

National Reference Laboratory: Annual report

FS616026/C8333 - Bacterial contamination of bivalve molluscs

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Contents

1. Executive summary	5
2. Glossary	6
3. Introduction	7
4. Core function: secretariat services.....	7
5. Core function: advice and representation within the UK and internationally (including a summary of meetings attended and any international collaboration activities)	9
6. Core function: production of standard operating procedures, codes of practice and guidance documents.....	10
7. Core function: compliance assessment via audits and ring trials.....	11
8. Core function: co-ordination within the UK of international initiatives	13
9. Core function: communication of results and data use and proficiency testing.....	13
10. Discussion of specialised areas e.g. research activities	14
11. Link to NRL website.....	15
12. Annexes – documents produced from NRL activities	15

1. Executive summary

This report outlines the key delivery outputs from the Cefas National Reference Laboratory (NRL) FS616026/C8333 - Bacterial contamination of bivalve molluscs for the period April 2022 to March 2023.

Key outputs were:

- Maintenance of expertise in bacteriological analysis of bivalve molluscs including maintenance of accreditation for all relevant methods pertaining to the examination of live bivalve shellfish.
- Successful participation in all relevant proficiency testing schemes.
- Maintenance of website for the NRL.
- Provision of NRL network meeting, December 2022.
- Active participation in committees and working groups addressing standardisation of methods relevant to the bacteriological examination of live bivalve shellfish.
- Provision of advice to official laboratories and competent authorities on methodologies relevant to the examination of live bivalve shellfish.
- Contribution to review of official methodology and capabilities within the UK for *E. coli* testing of shellfish.
- Provision of technical assessment of the feasibility of the colony count (pour plate) method for use in a UK context.

2. Glossary

BSI	British Standards Institution
CA	Competent Authority
Cefas	Centre for the Environment, Fisheries and Aquaculture Science
CEN	European Committee for Standardisation
<i>E. coli</i>	<i>Escherichia coli</i>
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
ISO	International Organisation for Standardisation
KPI(s)	Key Performance Indicator(s)
OCR	Official Controls Regulations
OL(s)	Official Laboratory (ies)
LOD	Limit of Detection
LOQ	Limit of Quantitation
NRL	National Reference Laboratory
MALDI ToF MS	Matrix assisted laser desorption ionisation time of flight mass spectrometry
MPN	Most Probable Number
PT	Proficiency Testing
SSWG	Shellfish Stakeholder Working Group
UKAS	United Kingdom Accreditation Service
UK HSA	UK Health Security Agency (previously known as Public Health England)

3. Introduction

This annual technical report summarises the activities carried out by the NRL during the financial year 2022-23 (April 2022 - March 2023). Delivery of the responsibilities of the NRL have been divided into the following key objectives of the Agreement signed between FSA and Cefas:

1. Provision of core functions/secretariat services (Section 4)
2. Advice and representation within the UK and internationally (Section 5)
3. Production of standard operating procedures, codes of practice and guidance documents (Section 6)
4. Compliance assessment via audits and PT (Section 7)
5. Co-ordination within the UK of International initiatives (Section 8)
6. Communication of results and data use (Section 9)
7. Discussion of specialised areas e.g. research activities (Section 10)
8. Link to NRL website (Section 11)

4. Core function: secretariat services

Item	Activity in period
Disseminating relevant information to the CA and OLS	In addition to the scheduled project review meetings with the FSA/FSS on 30th June, 21st October, 15th December and 22nd March, the NRL Director and team have been in regular contact with the FSA, OLS and industry on the following topics: 1. Microbiological testing, with information on processing of shellfish and pour plate method as well as MALDI ToF MS. 2. Request for information on risk assessment/testing of vibrios by UK HSA laboratories. 3. Attendance at a shellfish stakeholder working group meeting in June and December 2022 and input into discussions regarding application of methods. The NRL participated in a number of additional meetings with FSA and FSS staff including the Cefas Shellfish Safety Familiarisation day on 21/09/22 (contributing to sessions on National Reference

	Laboratory functions, and Viruses and Other Emerging Risks), the FSA NRL Symposium on 05/10/22 (delivering a short overview of the work undertaken by the NRL and participating in general discussions) and the Cefas/FSA Incidents Team Familiarisation day on 14th October (providing information on the possible assistance Cefas (including as the NRL). The NRL network meeting was held on 07/12/22 where relevant information regarding the application and changes to methods, application of new testing methods etc were communicated to the OL network.
Co-ordinating the activities of OLs responsible for analysis of official control samples to ensure verification of compliance with feed and food law	No activity delivered/requested in period.
Providing regular updates to the CA, OLs and other labs	As per the FSA/Cefas agreement, formal updates are in the form of monthly technical and financial reports plus more in-depth interim and final reports submitted in the Autumn and Spring of each year. Regular contact has been maintained with the FSA to provide updates on progress with delivery of research tasks etc. See section 12 for list of reports submitted in reporting period.
Creation & maintenance of NRL website	Some edits to specific content areas of the NRL website have been carried out in the last 12 months. Edits have included addition of new technical reports, and removal of reference to EU regulations, retained UK legislation etc. Specific maintenance of sections such as methods, current activities, PT, laboratory network information, and an information centre page with reports, publications and other relevant documents relating to bivalve shellfish sanitation were also carried out. Notes from the NRL network meeting were also added to the NRL website (restricted section). See section 11 of this report for link to the website

5. Core function: advice and representation within the UK and internationally (including a summary of meetings attended and any international collaboration activities)

Item	Activity in period
Providing impartial advice to the NRL laboratory network on analytical methodology and risk assessment	Advice to OL regarding identification of <i>Vibrio</i> species in seafoods. Various discussions with OLs on echinoderm and gastropod testing for <i>E. coli</i>, including input into best practise and possible changes to methodology. Provided input to various discussions regarding the use of alternative methods (e.g. colony-count) with various labs. Provided a questionnaire on alternative testing methods, which were circulated and collated from the laboratory network (presented at NRL network meeting on 07/12/22). Various communications with OLs regarding use of MALDI ToF MS for rapid identification of bacterial strains e.g vibrios etc.
Representing the UK at relevant international meetings and working groups	The NRL attended (virtually) the ISO/TC 34/SC 9 plenary meeting in June and August 2022 and presented at this meeting on method standardisation efforts regarding vibrios. Craig Baker-Austin hosted a meeting of ISO WG 27 (pathogenic vibrios, see details in standardisation section). The NRL also attended (virtually) the BSI AW/9 meetings (UK committee on standardisation of Food Microbiology Methods) in May and August 2022.
Participating in other international activities	The NRL director (Dr Craig Baker-Austin) was invited by EFSA to participate in an international working group regarding vibrios in seafood and their associated risks (December 2022). As agreed with FSA, attendance at these meetings is outside of the scope of this NRL and therefore outcomes are not reported here.
Advising on best scientific practice	The NRL provided miscellaneous advice to FSA and FSS on a number of matters during the last year, including a significant amount of advice on colony count method (e.g. update meetings on 17/07/22 and 23/02/23), and the use of specific methods used by official labs.
Maintaining expertise	See below (involvement in standardisation activities) for attendance at national and international scientific conferences, workshops and for activities relating to standardisation (e.g. <i>Vibrio</i> work through ISO and

	more general microbiological work through BSI AW/9, Section 5.6). Laboratory activities have been undertaken towards maintenance of UKAS accreditation of the NRL.
Involvement in standardisation activities relevant to work area	The NRL has been actively involved in method standardisation activities linked to general areas of competence. Craig Baker-Austin participated in ISO WG standardisation efforts (vibrios) throughout 2022 and early 2023. These included reviewing the final drafts of International Standards (ISO) documents and providing comments to the secretariat, clarifying issues related to the ISO 21872-1 (vibrio) test methods. Craig Baker-Austin convened at ISO WG27 (Vibrio) meeting on 11/10/22.
Supporting FSA/FSS with emergency situations	No activity delivered/requested in period.

6. Core function: production of standard operating procedures, codes of practice and guidance documents

Item	Activity in period
Contributing to the development of standardised protocols and advisory documents	Liaison with FSA & FSS, contribution to discussions and initiation of laboratory work on echinoderm and gastropod testing with the aim to develop an NRL SOP for <i>E. coli</i> testing in these species. These were forwarded for discussion at the NRL network meeting on 07/12/22, with the aim of outlining lab needs prior to changes to current SOPs. To this end, the NRL has initially drafted a working document on shucking methods for echinoderms and gastropods, based on request from FSA (submitted to FSA 12/05/22). The NRL has also prepared an SOP for the use of the colony-count methodology (completed February 2023). This SOP will be sent to FSA (March 2023) as an appendix to the colony count work report, and following feedback will be placed on NRL website and circulated to OLS.
Validating reagents	No activities in this period.

7. Core function: compliance assessment via audits and ring trials

Item	Activity in period
Ensuring consistency and quality of testing approaches	The NRL provided a 12-monthly desk-based review of the accreditation status of UK approved testing laboratories for statutory testing and found all laboratories in the network with the current and necessary accreditation status for classification testing of live bivalve shellfish. The NRL provides appropriate support for OLs in relation to problems experienced in the examination of foods within scope or the bacteriological examination of bivalve molluscs. During this 12-monthly period one issue was observed with OL 166 during an EQA distribution where both replicate <i>E. coli</i> MPN results reported were outside the median +4SD values and the tube combination reported were inconsistent with the MPN result reported for both replicates and the rules given in ISO 7218:2007/Amd 1:2013. An underperformance letter was issued, and the OL completed an internal investigation which concluded a laboratory transcription error occurred when reporting the results. Tayside Scientific Services were added by FSS as an additional official testing laboratory to the network in December 2022.
Organising PT for GB OLs & ensuring appropriate follow up	PT 91 (whole animal <i>E. coli</i> and Salmonella) was circulated to all UK OLs in November 2022. Ten OLs received the samples within 24 hours of dispatch as recommended by the NRL. Eight OLs reported satisfactory results for both <i>E. coli</i> and Salmonella spp. OL 9 and OL 145 had scores deducted for sample 1. On receiving the material OL 243 informed the NRL that they could not examine the material as they were not aware of its arrival and that the laboratory was carrying out staff training during the same week. On further investigation it was identified that the staff member on the NRL contact list was on long term sick and no forwarding email was in place. This was raised with the FSA.
Co-ordinating the participation of GB OLs and other relevant laboratories in international method validation studies and other initiatives	No activity delivered/requested in period

Participating in PT and method validation studies organised by international organisations	EURL Salmonella PT – No PT distribution has been organised for shellfish over the 12-month period. EURL <i>E. coli</i> PT – PT 32 distribution was organised in May 2022 and comprised of one shellfish sample. The EURL noted a low level of natural contamination present in the sample. Due to this and the results reported by participants no statistical assessment could be made. The EURL concluded all participants reporting a >20 MPN result obtained a satisfactory score. The NRL obtained a satisfactory result for this PT exercise. EQA Shellfish scheme: • Results received for SF070 (April 2022). Full marks were recorded for the enumeration of <i>E. coli</i> and the detection of <i>Salmonella</i> spp. • Participated in SF071 (July 2022). Only 1 replicate <i>E. coli</i> result was reported for both samples. Full marks were recorded for the enumeration of <i>E. coli</i> and the detection of <i>Salmonella</i> spp. • Participated in SF072 (December 2022). Full marks were recorded for the enumeration of <i>E. coli</i> and the detection of <i>Salmonella</i> spp. EQA <i>Vibrio</i> scheme: • Participated in V062 (April 2022). <i>Vibrio parahaemolyticus</i> was reported correctly for both samples. • Participated in V063 (July 2022). <i>Vibrio parahaemolyticus</i> was reported correctly for both samples. • Participated in V064 (December 2022). <i>Vibrio parahaemolyticus</i> was reported correctly for both samples.
Co-ordinating training exercises to promote best laboratory practice in respect of analysis	No activity delivered/requested in period. Training and/or demonstration activities are delivered in conjunction with the annual NRL network meeting.
Providing OLs advance notice of PT	Notification for PT 91 sent out to all designated shellfish UK OLs on the 17/10/22 for a distribution date of 21/11/22.

8. Core function: co-ordination within the UK of international initiatives

Item	Activity in period
Co-ordinating the implementation in the UK of international initiatives	The NRL was solicited for advice to FSA regarding a CODEX update on <i>Vibrio</i> guidance (21/09/22). The NRL provided a detailed response to FSA food policy team request on 31/10/22.

9. Core function: communication of results and data use and proficiency testing

Item	Activity in period
Providing regular updates to the CA	The NRL has provided regular reporting to the FSA, through the means of email, and via monthly summaries of costs showing staff effort and non-pay costs throughout the year, and monthly technical delivery summaries. Quarterly review meetings were held on 30/06/22, 21/10/22, 15/12/22 and 22/03/23.
Notification of deviations or unusual occurrences	No deviations identified in this reporting period.
Completing annual reports	The annual report for FY21/22 was submitted to the FSA on 29/04/22. It was subsequently approved and posted on the NRL website on 01/07/22. The interim report for the first half of FY22/23 was submitted to the FSA on 31/10/22. The draft annual report for FY22/23 was submitted to the FSA on 30/03/23.
Managing data and information	Data and documents associated with the NRL function have been stored in accordance with Cefas' data management systems.
Providing meeting	Notes from contractual update meetings were provided to FSA within

reports	the agreed timeframe. See list of reports in Section 12.
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10. Discussion of specialised areas e.g. research activities

Note: the NRL workplan for FY22/23 was drafted for review by FSA at the Q1 review meeting but only formally agreed at the Q2 review meeting held on 20/10/2022 (as listed in the below table). Given that resources allocated to research activities are limited each year, the volume of activity delivered in Q1 and Q2 was deliberately kept minimal to ensure that no resources were spent on items which may not be approved by FSA or which may yet be rescoped before formal approval.

Agreed priority for FY22/23	Activity in period
Analysis of the pour plate method for <i>E. coli</i> testing purposes	The NRL received a request from the FSA to carry out in-depth analysis regarding the potential use of the colony count (pour plate) method for routine/official use in a UK context. Briefly, this feasibility study involved: a). A review of the feasibility of lowering LOQ (limit of quantitation) of the pour plate method.; b). Assessing feasibility of increasing the LOQ using additional dilutions., c) A comparison study between pour plate method and reference method: for maximum 40-50 Scottish official control samples, to be selected from list of areas currently monitored. As the pour plate method is not used routinely for testing in the UK, the NRL was tasked with testing a limited number of official samples using both methods (pour plate and MPN). The focus of this work was to gain direct practical experience of the method, ascertain key practical and logistical issues from its routine use and to subsequently develop and refine an SOP based on this work. The NRL provided regular updates to the FSA on this work including a meeting on 23/02/23 to provide information and an update on progress of this work. A report of the outcomes is in draft and will be delivered to FSA by 31/03/23.
Validation of current <i>E. coli</i> MPN method for gastropods /	The NRL conducted a significant amount of work on the use of echinoderms and gastropods for routine microbiological testing. Various practical work using existing protocols as the basis for initial

echinoderms	validation work on two species – periwinkles and sea urchins was completed, which consisted of trialing various approaches for the processing of these species. The work was completed in April 2022 and the report based on this work sent to FSA on 12/05/22 (internal report, unpublished). The work was also presented at the December 2022 NRL network meeting.
Effects of different storage temperatures on media	We have designed a preliminary experimental approach to study the impact of media temperatures. Preliminary work has been completed and a draft of this has now been written. Work was presented and discussed at the NRL network meeting in December 2022.

11. Link to NRL website

[UK National Reference Laboratory \(NRL\) for bacteriological contamination of bivalve molluscs - Cefas \(Centre for Environment, Fisheries and Aquaculture Science\)](#)

12. Annexes – documents produced from NRL activities

Date produced	Title of document
Monthly	Monthly financial and technical updates
29.04.22	Draft annual report 2021-22
12.05.22	Internal report: Use of the MPN method in periwinkles and sea-urchins submitted to FSA.
24.06.22	NRL draft work programme (FY22/23) sent to FSA.
01.07.22	Final annual report 2021-22 published
15.07.22	Draft minutes of NRL Q1 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA

28.09.22	PT 92 (EQA) report circulated to all official laboratories
27.10.22	Draft minutes of NRL Q2 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
31.10.22	Draft interim report 2022-23
07.12.22	Final interim report 2022-23
06.01.23	Draft minutes of NRL Q3 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
26.01.23	Notes from NRL meeting circulated (to FSA and OLs) and placed on NRL website
27.01.23	PT 91 (whole animal) report circulated to all official laboratories
28.03.23	Draft minutes of NRL Q4 meeting submitted to FSA within the agreed turnaround time.
30.03.23	Draft annual report 2022-23
31.03.23	Internal report: Pour plate methodology report submitted to FSA

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We are the government's marine and freshwater science experts. We help keep our seas, oceans and rivers healthy and productive and our seafood safe and sustainable by providing data and advice to the UK Government and our overseas partners. We are passionate about what we do because our work helps tackle the serious global problems of climate change, marine litter, over-fishing and pollution in support of the UK's commitments to a better future (for example the UN Sustainable Development Goals and Defra's 25-year Environment Plan).

We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge. Together we can understand and value our seas to secure a sustainable blue future for us all, and help create a greater place for living.

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