Centre for Environment Fisheries & Aquaculture Science

Cefas

National Reference Laboratory: Annual report

FS430551/C8351 Foodborne Viruses

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Contents

1. Executive summary

This report outlines the key delivery outputs from the Cefas National Reference Laboratory (NRL) FS430551/C8351 – Foodborne Viruses for the period April 2023 to March 2024.

Key outputs were:

- Maintenance of expertise and accreditation for quantification of norovirus and hepatitis A virus (HAV) in bivalves.
- Quantification of norovirus and HAV in soft fruit and vegetables was added to the scope of accreditation for the NRL.
- Completion of a research project examining methods for detection of norovirus in seaweed products, indicating the suitability of the ISO 15216-1 method (with appropriate modifications) for testing seaweed samples in most cases.
- Initial investigations into two methods for estimation of infectious norovirus (PMAxx treatment and long-range RT-qPCR) which showed promise in terms of demonstration of the practicability and utility of the methods.
- Testing for a small number of norovirus outbreak-related oyster samples (RT-qPCR and amplicon sequencing for norovirus, RT-qPCR for sapovirus) to increase the dataset with the aim of improving understanding of the value of testing in outbreak management.
- The NRL director actively participated in committees and working groups addressing standardisation of methods relevant to foodborne viruses.
- The NRL participated in five proficiency testing schemes.
- The website for the NRL was maintained.

2. Glossary

BSI	British Standards Institution
CA	Competent Authority
Cefas	Centre for the Environment, Fisheries and Aquaculture Science
CEN	European Committee for Standardisation
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Office of the United Nations
FSA	Food Standards Agency
FSS	Food Standards Scotland
FY	Financial Year
HAV	Hepatitis A Virus
IEC	International Electrochemical Commission
ISO	International Organisation for Standardisation
NRL	National Reference Laboratory
OL	Official Laboratory
PCR	Polymerase Chain Reaction
PT	Proficiency Testing
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcription - Quantitative Polymerase Chain Reaction
SC	(in standardisation) Sub-committee
TC	(in standardisation) Technical Committee
UKHSA	UK Health Security Agency
UKAS	United Kingdom Accreditation Service
UV-C	Ultraviolet C
WG	(in standardisation) Working Group

3. Introduction

This annual technical report summarises the activities carried out by the NRL during the financial year 2023-24 (April 2023 - March 2024). 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 06/07/23, 20/09/23 and 19/01/24, the NRL Director and team have been in regular contact with the CA throughout the year on topics including whole genome sequencing for norovirus infectivity assessment, digital PCR for norovirus quantification and hepatitis E virus in wastewater. The NRL attended a meeting of the Shellfish Stakeholder Working Group on 08/02/24 to support the FSA on discussion of technical matters, including those related to norovirus in oysters and other shellfish. The NRL provided a standalone presentation on virus infectivity methods to the FSA risk assessment team.
Co-ordinating the activities of OLs responsible for analysis of official control samples to ensure verification of compliance with feed and food law	No designated OLs in network.
Providing regular updates to the CA, OLs and other labs	No designated OLs in network. As per the FSA/Cefas agreement, formal updates are in the form of monthly technical and financial reports plus the annual report submitted at the end 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	Routine maintenance of the NRL website has been carried out during the year including addition of new or updated documents, review and correction of minor errors etc. 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	In addition to ad hoc advice on virus methods provide to the CA, the NRL provided formal peer review of the FSA Risk assessment to support development of advice and guidance to manage outbreaks of norovirus in oysters, published during the year.
Representing the UK at relevant international meetings and working groups	The NRL attended (virtually) the CEN/TC463 and ISO/TC34/SC9 food microbiology standardisation plenary meetings in June 2023 as part of the delegation representing BSI.
Participating in other international activities	No activity delivered/requested in period.
Advising on best scientific practice	The NRL has maintained method protocols on its website.
Maintaining expertise	See below (involvement in standardisation activities) for activities relating to standardisation. The Cefas Food Safety Scope of Accreditation to ISO/IEC 17025 standard (including quantification of norovirus and hepatitis A virus in bivalve molluscs) was confirmed following the UKAS annual visit in October 2023. Quantification of norovirus and hepatitis A virus in soft fruits, leaf, stem and bulb vegetables was added to the Cefas Food Safety Scope of Accreditation after successful assessment of the dossier of evidence submitted to UKAS.
Involvement in standardisation activities relevant to work area	The NRL continued to actively participate in a number of relevant standardisation groups including ISO/TC34/SC9/WG31 (Hepatitis E virus), the Project Group of ISO/TC 34/SC 9/WG 3 (Method validation) covering “Viruses and Parasites”, BSI AW/009 (UK mirror group on food microbiology standardisation) and CEN/TC463/WG1 (basic requirements for PCR methods in food microbiology).

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	No activity delivered/requested in period.
Validation of reagents	No activity delivered/requested in period.

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

Item	Activity in period
Ensuring consistency and quality of testing approaches	No designated OLs in network.
Organising PT for GB OLs & ensuring appropriate follow up	No designated OLs in network.
Co-ordinating the participation of GB OLs and other relevant	No designated OLs in network. No activity delivered/requested in period.

laboratories in international method validation studies and other initiatives	
Participating in PT and method validation studies organised by international organisations	During the reporting period, the UK NRL participated in 5 relevant proficiency testing (PT) schemes: • NHV012 (norovirus and HAV in lenticule discs – April 2023) organised by UKHSA • PT 94 (norovirus and HAV in oysters - July 2023) organised by the FAO Reference Centre for Bivalve Mollusc Sanitation • EFV10 (norovirus and HAV in raspberries – September 2023) organised by the EURL for foodborne viruses • EFV11 (norovirus and HAV in oysters – December 2023) organised by the EURL for foodborne viruses • NHV013 (norovirus and HAV in lenticule discs – December 2023) organised by UKHSA We have received final reports for PT94, NHV012 and NHV013; in all cases we correctly identified presence/absence of all viruses in all samples, and in addition scored 100% for quantification performance where this was assessed. For EFV10 and EFV11, we have received intended results indicating that we correctly identified presence/absence of all viruses in all samples. Full reports for these schemes (possibly including assessment of quantification performance) are awaited.
Co-ordinating training exercises to promote best laboratory practice in respect of analysis	No designated OLs in network.
Providing OLs advance notice of PT	No designated OLs in network.

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 responded to the European Metrology Network survey on priorities for metrology in food safety and sustainability.

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 06/07/23, 20/09/23 and 19/01/24.
Notification of deviations or unusual occurrences	No deviations identified in this reporting period.
Completing annual reports	The final version of the annual report for FY22/23 (taking into account comments on the draft) was submitted to the FSA on 25/04/23. It was subsequently approved and posted on the NRL website. The draft annual report for FY23/24 was submitted to the FSA on 26/03/24.
Managing data and information	Data and documents associated with the NRL function have been stored in accordance with Cefas' data management systems.
Providing meeting reports	Notes from contractual update meetings were provided to FSA within the agreed timeframe. See list of reports in Section 12.

10. Discussion of specialised areas e.g. research activities

Agreed priority for FY23/24	Activity in period
Method development for norovirus in seaweed	The NRL continued research work begun in FY22/23 on methods for detection of norovirus in seaweed products. After initial application of the ISO 15216-1 method for vegetables to a number of fresh seaweed products (kombu kelp, sea lettuce, egg wrack, dulse) had indicated some issues in particular with RT-qPCR inhibition, two additional virus extraction methods, namely direct lysis, and swabbing, were identified from the literature and trialled in comparison to the ISO method. The direct lysis method was unable to provide improved results compared with the ISO method for any sample type. Results for the swabbing method were mixed, however concerns over the practicability of this method, in addition to a lack of information as to whether norovirus is likely to contaminate the surface or internal tissues of seaweed in real situations meant that this method was overall assessed as unsuitable. The overall conclusion was that in the event of an emergency situation or structured survey, testing of fresh seaweed products with a modified version of the ISO 15216-1 method would be most suitable, but subject to the caveat that for a reasonable proportion of samples RNA dilution to reduce inhibition could be necessary (lowering the sensitivity), and that in a smaller proportion of samples invalid results due to inhibition could be obtained even with RNA dilution.
Practical investigation of infectivity methods for norovirus in foods	The NRL carried out initial investigations into two methods for estimation of infectious norovirus, namely PMAxx treatment (application of a photo-inducible dye that binds irreversibly to exposed nucleic acid, reducing PCR signal associated with virus particles with damaged capsid, or free RNA), and long-range RT-qPCR (a two-step RT-qPCR method including reverse transcription primed at a region of the genome remote from the qPCR target region, reducing PCR signal associated with short fragments of the viral genome). Initial results were promising, showing almost complete removal of qPCR detection from norovirus positive shellfish samples subjected to inactivatory heat treatment when PMAxx treatment was included in the test method. Application of long-range RT-qPCR to UV-C treated norovirus positive shellfish samples did not provide significantly

	different results to the standard short-range RT-qPCR, indicating that the long-range RT was working efficiently, but that the UV-C treatment used was possibly not strong enough to elicit significant genome damage. Overall, these initial investigations provide a strong basis for additional work on these methods, possibly involving combining them into a single test for estimation of infectious norovirus which could be run alongside the standard method to provide extra information.
Testing of outbreak-related oyster samples to reduce uncertainty and assist in future incident management	In agreement with the FSA Risk Assessments team, the NRL tested a small number of oyster samples linked to a norovirus outbreak in the summer and autumn of 2023. Samples were tested for norovirus using the standard quantitative method with the aim of increasing the dataset of outbreak-related sample results to reduce the uncertainty associated with the links between human health risks and different levels of norovirus. For additional information, the samples were subjected to norovirus sequencing (revealing close sequence matches with associated clinical samples) and testing for sapovirus (as a potential co-infection in some of the oyster consumers). During the reporting period the NRL found out about a number of additional outbreaks; contact was made with UKHSA and local authorities with the aim of securing additional samples for norovirus testing, however no suitable samples were available. The NRL has taken several opportunities to raise awareness of this capacity to test in outbreak situations with UKHSA, local authorities and producers with the aim of increasing the likelihood of securing samples in future.

11. Link to NRL website

UK National Reference Laboratory (NRL) for foodborne viruses - 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
25/04/23	Final annual report 2022-23 published
05/07/23	NRL draft work programme (FY23/24) sent to FSA
27/07/23	Draft minutes of NRL Q1 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
10/10/23	Draft minutes of NRL Q2 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
15/02/24	Draft minutes of NRL Q3 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA.
26/03/24	Draft annual report 2023-24 submitted
13/05/24	Final annual report 2023-24 completed

Centre for Environment Fisheries & Aquaculture Science

Cefas

World Class Science for the Marine and Freshwater Environment

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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