Summary of Results

Norovirus and Hepatitis A virus Scheme

External Quality Assessment for Microbiology

Distribution Number: **NHV016**
Sample Numbers: **NHV0031 & NHV0032**

Distribution Date:	**10 March 2025**
Results Due:	**11 April 2025**
Report Date:	**15 May 2025**
Samples prepared and quality control tested by:	**Cefas**
Data analysed by:	**Joanna Donn** **Nita Patel**
Report compiled by:	**Joanna Donn** **Nita Patel**
Authorised by:	**Nita Patel**

UK Health Security Agency
Food and Environmental Proficiency Testing Unit (FEPTU)
61 Colindale Avenue
London
NW9 5EQ
Tel: +44 (0) 20 8327 7119
Email: foodeqa@ukhsa.gov.uk

Overview:
This Scheme provides external quality assessment samples for laboratories that examine food products or waters for hepatitis A virus and norovirus using the reverse-transcription polymerase chain reaction (RT-PCR). Regulation (EC) No 669/2009 sets out specifications for an increased level of official controls on imports of certain feed and food of non-animal origin and via Implementing Regulation (EU) 2016/2107 currently applies to frozen raspberries imported from Serbia.

According to the European Food Safety Authority (EFSA) foodborne viruses are the second most common cause of foodborne outbreaks in the European Union (EU) after *Salmonella.* EFSA has identified that removal of viral contamination is extremely difficult. It therefore recommends that the focus on control of viruses in the food chain needs to be based on preventing contamination and cross-contamination of food. Viruses cannot be cultured therefore molecular techniques are the methods of choice for the detection, identification and quantification of foodborne viruses.

ISO 15216-1:2017 Microbiology of the food chain -- Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR -- Part 1: Method for quantification is used by many laboratories. This ISO method covers pre-treatment steps to elute viruses from the different matrices.

This proficiency testing scheme challenges laboratories in detection and quantification (copies per sample) of hepatitis A virus (HAV) and Norovirus GI and GII. It has been organised in collaboration with Cefas[i] as the United Kingdom National Reference Laboratory (NRL) for monitoring bacteriological and viral contamination of bivalve molluscs.

FEPTU Quality Control:
The samples were prepared and quality controlled at Cefas.

To demonstrate homogeneity of the sample 20 LENTICULE® discs selected randomly from a batch were examined.

To demonstrate stability of the sample a minimum of Ten LENTICULE discs, selected randomly from a batch, were examined throughout the distribution period.

Cefas used a qRT-PCR using the RNA UltraSense™ One-Step Quantitative RT-PCR System (ThermoFisher), on a Stratagene Mx3005P real-time PCR machine.

The intended results letters provide guidance for participants regarding the intended result.

Guidelines and general advice:
If you experience difficulties with any of the examinations please refer to the Scheme Guide
Scheme Guide - Food and Environmental Proficiency Testing Unit

Please contact FEPTU staff for advice and information:

Repeat samples	Carmen Gomes or Kermin Daruwalla	**Tel: +44 (0)20 8327 7119**
Data analysis	Nita Patel	
Microbiological advice	Nita Patel	**E-mail: foodeqa@ukhsa.gov.uk**
General comments and complaints	Nita Patel	Website: Food and Environmental Proficiency Testing Unit
Scheme Advisors	James Lowther[i]	
Scheme Co-ordinator	Nita Patel	

Accreditation:
This scheme is not accredited, however all principles and practices of ISO/IEC 17043:2010 are followed.

[i]The Centre for Environment, Fisheries and Aquaculture Science, National Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs, Weymouth Laboratory, Dorset, DT4 8UB, United Kingdom

Total number of participants sent distribution NHV016	**21**
Number of laboratories not returning a result for NHV016	**1**
Number of laboratories not examining any samples in NHV016	**2**

Sample: NHV0031

Sample type: LENTICULE® discs prepared with known levels of norovirus GII from human faeces.

Request:
Examine for the presence of viruses (norovirus GI and GII, hepatitis A virus)
Quantify these viruses in the sample (if routinely done)

Contents and summary of results:

Examination		Expected result	Your Result	Score	Z-score
Virus	Norovirus GI	Negative	Your reported results and scores are shown on page 6		
	Norovirus GII	Positive			
	HAV	Negative			

Quantification results

Examination		Expected range	Your Result
Virus	Norovirus GI	-	Your reported results are shown on page 6 – see page 5 for comment on statistics
	Norovirus GII	Not determined – insufficient data sets	
	HAV	-	

Norovirus GI

Total participants reporting for Norovirus GI	18
Participants reporting correctly a not detected result	16 (89%)

Norovirus GII

Total participants reporting for Norovirus GII	18
Participants reporting correctly a detected result	17 (94%)
Number of laboratories reporting copies per sample	7
Range of copies per sample reported for GII	Min: $1.98x10^3$ ($3.30 \log_{10}$) Max: $1.40x10^4$ ($4.15 \log_{10}$)
FEPTU's QC median	$4.10x10^3$ copies per sample ($3.61 \log_{10}$)

HAV

Total participants reporting for HAV	16
Participants reporting correctly a not detected result	16 (100%)

Sample: NHV0032

Sample type: LENTICULE® discs prepared with known levels of norovirus GI from human faeces and known levels of Hepatitis A virus (HAV) cell culture supernatant.

Request:

Examine for the presence of viruses (norovirus GI and GII, hepatitis A virus)
Quantify these viruses in the sample (if routinely done)

Contents and summary of results:

Examination		Expected result	Your Result	Score	Z-score
Virus	Norovirus GI	Positive	Your reported results and scores are shown on page 7		
	Norovirus GII	Negative			
	HAV	Positive			

Quantification results

Examination		Expected range	Your Result
Virus	Norovirus GI	Not determined – insufficient data sets	Your reported results are shown on page 7 - see page 5 for comment on statistics
	Norovirus GII	-	
	HAV	Not determined – insufficient data sets	

Norovirus GI

Total participants reporting for Norovirus GI	18
Participants reporting correctly a detected result	17 (94%)
Number of laboratories reporting copies per sample	7
Range of copies per sample reported for GI	Min: $3.88x10^3$ ($3.59 \log_{10}$) Max: $9.70x10^4$ ($4.99 \log_{10}$)
FEPTU's QC median	$9.00x10^3$ copies per sample ($3.95 \log_{10}$)

Norovirus GII

Total participants reporting for Norovirus GII	18
Participants reporting correctly a not detected result	16 (89%)

HAV

Total participants reporting for HAV	16
Participants reporting correctly a detected result	16 (100%)
Number of laboratories reporting copies per sample	5
Range of copies per sample reported for GI	Min: $1.27x10^4$ ($4.10 \log_{10}$) Max: $1.60x5x10^5$ ($5.20 \log_{10}$)
FEPTU's QC median	$3.20x10^4$ copies per sample ($4.50 \log_{10}$)

General comments for this distribution

We apologise for any inconvenience caused by the delay in closing this distribution (original date 11 April 2025 and closed 12 May 25) and the subsequent publishing of this scheme report. This was due to an extension granted for some countries where there were shipment issues. FEPTU reference number: FPD25-1.

The samples in this distribution have been scored using the below scoring criteria. The quantification results have not been scored.

Presence/absence results
Participants correct results for detection are allocated scores up to a maximum of two points as follows:

Fully correct result for the intended result	2
False positive / false negative result	0

Non-return of results

Participants who do not return a result by the specified date are allocated a score of zero for all tests.

Quantification results
The expected range for each copies per sample result reported is calculated using the median absolute deviation from the median (*MADe***) values (see * below) which are determined from the median result reported by participants' and take into account the following criteria:

(1) median ± 2 *MADeS**
(2) median ± 3 *MADeS**
(3) median ± 0.5 log10 units

If the ranges in (1) and/or (2) are less than the value of the median ± 0.5 log10 units then the expected range is extended as described in (3).

Statistical evaluation for quantification results

Participants are advised that for a robust statistical evaluation for quantification data at least 20 results are required for each examination. When statistical calculation is based on 10 – 19 results, they should be interpreted with caution as they may be overly influenced by outlying results. When there are fewer than 10 reported results, the statistics are not considered robust to enable the data to be calculated or scored.

New website

We are pleased to announce the launch of our new website: https://www.feptu.org.uk/. Please refer to this website to obtain the latest information for your proficiency testing.

Information of importance

To understand more about the proficiency testing schemes, please use the following links for information on:

1. Report format explained: Annotated report

2. Performance rating: Performance-over-time and Scheme guide (section 16.0)

3. Scoring and statistics used: Scoring information and stats

4. Homogeneity and stability: Scheme guide (section 9.0)

5. Complaints and appeal process: Scheme guide (section 20.0 and 21.0)

For further information about the operation of the service including confidentiality and terms of participation, please refer to the Scheme Guide: Scheme guide

Reported results for laboratories and scores awarded for NHV0031

Lab ID	GI result	GI UKHSA score	GI z-score	GI Quantity	GII result	GII UKHSA score	GII z-score	GII Quantity	HAV result	HAV UKHSA score	HAV z-score	HAV Quantity
	Non-return											
	-	2	0		+	2	0	14000	-	2	0	
	-	2	0		+	2	0		-	2	0	
	-	2	0		+	2	0		-	2	0	
	Not examined											
	-	2	0		+	2	0		-	2	0	
	-	2	0		+	2	0	2110	-	2	0	
	-	2	0		+	2	0	1980	-	2	0	
	-	2	0	0	+	2	0	2256	-	2	0	
	-	2	0		+	2	0		-	2	0	
	Not examined											
	-	2	0		+	2	0		-	2	0	
	-	2	0		+	2	0		-	2	0	
	-	2	0		-	0	4		-	2	0	
	+	0	4		+	2	0					
	-	2	0	0	+	2	0	4722	-	2	0	0
	-	2	0	0	+	2	0	3764	-	2	0	0
	-	2	0	0	+	2	0	3094	-	2	0	0
	-	2	0		+	2	0		Not examined			
	-	2	0		+	2	0		-	2	0	
	+	0	4		+	2	0		-	2	0	

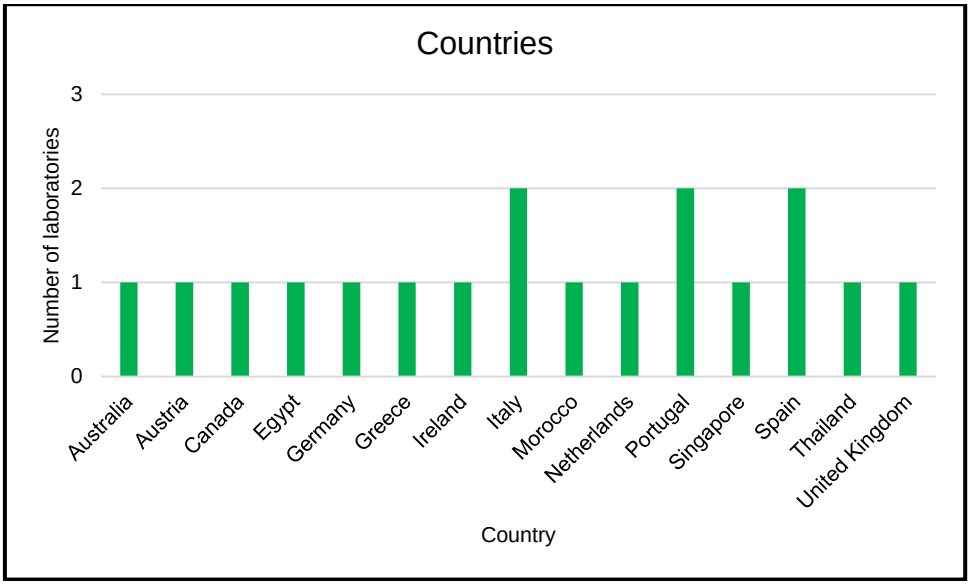
Reported results for laboratories and scores awarded for NHV0032

Lab ID	GI result	GI UKHSA score	GI z-score	GI Quantity	GII result	GII UKHSA score	GII z-score	GII Quantity	HAV result	HAV UKHSA score	HAV z-score	HAV Quantity
	Non-return											
	+	2	0	15000	-	2	0		+	2	0	
	+	2	0		-	2	0		+	2	0	
	+	2	0		-	2	0		+	2	0	
	Not examined											
	+	2	0		-	2	0		+	2	0	
	+	2	0	97000	-	2	0		+	2	0	44600
	+	2	0	12557	-	2	0		+	2	0	25159
	+	2	0	5734	-	2	0	0	+	2	0	
	+	2	0		-	2	0		+	2	0	
	Not examined											
	+	2	0		-	2	0		+	2	0	
	+	2	0		-	2	0		+	2	0	
	-	0	4		-	2	0		+	2	0	
	+	2	0		+	0	4					
	+	2	0	14597	-	2	0	0	+	2	0	160307
	+	2	0	13150	-	2	0	0	+	2	0	19930
	+	2	0	3883	-	2	0	0	+	2	0	12753
	+	2	0		-	2	0		Not examined			
	+	2	0		-	2	0		+	2	0	
	+	2	0		+	0	4		+	2	0	

Questionnaire results:
Please note that not all participants provided the relevant information. The data analysed does not evaluate or associate the results with a failure with PT to a method/process used nor does it attempt to compare performance of the various molecular kits/processes with each other.

Please note that not all participants provided the relevant information. 18 laboratories returned a result for this distribution.

The graph below shows the countries that returned a result (n=18).



17/18 (94%) of the laboratories used a real-time PCR.
1/18 (6%) of the laboratories used a droplet digital RT-PCR

The RNA extractions used is shown in the table below (n=16):

	No of users
BioMérieux NucliSENS® miniMag®	6
BIOTECON Diagnostics Extraction Kit	2
CONGEN SureFast® Prep DNA/RNA virus	1
In-house	1
Promega Maxwell® RSC PureFood GMO and Authentication Kit	1
Qiagen QIAamp® Viral RNA	2
VIR*Seek* RNA Extractor Food	1
Zinexts MagPurix Viral Nucleic Acid Extraction Kit	1
Zybio Nucleic Acid Extraction Kit (Magnetic Bead Method)	1

The PCR reagents used is shown in the table below (n=15):

	No of users
Applied Biosystems™ TaqMan™ Fast Virus One-Step Master Mix	2
Bio-Rad RT-ddPCR	1
BIOTECON foodproof® Norovirus (GI, GII, HAV)	1
CeeramTools® GI, GII and HAV detection kits	1
CONGEN SureFast® Norovirus PLUS	1
CONGEN SureFast® Norovirus/Hepatitis A 3plex kit	1
hygiena foodproof® Norovirus (GI, GII) Detection Kit	1
Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System	4
QIAGEN® OneStep RT-PCR Kit	1
PCR LightCycle® Multiplex RNA virus master	1
VIRSeek Norovirus Genotype I/II/Hep A real time RT-PCR kit	1

End of report