Centre for Environment Fisheries & Aquaculture Science

Cefas

National Reference Laboratory: Annual report

FS430551/C8351 - Foodborne Viruses

Author: Dr James Lowther

Date: April 2022

www.cefas.co.uk

Cefas Document Control

Submitted to:	Valerie McFarlane, Food Standards Agency
Date submitted:	29/06/2022
Project Manager:	Myriam Algoet
Report compiled by:	Dr James Lowther
Quality control by and date:	Michelle Price-Hayward, 20/04/2022
Approved by and date:	Dr Myriam Algoet, 29/06/2022
Report sensitivity:	Official
Version:	Final V1
Recommended citation for this report:	Lowther, J. (2022). National Reference laboratory (Foodborne Viruses): Annual Report (April 2022). FS430551/C8351 Report for FSA/FSS, 21 pp.

Version control history

Version	Author	Date	Comment
Draft V1	J. Lowther	12/04/2022	First draft for QC
Draft V2	M. Price-Hayward	20/04/2022	QC
Draft V3	J. Lowther	20/04/2022	Revised draft addressing QC comments
Draft V4	T. Jones	25/04/2022	Proof reading - minor editorial changes
Draft V5	M. Algoet	29/04/2022	Minor editorial changes. Draft submitted for FSA review
Final V1	M. Algoet & J. Lowther	29/06/2022	FSA comments received 28/06/22 addressed. Final report as submitted to FSA and published

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 2021 to March 2022.

Highlights include:

- Maintenance of expertise in analysis of foodborne viruses including maintenance of accreditation for quantification of norovirus and hepatitis A virus (HAV) in bivalves and production of standard operating procedures (SOPs), worksheets etc. for quantification of norovirus and HAV in soft fruit and vegetables
- Validation of alternative real-time Polymerase Chain Reaction (PCR) machines and training of additional analysts to improve resilience
- Support to the Food Standards Agency (FSA) during a large outbreak of oyster-related gastroenteritis
- Active participation in committees and working groups addressing standardisation of methods relevant to foodborne viruses
- Successful completion of four proficiency testing schemes
- Participation in an international method comparison trial for quantification of norovirus using digital PCR
- Preparation of an opinion paper on bivalve norovirus testing as a risk management tool
- Preparation of a report on the current state of methods for detection of infectious foodborne viruses
- Investigations into novel methods for genotyping norovirus using next generation sequencing technology
- Development and launching of a standalone website for the NRL

2. Glossary

AHG	(in standardisation) Ad Hoc Group
BSI	British Standards Institute
CA	Competent Authority
Cefas	Centre for the Environment, Fisheries and Aquaculture Science
CEN	European Committee for Standardisation
dPCR	Digital Polymerase Chain Reaction
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Office of the United Nations
FBV	Foodborne Viruses
FSA	Food Standards Agency
FSS	Food Standards Scotland
HAV	Hepatitis A Virus
HEV	Hepatitis E 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
qPCR	Quantitative Polymerase Chain Reaction
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SC	(in standardisation) Sub-committee

SOP(s)	Standard Operating Procedure(s)
TC	(in standardisation) Technical Committee
UKAS	United Kingdom Accreditation Service
UKHSA	UK Health Security Agency (previously known as Public Health England (PHE))
WG	(in standardisation) Working Group

3. Introduction

Cefas was redesignated as the National Reference Laboratory (NRL) for Foodborne Viruses by the FSA in the Spring of 2021 following on directly from previous designations covering the period February 2018 to March 2021.

The roles and responsibilities of the NRLs have changed significantly in recent years. The services required to be delivered under the scope of the current Cefas/FSA agreement include duties based on Articles 100-101 of Regulation (EU) 2017/625 (retained from January 1st 2021 in UK law subject to amendments through Statutory Instrument 2019 No. 665; The Official Controls for Feed, Food and Animal Health and Welfare (Amendment etc.) (EU Exit) Regulations 2019):

(a) cooperate internationally in their area of competence (and where possible, the relevant European Union Reference Laboratory (EURL);

(b) collaborate with international laboratories (where possible with the relevant EURL) and participate in training courses and inter-laboratory comparative tests organised by these laboratories;

(c) coordinate the activities of laboratories designated by FSA and Food Standards Scotland (FSS) (the competent authorities (CA)) as official laboratories (OLs) under Article 37 of Retained Regulation (EU) 2017/625 (OCR);

(d) where appropriate, organise comparative tests between OLs and ensure an appropriate follow-up of such comparative testing;

(e) ensure the dissemination of any information required by the CA;

(f) provide scientific and technical assistance to the CA for the implementation of multi annual national control plans (MANCP) in accordance with Article 109 and of co-ordinated control plans adopted in accordance with Article 112 of (retained) Regulation (EU) 2017/625;

(g) where necessary, conduct training courses for OL staff;

(h) upon request by the competent authority, actively assist in relevant emergency situations and in cases of non-compliance of consignments, carry out confirmatory analysis;

(i) be responsible for carrying out other specific duties as required by the CA, where appropriate and by prior agreement.

This technical report summarises the activities carried out by the NRL during the financial year 2021-22 (April 2021 - March 2022). 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 29th July, 6th October, 9th December and 9th March, and an informal meeting with the FSA to provide them with information on participation in the BSI AW/009 committee, the NRL Director and team have been in regular contact with the FSA, on topics including relevant developments in standardisation, MANCP priorities, the FSA National Capacity review etc.
Co-ordinating the activities of OLs	No designated OLs in network.

responsible for analysis of official control samples to ensure verification of compliance with feed and food law	
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 finance updates and (from November 2021) activity logs as well as 6-monthly reports submitted in the Autumn and Spring of each year. Regular contact has been maintained with the FSA to provide updates on interactions with the EURL, performance in Proficiency Testing etc.
Creation & maintenance of NRL website	A standalone website for the NRL for foodborne viruses (previously the foodborne viruses (FBV) and live bivalves' websites were integrated) was developed and launched in July 2021, including information on legislative underpinning, method protocols etc. The website was modified based on feedback from FSA and is subject to continuous informal improvements. The content of the website was checked for compliance with accessibility rules and corrections made where required. 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 UK NRL prepared and submitted an opinion paper on Norovirus testing as a shellfish risk and incident management tool for the FSA (submitted in October 2021). The UK NRL contributed to the review of the FSA MANCP for FY22-23 (submitted in January 2022).

	The UKNRL responded to the FSA review of National Capacity in January 2022.
Representing the UK at relevant international meetings and working groups	The UK NRL Director attended sessions of the EURL for Foodborne Viruses annual workshop in June 2021 covering proficiency testing however, due to exit from the EU, the UK NRL Director was unable to participate in the entire meeting. The NRL attended (virtually) the ISO/TC34/SC9 plenary meeting in June 2021.
Participating in other international activities	No activity delivered or requested in period.
Advising on best scientific practice	In addition to maintenance of method protocols, the NRL has provided FSA with guidance notes on requirements for norovirus testing laboratories.
Maintaining expertise	See other sections for attendance at national and international meetings, workshops and for activities relating to standardisation. In addition to regular scanning of the literature covering foodborne viruses, 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. The UK NRL for Foodborne Viruses has continued to collaborate closely with the 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). The UK NRL engaged with UKAS and FSA/FSS prior to & following LAB33 consultation.
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/AHG ad hoc working group covering the amendment of ISO 15216-1:2017 (the international

	standard method for quantification of viruses in foods – director of the UK NRL was convenor of this AHG) • 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	The UK NRL provided support in an investigation into a large outbreak of oyster-related gastroenteritis between November 2021 – January 2022. This support included accredited RT-qPCR testing for norovirus of 29 shellfish samples (16 on behalf of FSA), further genotyping analysis for 6 samples most closely implicated with illness in consumers, and attendance at Incident Management Team and Incident Management Team sub-group meetings.

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	The UK NRL has maintained a variety of method protocols and other supporting resources on its website: • Generic protocol for quantification of norovirus and hepatitis A virus in bivalve molluscan shellfish • Calculation spreadsheet for quantification of norovirus and hepatitis A virus in bivalve molluscan shellfish • Generic protocol for quantification of norovirus and hepatitis A virus in soft fruit • Calculation spreadsheet for quantification of norovirus and hepatitis A virus in soft fruit and vegetables • Generic protocol for detection of norovirus and hepatitis A virus on surfaces

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. The UK NRL participated in a method comparison trial for norovirus quantification using digital RT-PCR and quantitative RT-PCR (see below).
Participating in proficiency tests and method validation studies organised by international organisations	During the reporting period, the UK NRL has participated in 4 relevant proficiency testing (PT) schemes: • EFV06 (norovirus and HAV in oysters – April 2021) and • EFV07 (norovirus and HAV in strawberries – September 2021) organised by the EURL for foodborne viruses • PT 82 (norovirus and HAV in oysters - September 2021) organised by the FAO Reference Centre for Bivalve Mollusc Sanitation • NHV009 (norovirus and HAV in lenticule discs - November 2021) organised by UKHSA We have received final reports for EFV06, PT82 and NHV009; in all cases we correctly identified presence/absence of all viruses in all samples, and in addition scored 100% for quantification performance. For EFV07 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. In addition, during this reporting year we have received final reports for three additional schemes we participated in during the previous reporting year: • EFV04 (norovirus and HAV in lettuce – October 2020) and • EFV05 (norovirus and HAV in oysters – November 2020) organised by the EURL for foodborne viruses • NHV008 (norovirus and HAV in lenticule discs - January 2021) organised by UK PHE (now UKHSA) In all cases viruses were correctly identified in all samples, and, where applied, quantification scores were satisfactory, except for the result for NoV GII in one lenticule disc (scheme NHV008), where a questionable result for quantification was obtained. This result was investigated, and it was decided to monitor our performance in subsequent schemes, particularly those involving shellfish matrix samples. As above, no further issues with GII quantification (in shellfish or lenticule samples) have been experienced.
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 recommendations were received by the NRL during this reporting period.

9. Core function: communication of results and data use

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, telephone (where appropriate) and via monthly and 6-monthly reports. Because of the current Covid-19 pandemic no face-to-face meetings were conducted in the last year. Quarterly review meetings were held on 29/07/2021 (Q1), 06/10/2021 (Q2), 09/12/2021 (Q3) and 09/03/22 (Q4). In addition, Cefas provided FSA with monthly summaries of costs showing staff effort and non-pay costs throughout the year, and with monthly technical delivery summaries from November 2021 onwards.
Notification of deviations or unusual occurrences	No deviations/issues identified in this reporting period.
Completing annual reports	The annual report for the previous contract was submitted to the FSA on the 9th July 2021. It has since been approved and posted on the NRL website.
Managing data and information	Data and documents associated with the NRL function has 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 by the NRL on behalf of the

	FSA were also provided to FSA in the agreed period, with the exception of UKAS Lab 33 meetings which were also attended by FSA. See list of reports in Section 12.

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

Note: the NRL workplan for FY2122 was drafted for review by FSA at the Q1 review meeting but only formally agreed at the Q2 review meeting held on 06/10/2021. The table below lists the activities delivered against each of the agreed priorities for FY21/22.

Agreed priority for FY2122	Activity in period
Review paper/expert opinion on future of infectivity assays	A report on the current state of methods for detection of infectious foodborne viruses, including a review of all current literature, was forwarded to FSA during March 2022.
Incident preparedness - small literature review of published methods for emerging viruses including TBEV	Postponed to Q1 of FY22/23 due to staff unavailability.
Focus on NoV & HAV in high-risk foods – accreditation of methods per section 7.a of original agreement	Accreditation by UKAS to ISO 17025 standard of quantification of norovirus and HAV in bivalve shellfish following ISO 15216-1 has been maintained following successful internal and external audits. Validation studies comparing the current Stratagene Mx3005 qPCR machines and the alternative Applied Biosystems Quantstudio 3 qPCR machines have been completed, and the latter have been added as an option in both the UK NRL SOPs and the Cefas scope of accreditation, providing both extra capacity and increased flexibility. The UK NRL continues to work towards accreditation of quantification of norovirus and HAV in soft fruits, leaf, stem, and bulb vegetables. A new SOP 3153 for virus extraction from fruit and vegetables has been developed by the NRL and published by the Cefas Quality System, and other existing SOPs (for RNA extraction, PCR, preparation of reagents etc.) have been modified

	to accommodate fruit and vegetable testing. A complete set of worksheets to ensure complete traceability and uniformity of test methods has been published on the Quality System. A plan for validation experiments during the first part of financial year 2022-2023 has been drawn up.
Method development for HAV in dates	Initial investigations into methods for detection of HAV in dates, including a review of the available literature and informal approaches to laboratory contacts including the EURL were made in March 2022. Work will be completed in Q1 of FY22/23.
Implementation of Digital PCR methodology	In parallel with EURL PT scheme EFV06 (see Section 7 above) the UK NRL took part in an interlaboratory trial comparing dPCR and qPCR for the quantification of NoV in oysters. This was organised within the network of European NRLs and associated labs by Wageningen University (WU; the Netherlands) assisted by the EURL. The UK NRL initially implemented the method in use at WU, comparing their parameters for certain reagent concentrations (validated on a different dPCR platform) with the default parameters for the platform we had access to. This demonstrated that the WU parameters were suitable for use in the trial itself. This consisted of analysing in parallel a series of oyster RNA extracts and associated control materials (distributed alongside the PT distribution) on dPCR and qPCR platforms. Results were submitted to WU and full analysis is awaited. As well as enabling us to trial and implement a dPCR method in the lab, which could have research benefits in the future, our results will contribute to a thorough multi-lab evaluation of the technology in comparison to the existing ISO 15216 standard.
Development of sequence characterisation methodology using Next Generation technology	The UK NRL continued to refine its method for norovirus metabarcoding, i.e. deep sequencing using NGS technology of short phylogenetically informative stretches of the viral genome (the polymerase and capsid genes). During the reporting year this method was applied successfully to genotype several oyster samples implicated in outbreaks, and enabled genotype and strain matches with associated faecal samples. In addition, novel Nanopore sequencing assays to genotype GI and GII norovirus, and in particular to generate longer sequence stretches enabling identification of recombinant strains have been investigated. Four different sequencing approaches were tested: 1) non-overlapping short amplicons of VP1 and RdRp of GI and GII obtained by semi-nested PCR (<400 bp)

	2) overlapping short amplicons of VP1 and RdRp GI and GII obtained by semi-nested PCR (<400 bp) 3) medium length amplicons of VP1 and RdRp GI and GII obtained by semi-nested PCR (ca. 1000 bp) 4) long-range amplicons of GII obtained by semi-nested PCR for whole genome sequencing (ca. 2500 – 7500 bp) The short amplicon approaches were successfully used to genotype GI and GII in shellfish samples, whereas medium and long-range amplicons were only successful using wastewater and faecal samples with a high viral load. The long amplicon approach allowed characterisation of the whole genome of a norovirus GII strain from a faecal sample used to produce lenticules, however application of this type of methods to food samples will be challenging due to the low viral loads these samples generally contain.

11. Link to NRL website

UK National Reference Laboratory (NRL) for foodborne viruses - Cefas (Centre for Environment, Fisheries and Aquaculture Science)

12. Annex – Summary of documents produced from NRL activities

Date produced	Title of document
04.06.21	PT 84 – PHE EQA shellfish scheme final report submitted to FSA (PT exercise conducted under previous NRL contract)
26.07.21	Draft workplan for FY21/22 submitted to FSA
03.08.21	Summary report of annual plenary meeting of CEN/TC463 and ISO/TC34/SC9 (June 2021) submitted to FSA
05.08.21	Summary report of BSI AW/9 May 2021 meeting (UK committee on standardisation of food microbiology methods) submitted to FSA
24.08.21	Summary report of BSI AW/9 August 2021 meeting (UK committee on

	standardisation of food microbiology methods) submitted to FSA
Monthly	Summary of cost breakdown submitted to FSA
05.08.21	Draft minutes of NRL Q1 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
30.09.21	Draft agenda and documentation for Q2 review meeting submitted to FSA
04.10.21	Research priority breakdown for FY21/22 submitted to FSA
19.10.21	Draft minutes of NRL Q2 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
27.10.21	Norovirus testing as a shellfish risk and incident management tool – Opinion paper submitted to FSA
28.10.21	Draft National Reference Laboratory for Foodborne Viruses Interim report submitted to FSA
09.11.21	Update on relevant developments on HEV standardisation from ISO/TC34/SC9/WG31
12.11.21	Update on meetings of standardisation groups ISO/TC34/SC9/WG31 and CEN/TC463/WG1 November 2021 meetings
30.11.21	Final National Reference Laboratory for Foodborne Viruses Interim report submitted to FSA
Monthly (from November onwards)	Technical update reports submitted to FSA
03.12.21	Draft agenda and documentation for Q3 review meeting submitted to FSA
12.12.21	Update on meetings of standardisation groups ISO/TC34/SC9/WG31 and BSI/AW009 December 2021 meetings
04.01.22	Draft minutes of NRL Q3 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments

	by FSA
19.01.22	Summary report of 1st meeting of ISO/TC34/SC9/WG31 (Jan 2022) on hepatitis E virus submitted to FSA
16.02.22	UK NRL submitted contribution to MANCP
Feb 22	UK NRL submitted response to Defra online survey on EURL engagement
08.03.22	Draft agenda and documentation for Q4 review meeting submitted to FSA
11.03.22	Draft minutes of NRL Q4 meeting submitted to FSA within the agreed turnaround time. Minutes amended following submission of comments by FSA
28.03.22	Report on current state of methods for detection of infectious foodborne viruses submitted to FSA
29.04.22	Draft National Reference Laboratory for Foodborne Viruses Annual report submitted to FSA

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.

OGL

Pakefield Road, Lowestoft, Suffolk, NR33 0HT

The Nothe, Barrack Road, Weymouth DT4 8UB

www.cefas.co.uk | +44 (0) 1502 562244

disability confident EMPLOYER

CERTIFIED ISO 9001

CERTIFIED OHSAS 18001

SGS