practical laboratory-based session focussing on the different EU approved methods for detecting and enumerating *E. coli* in shellfish, namely pour plate, impedance and MPN-based methodologies. A wide variety of topics were covered on the second day. These included OCL performance in proficiency testing (PT), the experience of different OCLs (e.g. in England, Northern Ireland, Scotland etc) on testing, amendments to the current MPN calculator, background, use and variability of shellfish testing methods for *E. coli,* progress at ISO on revisions to the *E. coli* and Salmonella standards, 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 provided advice to several OCL labs regarding EPT testing procedures and issues with methodological discrepancies at different OCL's. This advice was required following anomalous MPN results from EPT samples provided to industry during the summer of 2016, which had unforeseen impacts, such as the rejection of samples for EPT purposes. As this involved OCLs, it was deemed to fall within the remit of the NRL. Investigations were carried out at Colindale and information provided to the FSA on this issue. Dissemination of information to prevent re-occurrence of these issues was given to the laboratory network in September 2016. The NRL also provided assistance to two OCLs regarding discrepancies in reporting of *E. coli* MPN tube combinations from the most recent PT round (Winter 2016).

The NRL has participated in the BSI Food Microbiology Committee during 2016-2017 and provided oral and written comments on standards relevant to the area of shellfish microbiology. The NRL also circulated key information on standards to the laboratory network and the competent authority, including changes to methods that impinged on current protocols. Future potential changes to relevant protocols were also discussed at the September OCL network meeting.

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. Information and documents were provided to PHE in support of its development of the general food microbiology NRL. The NRL has provided continued assistance in this area.

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

i. Appropriate methods for EPT purposes in LBS.
ii. Risks associated with vibrios in seafood produce such as prawns.
iii. The application and use of new MPN calculator, with assistance where necessary.
iv. Adequate reporting of results, with imapacts for classification purposes.
v. Advice on sample transport criteria, in particular time and temperature-related issues.
vi. Possible impacts of new class A criteria on testing approaches.

3.2 NRL website

The NRL website (**https://www.cefas.co.uk/nrl.aspx?RedirectMessage=true**) was maintained during the period by adding new material and removing obsolete material. Several additions to the NRL website took place in 2016-2017, including the following:

- Edits to 'Current activities' page.
- PT 62 results report added.
- Changes of address and contact details of OCL's identified from the September 2016 network meeting.
- Addition of the new EURL good practice guide (February 2017) to the NRL website.
- Addition of network meeting notes from the 2016 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.

Recent instances of issues related to methodological problems associated with *E. coli* testing approaches have been relayed to FSA. These included EPT testing issues (Colindale) in August 2016. The NRL subsequently provided a guidance document for EPT purposes to Beverley Kuster (FSA, Winter 2016) that provides a succinct summary for industry use. The NRL contributed to a FSA-led stakeholder meeting, May 2016, 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. *Ad hoc* advice was provided, including the provision of advice to FSA on MPN method variability (April 2016). Significant assistance and technical advice and oversight was provided to the CA with regards to the UK-side of the EU norovirus baseline survey, which began in November 2016.

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:

- Report consisting of notes and resolutions from the 15th Workshop of NRLs for meeting provided at the NRL workshop meeting, September 2016.
- 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.
- Class A criterion changes (January 2017).

4.3 Other advice

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

- Current or upcoming amendments to ISO methods and the associated impact on shellfish-associated methods (provided with quarterly RAG reports).
- Proposals for amending the EU standards for class A areas and potential impacts on laboratory testing.
- Human health risks associated with vibrios in prawn produce.

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

5.1 Assessment of OCL performance in whole animal distribution

In November 2016, the NRL organised a distribution comprising of Pacific oysters (*Crassostrea gigas*) for enumeration of *E. coli* and the detection of *Salmonella* spp.. Whole matrix and shellfish homogenate 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 internal temperature did not exceed recommended transport temperature of <10°C. All OCLs returned results for this distribution. Participation and performance assessments for *E. coli* and *Salmonella* spp. are given in Tables 1, 2 and 3. For sample 1, 10 OCLs returned replicate *E. coli* results between ±3 SD of the participants' median, with 7 receiving a maximum score of 12. OCLs 271 and 578 reported one replicate result and OCL 9 reported both replicate results between ±3 and ±5 SD of the participants' median and scored 9 and 2 respectively. OCLs 7, 9, 166 and 532 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. For sample 3, 12 OCLs returned replicate *E. coli* results between ±3 SD of the participants' median, with 11 received a maximum score of 12. OCL 532 had 2 points deducted as the tube combination selected and the MPN value reported for 1 replicate was not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL. For sample 4, 12 OCLs returned replicate *E. coli* results between ±3 SD of the participants' median, with 11 receiving a maximum score of 12. OCL 532 had 4 points deducted as the tube combination selected and the MPN value reported for both replicates were not consistent with rules given in ISO 7218:2007/Amd 1:2013 or MPN tables provided by the NRL. S*almonella* spp. results for sample 2, 11 OCLs returned results and reported the absence of *salmonella* spp. in this sample and received a score of 2. For sample 3 and 4, 12 OCLs returned results and reported the presence of *Salmonella* spp. in both samples and received a score of 2. OCL 532 did not examine samples for the presence of *Salmonella* spp. as they do not undertake *Salmonella* spp. testing.

Table 1. Summary statistics of OCLs' results – *E. coli*

E. coli	**Sample 1 - Oysters**	**Sample 3 - Homogenate**	**Sample 4 - Homogenate**
Participants reporting duplicate results for *E. coli* MPN	13	12	12
Participants reporting single MPN results only	0	0	0
Participants reporting MPN results within the expected range for both replicates	10	12	12
Participants reporting MPN results outside the expected range for one replicate	2	0	0
Participants reporting MPN results outside the expected range for both replicates	1	0	0
Participants reporting MPN results as censored results for one or both replicates	0	0	0

Table 2. Participants results and allocated scores (Whole animal matrix)

Lab ID	Sample 1 - Oysters			Sample 2 - Oyster	
	E. coli MPN/100g			*Salmonella* spp. in 25g	
	Rep. 1	Rep. 2	Score	Result	Score
7	230	310	10	Not detected	2
9	20	20	2	Not detected	2
14	230	170	12	Not detected	2
67	130	170	12	Not detected	2
97	220	78	12	Not detected	2
145	490	170	12	Not detected	2
166	170	110	8	Not detected	2
243	92	68	12	Not detected	2
271	40	20	9	Not detected	2
532	170	170	8	NE	-
578 [1]	45	20	9	NE	-
1160	45	45	12	Not detected	2
1817	170	40	12	Not detected	2

NE – Not Examined

[1] Sample 2 was not analysed as stated in the accompanying paperwork. Sample 2 was analysed for *E. coli* only.

Table 3: Participants results and allocated scores (Homogenate)

Lab ID	Sample 3 - Homogenate					Sample 4 - Homogenate				
	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	4900	11000	12	Present	2	13000	7900	12	Present	2
9	4900	2300	12	Present	2	1700	4600	12	Present	2
14	3300	3300	12	Present	2	4900	7900	12	Present	2
67	3300	7900	12	Present	2	2300	3300	12	Present	2
97	2300	4900	12	Present	2	7900	4900	12	Present	2
145	2300	2600	12	Present	2	4900	3100	12	Present	2
166	2300	3300	12	Present	2	4900	2300	12	Present	2
243	3300	4900	12	Present	2	4900	3300	12	Present	2
271	7900	7900	12	Present	2	7900	7900	12	Present	2
532	5400	4600	10	NE	-	3500	2400	8	NE	-
578 [1]	NE	NE	2	Present	2	NE	NE	2	Present	2
1160	2300	3300	12	Present	2	7900	4900	12	Present	2
1817	1700	7900	12	Present	2	3300	3300	12	Present	2

NE – Not Examined

[1] Samples 3 and 4 were not analysed as stated in the accompanying paperwork. Samples 3 and 4 were analysed for *Salmonella* spp. only.

5.2 Assessment of OCL 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 (SF054, SF055 and SF056). Scores were allocated in accordance with the Cefas/PHE shellfish EQA scheme scoring system. Twelve 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 4 and 5. All 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. Laboratory 14 was unable to complete all 3 distributions as the laboratory informed the NRL of its closure in April 2017.

Table 4. Performance of UK OCLs in Cefas/PHE EQA distributions for *E. coli*

OCL ID	Distribution SF054		Distribution SF055		Distribution SF056		All distributions		
	SF0116	SF0117	SF0118	SF0119	SF0120	SF0121	Cumulative score	Max score	%
7	12	12	12	12	12	12	72	72	100
9	12	12	8	7	12	12	63	72	88
14	12	12	12	12	NE	NE	48	48	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	8	8	64	72	89

[a] This laboratory closed in 2017
[b] This laboratory does not undertake *Salmonella* testing of official control samples.
NE – Not examined.

Table 5. Performance of UK OCLs in Cefas/PHE EQA distributions for *Salmonella* spp.

OCL ID	Distribution SF054		Distribution SF055		Distribution SF056		All distributions		
	SF0116	SF0117	SF0118	SF0119	SF0120	SF0121	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 [a]	2	2	2	2	NE	NE	8	8	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 [b]	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 closed in 2017
[b] 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 2016 (SF0116, SF0117), November 2016 (SF0118, SF0119) and February 2017 (SF0120, SF0121). 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 2016 (V0134, V0135), November 2016 (V0136, V0137) and February 2017 (V0138, V0139). The NRL results for the detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* were satisfactory for all samples.

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

The NRL participated in the EURL PT distribution (PT 64) for *E. coli* enumeration in the whole animal distribution comprising of three Pacific oysters (*Crassostrea gigas*) in November 2016. The NRL achieved performance assessment of 100% for *E. coli* and Salmonella for all samples.

5.6. 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 2016 (PT 65) and September 2016 (PT 61). PT 65 distribution comprised of two laboratory constructed (LENTICULE) samples. The NRL scored a satisfactory (100% accuracy) for all performance measures (presence/absence and Quantification). PT 61 distribution comprised of four shellfish matrix and two constructed (LENTICULE) samples. The NRL scored a satisfactory (100% accuracy) for presence/absence data for both sample types. For quantification, an unsatisfactory for Norovirus Genogroup II (two or more results fall outside ±2 δMAD of participants' median, or one or more result falls outside ±2.58 δMAD of participants' median) was recorded. Follow-up action regarding this poor performance was undertaken by the NRL, including a review of the SOPs and a re-test of sample.

5.7 Meetings, workshops and task forces

The NRL director participated in the 16th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held in Split, Croatia, May 2016. Minutes and an overview report detailing participation and major outcomes will be 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, EA and industry following the initiation of the shellfish working group.

Dr Craig Baker-Austin Date…18/05/17……

NRL Director

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