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

Annual Technical Report for 2012/13

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SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2012 - MARCH 2013

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 2012-13 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

2.1 Plan and organise proficiency tests between OCLs

i. Provision of proficiency tests

In November 2012, the NRL organised a distribution comprising common mussels (*Mytilus edulis*) and cockles (*Cerastoderma edule*) for enumeration of *Escherichia coli*. 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. Two cockle samples were included for a following year in the distribution. Material was distributed to 13 UK OCLs. Participation and performance assessments for *E. coli* for each sample are given in Tables 1, 2, 3 and 4. All OCLs returned results for this distribution. For the mussel sample (PT 47 – sample 1) (Table 1) all laboratories that returned results reported *E. coli* MPN/100g within the expected range and received a maximum score of 12. For both cockle samples (PT 47 – sample 2 and 3) (Table 2 and 3) 10 laboratories (77%) returned replicate results within ±3 theoretical standard deviations of the participant's median (expected range). For sample 2 three laboratories returned one replicate result outside the participants median ±3 theoretical standard deviations and for sample 3, 2 laboratories returned one replicate result outside the participants median ±3 theoretical standard deviations and 1 laboratory returned a single replicate outside ±5 theoretical standard deviations of the median. The observed variability in laboratories results for the cockle samples, which was similar to that observed in the 2011 PT distribution, was discussed at the annual official control network meeting in October 2012 and it was agreed to seek further information from the OCLs to facilitate further analysis of the PT results. A summary of the responses from OCLs, together with a summary analysis of those results, has been distributed. Further statistical analysis of the data will be undertaken and reported separately. Due to the marked variability in the cockle results from the distribution, follow-up action was not instituted for low scores for samples 2 and 3.

Table 1. Summary of participation by OCLs – PT 47 Sample 1 – Mussels

Participation summary - Sample 1 – Mussels	
Total participants reporting duplicate results for *E. coli* MPN	13
Participants reporting MPN results within the expected range[1]	13

[1]expected range = participants' median ± theoretical 3SD ($5.3 \times 10^{1} - 1.9 \times 10^{3}$)

Table 2. Summary of participation by OCLs – PT 47 Sample 2 – Cockles

Participation summary – Sample 2 – Cockles	
Total participants reporting duplicate results for *E. coli* MPN	13
Participants reporting MPN results within the expected range[1]	10
Participants reporting MPN results outside the expected range for one replicate	3
Participants reporting MPN results outside the expected range for both replicates	0

[1]expected range = participants' median ± theoretical 3SD ($3.7 \times 10^{1} – 1.4 \times 10^{3}$)

Table 3. Summary of participation by OCLs – PT 47 Sample 3 – Cockles

Participation summary – Sample 3 – Cockles	
Total participants reporting duplicate results for *E. coli* MPN	13
Participants reporting MPN results within the expected range[1]	10
Participants reporting MPN results outside the expected range for one replicate	2
Participants reporting MPN results outside the expected range for both replicates	1

[1]expected range = participants' median ± theoretical 3SD ($2.5 \times 10^{2} – 9.0 \times 10^{2}$)

Table 4. Performance assessment of returned participants' results

Lab ID	Sample 1 *E. coli* MPN/100g			Sample 2 *E. coli* MPN/100g			Sample 3 *E. coli* MPN/100g		
	Replicate 1	Replicate 2	Score	Replicate 1	Replicate 2	Score	Replicate 1	Replicate 2	Score
7	490	220	12	80	70	12	20	20	12
9	50	130	12	50	20	9	20	70	12
14	330	790	12	80	230	12	230	170	12
67	70	130	12	50	<20	9	110	<20	9
97	170	220	12	80	20	9	20	20	12
145	230	490	12	130	230	12	230	130	12
166	170	700	12	130	140	12	20	40	12
243	790	330	12	330	230	12	460	170	9
271	90	170	12	50	50	12	40	20	12
532	130	230	12	230	130	12	230	80	12
578	220	230	12	330	330	12	230	230	12
1160	230	310	12	330	130	12	70	80	12
1817	490	330	12	230	230	12	790	490	6

ii. Provision of 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/HPA EQA shellfish scheme (SF042, SF043 and SF044). Thirteen laboratories completed three distributions during this reporting period for *E. coli* enumeration and eleven for the detection of *Salmonella* spp. Laboratory performance is summarised in Tables 5 and 6. All laboratories apart from one achieved scores in excess of 70% over three distributions, the measure of performance considered by the scheme organisers to demonstrate satisfactory performance. Laboratory 243 did not examine distribution SF042 due to workload conflicts and did not examine distribution SF043 as the NRL whole animal PT was expected around the time of that distribution. 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. The NRL contacted Laboratory 243 to remind them of this. Laboratory 67 achieved a very low score in one of the samples of distribution SF043 and the NRL contacted the laboratory in

order to ensure that an investigation was undertaken. This was done and the outcome reported to the NRL.

Table 5. Performance of UK OCLs in Cefas/HPA EQA distributions for *E. coli*

Lab no.	Distribution SF042		Distribution SF043		Distribution SF044		All distributions		
	SF0092	SF0093	SF0094	SF0095	SF0096	SF0097	Cumulative score	Max score	%
7	12	12	12	12	12	12	72	72	100
9	12	10	7	7	12	12	60	72	83
14	12	12	12	12	12	12	72	72	100
67	12	12	2	12	12	12	62	72	86
97	12	12	8	12	12	12	68	72	94
145	12	12	12	12	12	12	72	72	100
166	12	12	12	12	12	12	72	72	100
243	NE	NE	NE	NE	12	12	24	72	33
271	12	12	10	12	12	12	70	72	97
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

NE – Not examined.

Table 6. Performance of UK OCLs in Cefas/HPA EQA distributions for *Salmonella* spp.

Lab no.	Distribution SF042		Distribution SF043		Distribution SF044		All distributions		
	SF0092	SF0093	SF0094	SF0095	SF0096	SF0097	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	NE	NE	NE	NE	2	2	4	12	33
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.

2.2 Supervision, liaison with and provision of advice to OCLs

i. Testing procedures

The NRL has been liaising with laboratories with respect to a major revision of the NRL generic protocol for the enumeration of *E. coli* in bivalve molluscs. This review is awaiting publication of ISO 16649-3 as a full standard, and an amendment to ISO 7218, before making any consequential amendments and circulating to the laboratory network for final comment.

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.

The NRL held separate liaison meetings with the Health Protection Agency 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.

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

i. Follow-up investigations and actions further to concerns with performance in proficiency testing (NRL whole animal PT and the Shellfish EQA Scheme).
ii. Problems with an unusually high proportion of void MPN tube combinations.

The NRL undertook a courtesy visit to the new shellfish testing unit at HPA Porton.

ii. 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.

iii. NRL laboratory network meeting

The fourth network meeting of laboratories undertaking microbiological testing of bivalve shellfish was held at the NRL on the 16th and 17th October 2012. Twenty-one delegates attended the two day event although a small number did not attend the first day. The first day (pm) comprised practical activities and seminars relating to detection and enumeration of pathogenic vibrios and viruses and microbial source tracking. Topics covered on the second day included OCL performance in proficiency testing, the effects of interrupted analysis on *E. coli* results (with respect to procedures at weekends), progress at ISO on revisions to standards relevant to E. coli, Salmonella spp. and pathogenic vibrios in bivalves, and developments at both the European and international levels.

3. Provision of assistance to the CA

3.1 Provision of technical advice and support to CA in development and management of the OCL network.

No major changes occurred in the laboratory network during the period (after several years of continuing change) but minor changes took place with regard to contact details for several laboratories. Revised details were placed on the NRL website. Details of UK laboratories accredited for the microbiological testing of shellfish (OCLs and others) were supplied to FSAS in response to a query.

3.2 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. Specific papers circulated are listed below:

- Report on the 11th Workshop of NRLs for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs.
- Thematic Agenda of 12th Workshop of Microbiological NRLs, 24th - 26th April 2012.
- Report of an EURL proficiency test distribution for *E. coli* in bivalve molluscs
- Report of an EURL proficiency test distribution for norovirus and hepatitis A
- Report of the 2nd International Workshop on Shellfish Classification and Management
- Community Guide to the monitoring and classification of bivalve mollusc harvesting areas

An invitation to participate in a EURL norovirus training workshop was circulated to OCLs. Information was also circulated in relation to methodology for the detection of viruses on soft fruit.

3.3 Other advice

The NRL gave *ad hoc* advice to FSA on a number of occasions in relation to the testing of bivalve shellfish for norovirus and also the collection of shellfish samples from outbreaks for norovirus testing.

4. Collaboration with the EURL for monitoring bacteriological and viral contamination of bivalve molluscs - Participation in proficiency testing (PT) and method validation studies organised by the EURL.

4.1 Participation in EURL/HPA EQA shellfish scheme for E. coli and Salmonella spp.

Performance assessments for EURL/HPA EQA shellfish scheme for *E. coli* and *Salmonella* spp. were undertaken for distributions SF042, SF043 and SF044 in 2012/13. 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.

4.2 Participation in HPA EQA for pathogenic Vibrio *spp.*

The NRL participated in the HPA FEPTU *Vibrio* scheme. Six samples were analysed across three distributions in July 2012 and March 2013 (V035, V036 and V037). The NRL results for detection of *V. parahaemolyticus* and *V. cholerae* were satisfactory on two occasions for both samples but detected the presence of *V. cholerae* in one sample from the last distribution.

4.3 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 Pacific oysters (*Crassostrea gigas*) and Common mussels (*M. edulis*) in September 2012. The NRL achieved performance assessment of 100%.

4.4 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 2012 (October (PT 46)). The distribution comprised of both matrix and

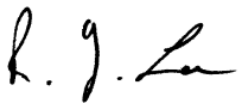
laboratory constructed samples (4 and 2 samples). The NRL scored 100% for all performance measures (relative accuracy, specificity and sensitivity).

4.5 Participation in EURL pre-validation studies in support of the development of new analytical methods for the detection of viruses in shellfish.

The NRL maintained accreditation for the CEN method. The ongoing competency of analysts to carry out the method has been assessed. Comparisons of real-time primer/probe combinations for norovirus GI were carried out. The performance of nuclisens magnetic extraction materials in the absence of the miniMag extraction station were assessed.

4.6 Meetings, workshops and task forces

The NRL director and a virology specialist participated in the 11th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held in Weymouth in April 2012. A report detailing participation and major outcomes was provided to the FSA and the laboratory network following the workshop.



Dr Ron Lee Date…20/06/13……

NRL Director

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