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 2022 to March 2023.

The main activities completed during the period are highlighted below:

- The NRL maintained expertise and accreditation for quantification of norovirus and hepatitis A virus (HAV) in bivalves.
- A dossier including summaries of verification and method uncertainty studies was completed in support of accreditation of the NRL for quantification of norovirus and HAV in soft fruit and vegetables.
- The NRL director actively participated in committees and working groups addressing standardisation of methods relevant to foodborne viruses.
- A review paper on incident preparedness/emerging food-virus combinations was completed and published on the NRL website.
- A method comparison was completed on two extraction methods for detection of HAV in dates.
- Initial studies evaluating the performance of the ISO 15216-1 method for detection of norovirus in fresh and dried seaweed were completed and changes to improve method performance were identified.
- The NRL participated in five proficiency testing schemes.
- The standalone website for the NRL was maintained.

2. Glossary

BSI	British Standards Institute
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
HAV	Hepatitis A Virus
ISO	International Organisation for Standardisation
MANCP	Multi annual national control plans
NGS	Next Generation Sequencing
NoV	Norovirus
NRL	National Reference Laboratory
OL(s)	Official Laboratory (ies)
PCR	Polymerase Chain Reaction
PG	(in standardisation) Project Group
PT	Proficiency Testing
RT-PCR	Reverse Transcription - Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SC	(in standardisation) Sub-committee
TC	(in standardisation) Technical Committee
UKAS	United Kingdom Accreditation Service
UKHSA	United Kingdom Health Security Agency
WG	(in standardisation) Working Group

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 has been divided into the following key objectives of the Agreement signed between FSA and Cefas:

1. Provision of 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 ring trials (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 on topics including developments in SARS-CoV-2 method standardisation, the possible food safety implications of avian influenza and virus method limits of detection and quantification. The NRL participated in a number of additional meetings with FSA and FSS staff including the Cefas Shellfish Safety Familiarisation day on 21st September (contributing to sessions on National Reference Laboratory functions, and Viruses and Other Emerging Risks), the FSA NRL Symposium on 5th October (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) is able to provide to FSA in investigations of human illness linked to consumption of bivalves and other foods).
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 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 performance in Proficiency Testing and progress with delivery of research tasks etc.
Creation & maintenance of NRL website	The standalone website for the NRL for Foodborne Viruses was maintained with relevant updates throughout the reporting period. 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	The NRL responded to comments received from FSA on an opinion paper on Norovirus testing as a shellfish risk and incident management tool (submitted during FY21/22). The NRL responded to an FSA call for advice and comments on discussion paper for the revision of the general principles of food hygiene to the control of viruses in food (CXG79-2012).
Representing the UK at relevant international meetings and working groups	The NRL attended (virtually) the CEN/TC463 and ISO/TC34/SC9 plenary meetings in June 2022. N.B: The UK NRL was not invited to the EURL for Foodborne Viruses annual workshop in line with updated guidance for participation of 3rd country representatives issued by the European Commission.
Participating in other international activities	No activity delivered or requested in period.
Advising on best scientific practice	The NRL has maintained method protocols on its website.
Maintaining expertise	See other subsections within this section (Representing the UK at relevant international meetings and working groups, Involvement in standardisation activities relevant to work area) for attendance at national and international meetings, workshops and for activities relating to standardisation. In addition to maintaining knowledge of current scientific developments relevant to foodborne viruses through regular scanning of journal publications and attendance at relevant

	conferences/symposia etc., the laboratory continues to maintain practical expertise in the most important methodologies. Quantification of norovirus and HAV in a variety of foods using ISO 15216-1 has been carried out regularly across the period for a variety of purposes, and additional staff have been trained. An internal audit of the method for quantification of noroviruses and HAV in bivalves, with particular attention on use of a new alternative real-time PCR machine (Quantstudio 3), was carried out during September 2022. Findings from the audit were generally positive, however the NRL is currently in discussion with the Cefas Quality Department about a single finding related to estimation of method uncertainty for quantification of viruses in shellfish samples. Once this finding is satisfactorily resolved the NRL will be free to use the Quantstudio 3 machine for accredited analysis. The UKAS annual accreditation inspection visit to the Cefas Weymouth laboratory was carried out in February 2022. No practical witnessing of foodborne virus methods was carried out however the NRL provided documents for assessment by the auditors. The final audit report is awaited but indications are that there were no findings of relevance to this area or relating to Cefas Quality Management System in general. The NRL for Foodborne Viruses has continued to collaborate closely with the UK NRL for bacterial contamination of live bivalve molluscs (also based at Cefas) where remits overlap (e.g., common aspects of bacterial and viral contamination of bivalve molluscs).
Involvement in standardisation activities relevant to work area	The Director of the UK NRL has participated in meetings and other activities (document review etc.) of: • ISO/TC34/SC9 international committee for standardisation in microbiology of food products • CEN/TC463 European committee for standardisation in microbiology of the food chain

	• BSI AW/009 UK mirror committee for standardisation in food microbiology • ISO/TC34/SC9/WG3/PG project group on validation of methods for viruses and parasites • ISO/TC34/SC9/WG31 working group on hepatitis E virus • CEN/TC463/WG1 working group on general requirements for PCR methods for food microbiology
Supporting FSA/FSS with emergency situations	No activity delivered or 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 or 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 or 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: • NHV010 (norovirus and HAV in lenticule discs – April 2022) organised by UKHSA • EFV08 (norovirus and HAV on food surfaces [bell peppers] – May 2022) organised by the EURL for foodborne viruses • PT 89 (norovirus and HAV in oysters - July 2022) organised by the FAO Reference Centre for Bivalve Mollusc Sanitation • EFV09 (norovirus and HAV in oysters – November 2022) organised by the EURL for foodborne viruses

	• NHV011 (norovirus and HAV in lenticule discs – November 2022) organised by UKHSA We have received final reports for PT89, NHV010 and NHV011; in all cases we correctly identified presence/absence of all viruses in all samples, and in addition scored 100% for quantification performance. For EFV08, we have received intended results indicating that we correctly identified presence/absence of all viruses in all samples. A full report for this scheme (possibly including assessment of quantification performance) is awaited. For EFV09, intended results distributed by the EURL indicate that for a single sample a false positive result was obtained for norovirus genogroup II (sample was positive at levels below the limit of quantification where the intended result was negative). An investigation was launched indicating no apparent significant underlying issues and this was reported to FSA in March 2023. A full report for this scheme (possibly including assessment of quantification performance) is expected.
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 relevant CEN/ISO international initiatives during this reporting period.

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

Item	Activity in period
Providing regular updates to the CA	The UK 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/issues identified in this reporting period
Completing annual reports	The annual report for FY21/22 was submitted to the FSA on the 29th April 2022. It was subsequently approved and posted on the NRL website on 1st July 2022. The interim report for the first half of FY22/23 was submitted to the FSA on 1st November 2022.
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. Notes from other meetings attended on behalf of the NRL were also provided to FSA in the period. See list of reports in Section 12.

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
Review paper/expert opinion on future of infectivity assays	NRL finalised a report on the current state of methods for detection of infectious foodborne viruses, including a review of all current literature (originally submitted at the end of FY 21/22) based on comments received from FSA. The final version was posted on the NRL website on 1st November 2022.
Incident preparedness - small literature review of published methods for emerging viruses including TBEV	A review paper on incident preparedness/emerging food-virus combinations was submitted to FSA during June 2022. After responding to comments received from FSA, the final version was posted on the NRL website on 1st November 2022.
Focus on NoV & HAV in high-risk foods – accreditation of methods per section 7.a of original agreement	A verification study on quantification of norovirus and HAV in soft fruits and vegetables, to generate limits of detection and quantification that can be compared with published validation data for ISO 15216-1, and a separate study to generate method uncertainty figures for the same method were completed during the reporting period. Data from these studies was combined with additional existing information on PT performance etc, to form a dossier of evidence submitted to the Cefas Quality Department in March 2023. This will shortly be submitted to UKAS as part of an extension to scope application.

Method development for HAV in dates	A small-scale method comparison exercise on two extraction methods for detection of HAV in dates (ISO 15216 and the direct lysis method) was carried out and reported to FSA in August 2022. This method comparison demonstrated that the ISO 15216 method for quantification of HAV in soft fruits (already implemented by the NRL) is suitable for testing dates and could be used in the event of an incident of HAV related to date consumption, if required.
Implementation of Digital PCR methodology	No activity delivered or requested in period.
Development of sequence characterisation methodology using Next Generation technology	A number of experimental approaches for long-read and whole genome sequencing for norovirus that will hopefully improve NRL capacity for norovirus sequencing in e.g. incident investigations have been have been designed and/or developed during the reporting year. A PCR tilling approach for whole genome sequencing of norovirus genogroup II has been designed using an in-silico approach. A long-read high throughput sequencing assay has been used to genotype norovirus reference material (faecal samples) intended to be used as positive controls for whole genome sequencing. Separately an experimental procedure for detection and characterization of HAV in food matrices using nanopore (high throughput) sequencing of amplicons obtained by nested PCR has been designed. The NRL already has the capacity to characterise norovirus in food samples using equivalent amplicon sequencing methods; this procedure will hopefully expand that capacity to cover HAV in addition.
Method development for norovirus in seaweed	NRL first discussed with FSA the possibility of developing or trialling methods for detection of norovirus in seaweed products in September 2022. Subsequent to this, practical investigations into application of the ISO 15216-1 method for vegetables into a number of fresh (kombu kelp, sea lettuce, egg wrack, dulse) and dried seaweed products (crispy nori thins) were carried out. For fresh products significant issues with RT-PCR inhibition were frequently noted however a number of modifications including addition of a specific

	inhibitor removal process and dilution of RNA prior to RT-PCR enabled acceptable performance of the method in most cases. For dried seaweed products, use of 25g samples was not practical due to absorption of elution buffer by the test sample; use of smaller 5g samples resolved this issue however. NRL has provided FSA with further details to enable them to decide whether further investigation of method modifications for fresh products may be necessary in FY23/24 to try and improve method performance.

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
19.04.22	Update on April 2022 meeting of standardisation group CEN/TC463/WG1 (PCR methods)
29.04.22	Draft annual report 2021-22
26.05.22	Summary report of second meeting of ISO/TC34/SC9/WG31 (Hepatitis E virus)
24.06.22	NRL draft work programme 2022-23
24.06.22	Review of food-virus combinations of concern or potential concern for food safety in the United Kingdom (Draft)
28.06.22	Agenda of NRL review meeting submitted to FSA for comments
29.06.22	National Reference Laboratory: Annual report - FS430551/C8351 - Foodborne Viruses (FY 21/22 Final version)
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
30.08.22	Method comparison trial for detection of hepatitis A virus in dates (Draft)
30.08.22	Current state of methods for detection of infectious foodborne viruses (Final version)

20.09.22	Update on September 2022 meeting of standardisation project group ISO/TC34/SC9/WG3/PG (validation of methods for viruses and parasites)
03.10.22	Method comparison trial for detection of hepatitis A virus in dates (Final)
18.10.22	Summary report on the virtual plenary meetings of the European (CEN/TC463) and International committees (ISO/TC34/SC9) on standardisation in food microbiology
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
01.11.22	Review of food-virus combinations of concern or potential concern for food safety in the United Kingdom (Final & website publication)
01.11.22	Draft interim report 2022-23
01.11.22	Current state of methods for detection of infectious foodborne viruses (Website publication)
09.12.22	Update on December 2022 meeting of standardisation project group ISO/TC34/SC9/WG31 (Hepatitis E virus)
06.12.22	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
27.03.23	Draft annual report 2022-23
28.03.23	Draft minutes of NRL Q4 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA

Centre for Environment Fisheries & Aquaculture Science

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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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