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) FS616026/C8333 - FS430551/C8351 – Foodborne Viruses for the period April 2024 to March 2025.

Key outputs were:

- Maintenance of expertise and accreditation for quantification of norovirus and hepatitis A virus (HAV) in bivalves
- Maintenance of expertise and accreditation for quantification of norovirus and hepatitis A virus (HAV) in soft fruit and vegetables.
- Completion of a research project examining methods for estimation of infectious norovirus using combined PMAxx treatment and long-range RT-qPCR.
- 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, including method protocols, ad hoc literature reviews and project reports and other information 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
LOD	Limit of Detection
LOQ	Limit of Quantification
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 2024-25 (April 2024 - March 2025). 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, OCLs and industry.	In addition to the scheduled project review meetings with the FSA/FSS on 02/05/24 and 13/12/24, the NRL Director and team have been in regular contact with the FSA/FSS throughout the year. The NRL attended two meetings of the Shellfish Stakeholder Working Group on 04/09/24 and 04/12/24 to support the FSA on discussion of technical matters including norovirus contamination of shellfish.
Co-ordinating the activities of OCLs responsible for analysis of official control samples to ensure	No designated OLs in network.

verification of compliance with feed and food law.	
Providing regular updates to the CA, OCLs 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 reports submitted at the end of each year. Regular contact has been maintained with the FSA to provide updates on progress. 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 provided to the FSA, the NRL participated in discussions organised by Seafish on the potential for use of industry norovirus data in relation to re-opening beds after illness outbreaks.
Representing the UK at relevant international meetings and working groups.	No activity delivered/requested in period.
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, soft fruit, and leaf, stem and bulb vegetables) was confirmed following the UKAS annual visit in October 2024.
Involvement in standardisation activities relevant to work area.	NRL director was appointed project leader for the revision of ISO 6887-3 (preparation of fish and fishery products for microbiological analysis) under ISO/TC34/SC9/WG8 (sample preparation) and began working with other WG members on the new text in the last part of the reporting period. 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 ,later replaced by ISO/TC34/SC9/WG37).
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 comparative testing for UK laboratories & ensuring appropriate follow up.	No designated OLs in network.
Co-ordinating the participation of UK OLs and other relevant laboratories in international method validation studies and other initiatives.	No designated OLs in network. No activity delivered/requested in period.
Participating in proficiency tests and method validation studies organised by international organisations.	During the reporting period, the UK NRL participated in 5 relevant proficiency testing (PT) schemes: • NHV014 (norovirus and HAV in lenticule discs – April 2024) organised by UKHSA • EFV12 (norovirus and HAV on food surfaces – April 2024) organised by the EURL for foodborne viruses • PT 99 (norovirus and HAV in oysters - July 2024) organised by Cefas as the FAO Reference Centre for Bivalve Mollusc Sanitation • NHV015 (norovirus and HAV in lenticule discs – November 2024) organised by UKHSA

	• EFV13 (norovirus and HAV in oysters – November 2024) organised by the EURL for foodborne viruses The NRL has received final reports for all schemes except EFV13; it scored full marks for presence/absence and quantification (where assessed) in all schemes. For EFV13, the NRL has received intended results but is awaiting the final report. This indicated that a false positive for norovirus GI was obtained for one sample, all other GI results for other samples and all results for norovirus GII and HAV were as intended. Investigation of this result indicated that the root cause was most likely cross contamination from one of the other artificially contaminated samples in the scheme; this contained levels of norovirus GI more than 4x greater than the NRL has ever recorded in a naturally contaminated sample, greatly increasing the likelihood of cross contamination within the laboratory.
Co-ordinating training exercises to promote best laboratory practice in respect of analysis.	No designated OLs in network.
Providing OLs advance notice of proficiency testing.	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	No activity delivered/requested in period

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. Review meetings were held on 02/05/24 and 13/12/24
Notification of deviations or unusual occurrences	No deviations identified in this reporting period.
Completing annual reports	The final version of the annual report for FY23/24 (taking into account comments on the draft) was submitted to the FSA on 13/05/24. It was subsequently approved and posted on the NRL website. The draft annual report for FY24/25 was submitted to the FSA on 24/03/25.
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
Practical investigation of infectivity methods for norovirus in foods	In the previous FY, the NRL started 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 fairly promising, showing reasonable performance of the methods in norovirus positive shellfish samples subject to heat and UV-C treatment designed to elicit damage to the viral capsid and genome respectively, although some calibration of the treatments to allow better demonstration of the methods' usefulness appeared necessary (this work is reported in the annual report for 23/24). In this reporting period, the NRL initially investigated alternative heat and UV-C treatment regimes to enable further investigation and demonstration of proof-of-principle of the methods in artificially inactivated samples, however results were ambiguous. In order to maximise available time and resources, the NRL therefore concentrated on testing a set of 10 naturally contaminated norovirus positive shellfish samples using a method combining both the PMAxx treatment and long-range RT-qPCR (that should in theory only allow detection of viruses with both intact capsids and largely undamaged genomes) alongside parallel testing with the ISO 15216-1 standard method as a reference. The samples tested included a range of different contamination levels as determined by the reference method (from <LOQ to >1000 copies/g). Five out of the 10 samples gave positive results with the PMAxx/long-range RT-qPCR method indicating contamination with intact, potentially infectious virus, however the results highlighted the multiple difficulties in interpretation of both positive and negative results using this method including:- • Complexity in the infectivity method meaning that reductions in detectable virus compared to the reference method could be down to a range of methodological factors including inefficiencies in RNA extraction or RT-qPCR. This could be partially tackled through introduction of appropriate method controls. • Difficulty in determining if viral damage in samples is occurring post-sample collection e.g. during sample transport, storage or processing before application of the infectivity methods (e.g. virus extraction using the proteinase K method) so that levels of potentially infectious virus detected are suppressed artificially compared to levels in fresh shellfish. This could be partially tackled through investigation and introduction of appropriate sample transport and storage conditions, alternative virus extraction methods etc.. • *A priori* reduced sensitivity of the infectivity method due to smaller amounts of shellfish equivalent material assayed in the detection part of the test, resulting from the use of different RNA extraction methods and extra treatment steps etc. This

	issue will (all other things being equal) increase both the LOD and LOQ of the method, making interpretation of negative results and quantitative interpretation of positive results more difficult. This issue could be partially tackled through modification of the infectivity method to increase the amount of material tested where possible, although this could also negatively impact method efficiency. • Lack of baseline data for the infectivity method meaning that it is difficult to interpret results in the context of knowledge on prevalence, levels and seasonality of positive results in production area, end product and outbreak-related samples.

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

Just list the reports/documents provided to FSA in the period (April to September or October to March) as well as name of SOPs reviewed

Date produced	Title of document
Monthly	Monthly financial and technical updates
13/05/24	Final annual report 2023-24 published
17/05/24	Draft minutes of NRL Q4 meeting submitted to FSA. Minutes amended following submission of comments by FSA
06/01/25	Draft minutes of NRL Q1-Q3 combined meeting submitted to FSA. Minutes amended following submission of comments by FSA.
05/03/25	Report on method development for norovirus in seaweed published on Cefas website.
24/03/25	Draft annual report 2024-25 submitted
23/04/25	Final report 2024-25 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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