UK Health Security Agency

Cefas

Summary of Results

Norovirus and Hepatitis A virus Scheme

External Quality Assessment for Microbiology

Distribution Number: **NHV011**
Sample Numbers: **NHV0021 & NHV0022**

Distribution Date:	**November 2022**
Results Due:	**9 December 2022**
Report Date:	**15 December 2022**
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**

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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 section 17.0 of the Scheme Guide
https://www.gov.uk/government/publications/food-and-water-proficiency-testing-schemes-scheme-guide

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	**FEPTU's website**
Scheme Advisors	Justin Avant[i] & 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 NHV011	**36**
Number of laboratories not returning a result for NHV011	**2**
Number of laboratories not examining any samples in NHV011	**0**

Sample: NHV0021

Sample type: LENTICULE® discs prepared with known levels of norovirus GI and GII 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 9		
	Norovirus GII	Positive			
	HAV	Positive			

Quantification results

Examination		Expected range	Your Result
Virus	Norovirus GI	$4.6x10^2$ – $8.4x10^4$ ($2.67 \log_{10}$ – $4.92 \log_{10}$)	Your reported results are shown on page 9
	Norovirus GII	$6.8x10^2$ – $3.0x10^3$ ($2.83 \log_{10}$ – $3.48 \log_{10}$)	
	HAV	$2.2x10^3$ – $8.9x10^4$ ($3.33 \log_{10}$ – $4.95 \log_{10}$)	

Norovirus GI (graph of results shown on page 4)

Total participants reporting for Norovirus GI	34
Participants reporting correctly a detected result	33 (97%)
Number of laboratories reporting copies per sample	9
Expected range as copies per sample	$4.6x10^2$ – $8.4x10^4$ ($2.67 \log_{10}$ – $4.92 \log_{10}$)
Assigned value (participants' median)	$6.2x10^3$ copies per sample ($3.79 \log_{10}$)
No. of outlying counts	0
Uncertainty of assigned value ($U(X_{pt})$ = $\log_{10}$)	0.24
Participants mean	$1.2x10^4$ copies per sample ($4.07 \log_{10}$)
Standard deviation of participants results	0.56 copies per sample $\log_{10}$
FEPTU's QC median	$5.8x10^3$ copies per sample ($3.76 \log_{10}$)

Norovirus GII (graph of results shown on page 5)

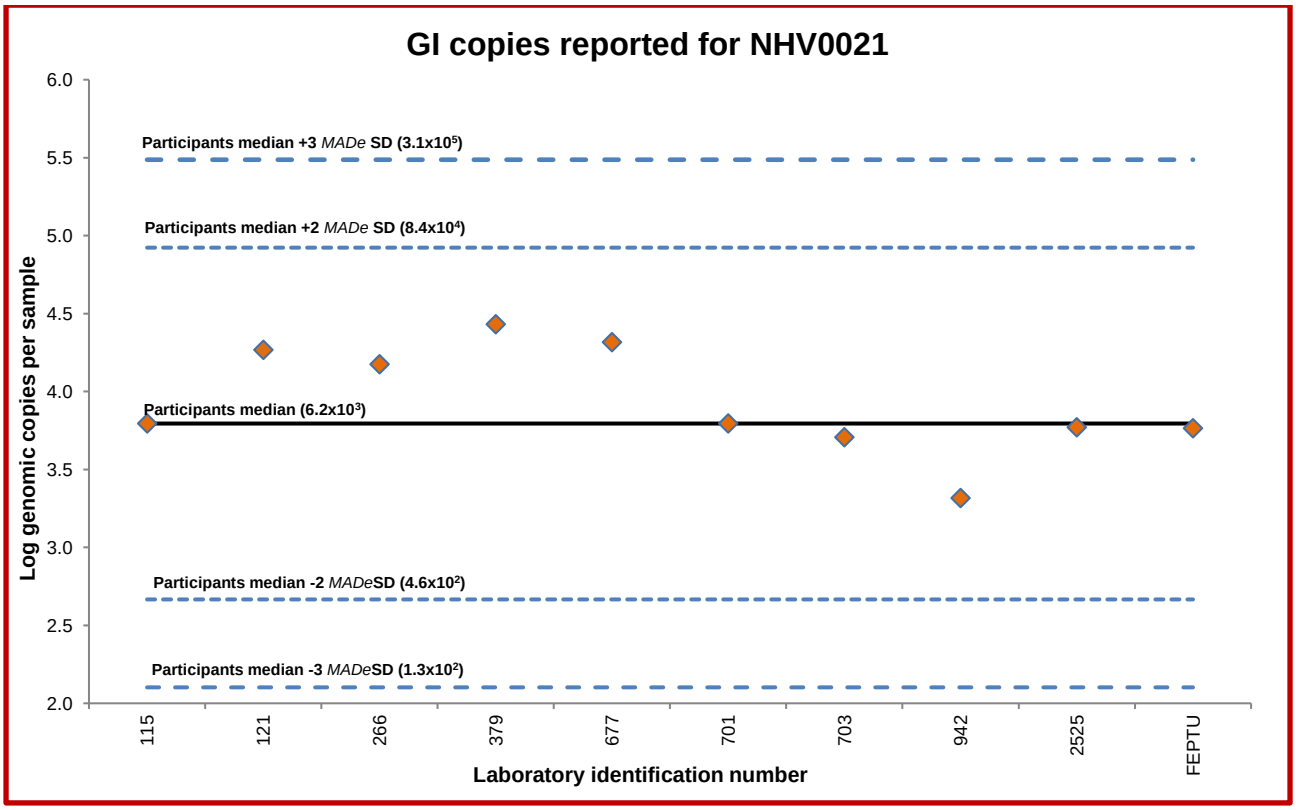
Total participants reporting for Norovirus GII	33
Participants reporting correctly a detected result	28 (85%)
Number of laboratories reporting copies per sample	10
Expected range as copies per sample	$6.8x10^2$ – $3.0x10^3$ ($2.83 \log_{10}$ – $3.48 \log_{10}$)
Assigned value (participants' median)	$1.4x10^3$ copies per sample ($3.15 \log_{10}$)
No. of outlying counts	3 (2 low, 1 high) – see page 10 for comment on statistics
Uncertainty of assigned value ($U(X_{pt})$ = $\log_{10}$)	0.06
Participants mean	$1.6x10^3$ copies per sample ($3.20 \log_{10}$)
Standard deviation of participants results	0.16 copies per sample $\log_{10}$
FEPTU's QC median	$7.40x10^2$ copies per sample ($2.87 \log_{10}$)

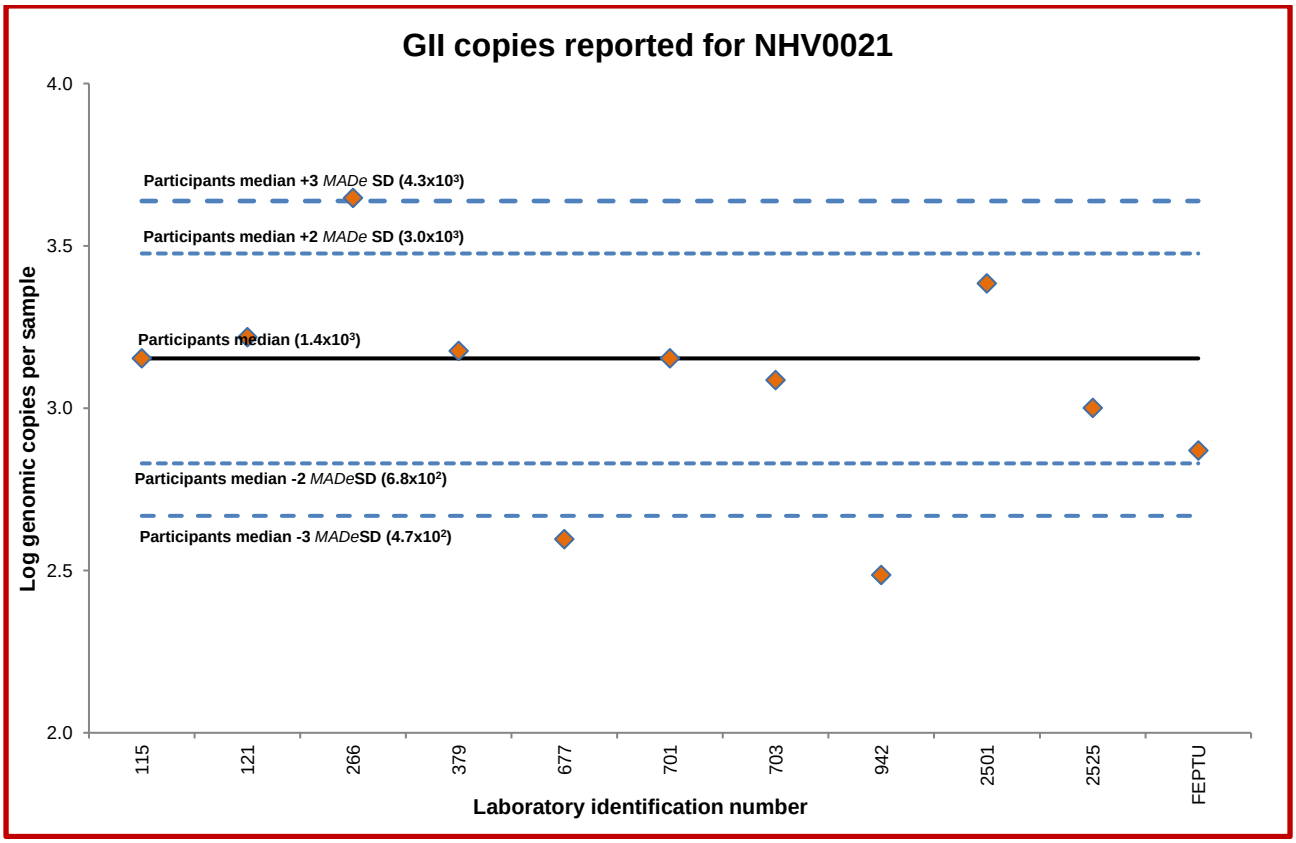
Examination specific comment: GII

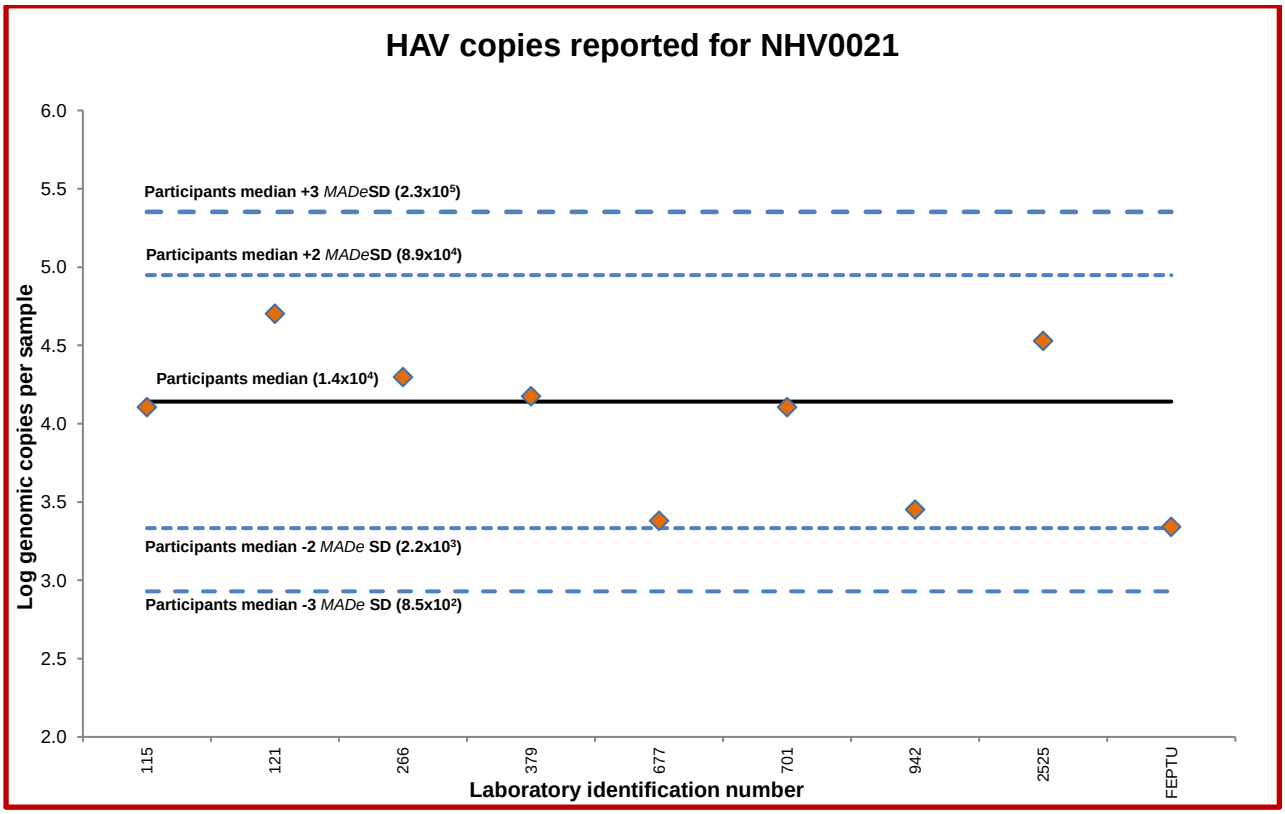
5/33 (15%) of the laboratories reported a false negative result for GII, levels were lower than other virus targets and more challenging, it is important for laboratories to understand the limit of detection for the assays they use. Further analysis of the method/kit information did not highlight an issue with any particular method/kit.

HAV (graph of results shown on page 5)

Total participants reporting for HAV	31
Participants reporting correctly a detected result	29 (94%)
Number of laboratories reporting copies per sample	8
Expected range as copies per sample	$2.2x10^3$ – $8.9x10^4$ ($3.33\ log_{10}$ – $4.95\ log_{10}$)
Assigned value (participants' median)	$1.4x10^4$ copies per sample ($4.14\ log_{10}$)
No. of outlying counts	0
Uncertainty of assigned value ($U(X_{pt})$ = log_{10})	0.18
Participants mean	$1.9x10^4$ copies per sample ($4.27\ log_{10}$)
Standard deviation of participants results	0.40 copies per sample log_{10}
FEPTU's QC median	$2.2x10^3$ copies per sample ($3.34\ log_{10}$)




GII copies reported for NHV0021
Log genomic copies per sample
4.0
3.5
3.0
2.5
2.0
Participants median +3 MADe SD (4.3x10³)
Participants median +2 MADe SD (3.0x10³)
Participants median (1.4x10³)
Participants median -2 MADeSD (6.8x10²)
Participants median -3 MADeSD (4.7x10²)
115
121
266
379
677
701
703
942
2501
2525
FEPTU
Laboratory identification number


HAV copies reported for NHV0021
Log genomic copies per sample
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Participants median +3 MADeSD (2.3x10⁵)
Participants median +2 MADeSD (8.9x10⁴)
Participants median (1.4x10⁴)
Participants median -2 MADe SD (2.2x10³)
Participants median -3 MADe SD (8.5x10²)
115
121
266
379
677
701
942
2525
FEPTU
Laboratory identification number

Sample: NHV0022

Sample type: LENTICULE® discs prepared with 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	Negative	Your reported results and scores are shown on page 10		
	Norovirus GII	Negative			
	HAV	Positive			

Quantification results

Examination		Expected range	Your Result
Virus	Norovirus GI	-	Your reported results are shown on page 10
	Norovirus GII	-	
	HAV	$1.7x10^3$ – $1.8x10^5$ ($3.24 \log_{10}$ – $5.26 \log_{10}$)	

Norovirus GI

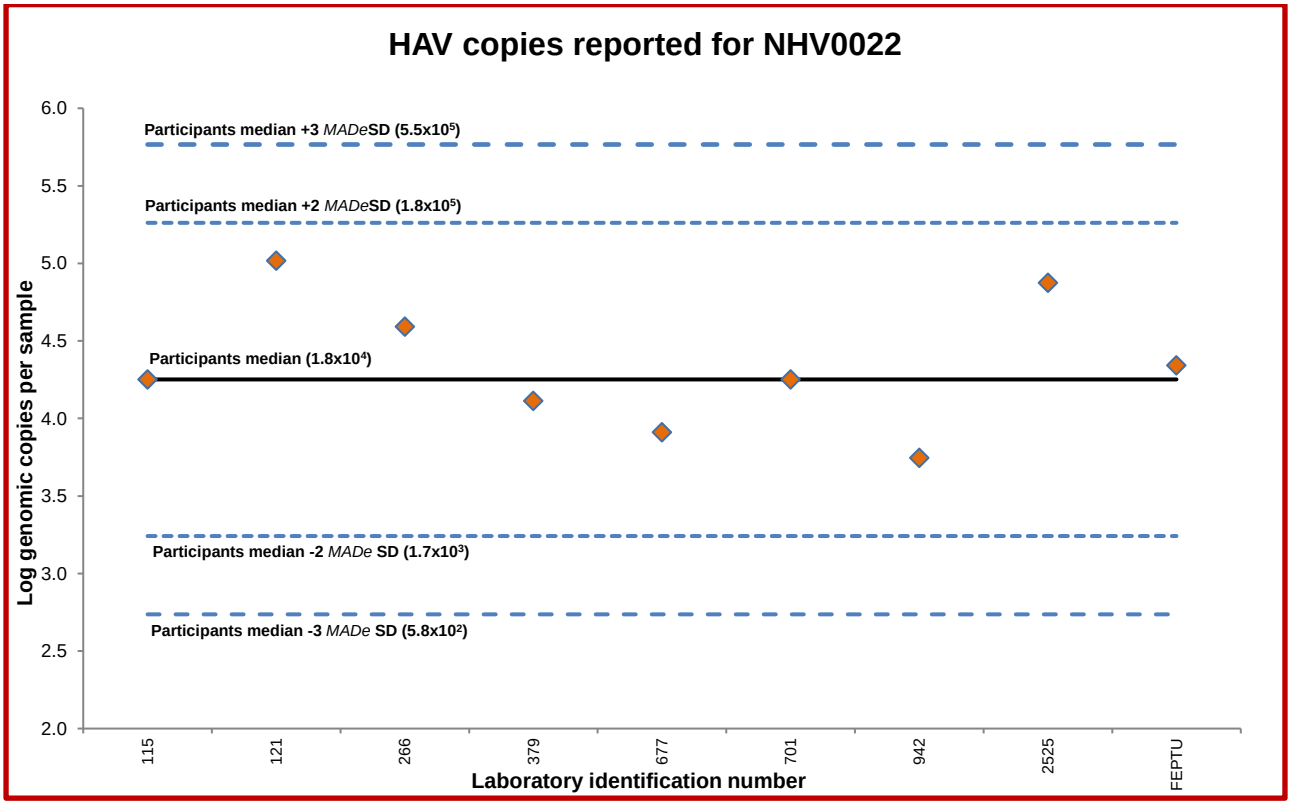
Total participants reporting for GI	34
Participants reporting correctly a not detected result	34 (100%)

Norovirus GII

Total participants reporting for GII	32
Participants reporting correctly a not detected result	31 (97%)

HAV (graph of results shown on page 7)

Total participants reporting for HAV	31
Participants reporting correctly a detected result	30 (97%)
Number of laboratories reporting copies per sample	8
Expected range as copies per sample	$1.7x10^3$ – $1.8x10^5$ ($3.24 \log_{10}$ – $5.26 \log_{10}$)
Assigned value (participants' median)	$1.8x10^4$ copies per sample ($4.25 \log_{10}$)
Uncertainty of assigned value (U(Xpt) = log10)	0.22
No. of outlying counts	0
Participants mean	$3.5x10^4$ copies per sample ($4.54 \log_{10}$)
Standard deviation of participants results	0.50 copies per sample $\log_{10}$
FEPTU's QC median	$2.2x10^4$ (copies per sample ($4.34 \log_{10}$)


HAV copies reported for NHV0022
Participants median +3 MADeSD (5.5x10⁵)
Participants median +2 MADeSD (1.8x10⁵)
Participants median (1.8x10⁴)
Participants median -2 MADe SD (1.7x10³)
Participants median -3 MADe SD (5.8x10²)
Log genomic copies per sample
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
115
121
266
379
677
701
942
2525
FEPTU
Laboratory identification number

General comments for this distribution

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 $\log_{10}$ units

If the ranges in (1) and/or (2) are less than the value of the median ± 0.5 $\log_{10}$ 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 examination to be scored.

Reported results for laboratories and scores awarded for NHV0021: NR: Non-return of results, N/A: Not applicable, NE: Not examined

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

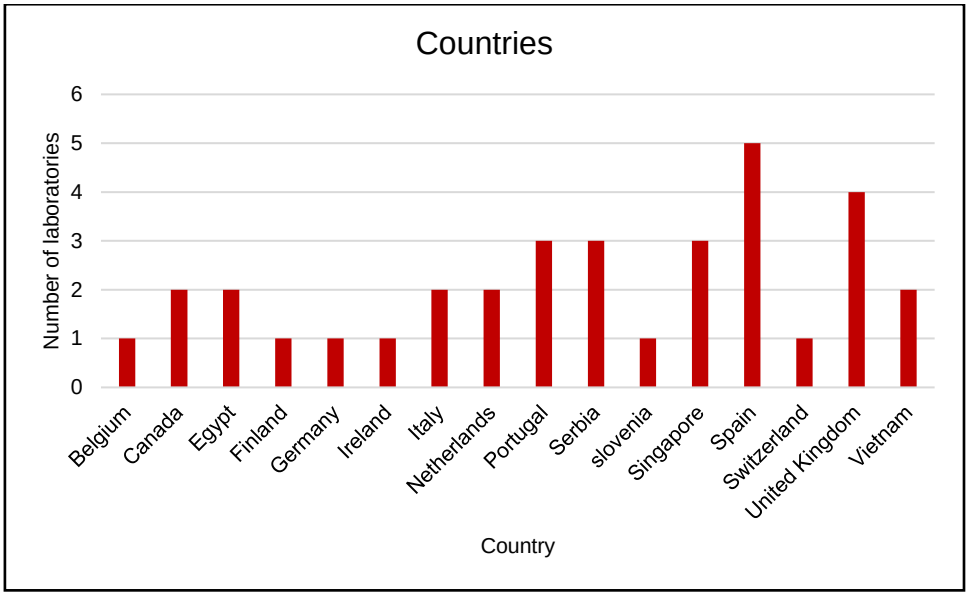
Reported results for laboratories and scores awarded for NHV0022: NR: Non-return of results, N/A: Not applicable, NE: Not examined

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

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. 34 laboratories returned a result for this distribution.

The graph below shows the countries that returned a result (n=34).



34/34 (100%) of the laboratories used a real-time PCR.

The RNA extractions used is shown in the table below (n=32):

	No of users
BioMérieux NucliSENS® miniMag®	15
BIOTECON Diagnostics Extraction Kit	3
BIOTECON Diagnostics foodproof® Magnetic Preparation Kit VI	1
BRUKER HAIN LIFESCIENCE GenoXtract®	1
CONGEN SureFast® Prep DNA/RNA virus	1
Geneaid Biotech Viral Nucleic Acid Extraction Kit II	1
KingFisher™ MagMAX™ CORE Nucleic Acid Purification Kit	1
Promega Maxwell® RSC PureFood GMO and Authentication Kit	1
Qiagen QIAamp® Viral RNA	5
Roche High Pure Viral RNA kit	1
Thermo Scientific GeneJET RNA Purification Kit	1
Zinexts MagPurix® Viral Nucleic Acid	1
Zymo Research Quick-DNA/RNA™ Viral Kit	1

The PCR reagents used is shown in the table below (n=32): some laboratories used more than one reagent.

	No of users
Applied Biosystems™ TaqMan™ Fast Virus One-Step Master Mix	2
Bio-Rad One-Step RT-ddPCR Advanced Kit for Probes	1
BIOTECON Diagnostics	1
BIOTECON Diagnostics foodproof® Norovirus G1, G2 and HAV Detection Kit	5
CeeramTools® Ceeram commercial Kit	1
CeeramTools® GI, GII and HAV detection kits	7
CeeramTools® GI, GII detection kits	1
CONGEN SureFast® Norovirus/Hepatitis A 3plex kit	2
Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System	9
nzytech NZYSpeed One-step RT-qPCR Probe Master Mix	1
OTHER - Generon - WHATfinder Norovirus GI, Norovirus GII e HAV detection	1
Promega GoTaq® Probe 1-Step qPCR Master mix.	1
Qiagen QuantiNova Pathogen + IC Kit	1
QIAGEN® QuantiTect® Probe RT-PCR kit	1

End of report