FAO Reference Centre for Bivalve Mollusc Sanitation

Norovirus (Genogroup I and II) and Hepatitis A virus Proficiency Testing (PT 104)

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Cefas Document Control

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Version control history

Version	Author	Date	Comment
0.01	L. Stockley	28.11.25	Sent to JL for QA
0.02	L. Stockley	18.12.25	After QA by JL
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Final V1	L. Stockley	22.01.26	Updated following participants comments

Contents

1. Preparation of sample material

1.1. Sample and virus strain origin

Materials dispatched consisted of whole Pacific oysters (*Magallana gigas*), blended digestive glands from the same species and dsDNA control solutions for quantification (1 x 10^5 copies/µl) for each target virus. The origin of the viruses used for preparing the samples are given in Table 1. All samples were held at <-15°C until required for quality control testing, dispatch and/or reference analysis.

Table 1 - Origin and strain/genotype of viruses used for shellfish contamination

Description	Source	Strain ID / genotype
Hepatitis A virus	Cell culture supernatant	HM175/43c
Norovirus genogroup I	Faecal material	GI.3 (based on capsid sequence)
Norovirus genogroup II	Faecal material	GII.4 (based on capsid sequence)

1.2. Preparation of digestive gland base material

A single batch of approximately 3000 Pacific oysters (*M. gigas*) was collected from a UK commercial harvesting area in August 2023. A proportion of the shellfish was shucked, and the digestive glands removed, pooled together and blended to form a homogenous mixture. The mixture was tested for norovirus (NoV) genogroups I and II (GI and GII) and hepatitis A virus (HAV) prior to being used to prepare different samples.

1.3. Sample preparation

1.3.1. Sample 1

Pacific oysters (*M. gigas*) collected from a UK commercial harvesting area in August 2023 were initially tested to demonstrate the absence of all 3 target viruses. The shellfish were then placed in a large sterile container and thoroughly mixed before subsamples of 10 oysters were randomly selected and placed in sample bags and stored at <-15°C.

1.3.2. Sample 2

Blended digestive gland base material (see above) was mixed with NoV GII to obtain the desired target levels before being split into 2 g aliquots. The samples were held at <-15 °C until required for quality control testing, dispatch and / or reference analysis (Table 2).

1.3.3. Sample 3

Blended digestive gland base material (see above) was mixed with NoV GI, NoV GII and HAV to obtain the desired target levels before being split into 2 g aliquots. The samples were held at <-15 °C until required for quality control testing, dispatch and / or reference analysis (Table 2).

1.3.4. Sample 4

Blended digestive gland base material (see above) was mixed with NoV GI and HAV to obtain the desired target levels before being split into 2 g aliquots. The samples were held at <-15 °C until required for quality control testing, dispatch and / or reference analysis (Table 2).

2. Sample distribution

Samples were dispatched on dry ice in accordance with IATA packing instructions for UN3373 'Diagnostic Specimens' on 21st July 2025 to 17 participating laboratories by the courier company Ihrglobal with an additional 2 boxes being dispatched on the 24th July. Major issues were experienced during the initial shipment which impacted the transport of 11 boxes being held at customs in Germany and Belgium. Regular communication with Ihrglobal identified customs resources was a significant factor in the delays and it was decided to arrange all outstanding boxes to be returned to Cefas. The samples were then resent to the remaining participants on the 7th October following consultation with Ihrglobal.

On arrival, participants were given a month to analyse the test samples using their routine method. Those laboratories using quantitative real-time RT-PCR were requested to calculate the quantity of target virus in each sample using both their own standard material and using the dsDNA control solutions provided with this PT distribution.

3. Results

3.1. Reference results

Reference analyses were performed by the FAO Reference Centre (FAO RC) for Bivalve Mollusc Sanitation on samples stored at <-15˚C. Six randomly selected samples from each sample type were extracted in duplicate and qRT-PCR (TaqMan™) was carried out using duplicate PCR reactions for each RNA extract and each target. Reference results for each sample are shown in Table 2, with box and whisker plots included in Appendix 1.

Table 2 - Reference results for PT 104 Proficiency testing material

Sample	NoV GI	NoV GII	HAV
Sample 1 (Whole animal)	-	-	-
Sample 2 (Digestive gland)	-	+ $2.41 \times 10^3 – 4.36 \times 10^3$	-
Sample 3 (Digestive gland)	+ $1.57 \times 10^3 – 4.14 \times 10^3$	+ $2.18 \times 10^2 – 2.29 \times 10^3$	+ $2.52 \times 10^2 – 1.55 \times 10^3$
Sample 4 (Digestive gland)	+ $1.06 \times 10^3 – 5.09 \times 10^3$	-	+ $1.29 \times 10^3 – 5.41 \times 10^3$

KEY: - = Negative, + = Positive, Quantities in copies / g, ranges based on a 95% confidence limit determined as 2 geometric standard deviations above and below the geometric mean.

3.2. Participants' results and scoring

Participants' results are tabulated in Appendices 2, 3 and 4 and quantitative results are shown in graphical form alongside the reference values in Appendix 5.

3.2.1. Presence / absence

Performance scoring was undertaken on each participant's presence / absence results. A single score for each sample and each target virus (NoV GI, NoV GII and HAV) was assigned as follows: Correct = 2 points, Incorrect = 0 points. For each laboratory an overall score is provided for each target virus, taking into account the results of all samples (Table 4).

3.2.2. Quantification

For those laboratories submitting quantitative results, an additional performance scoring for quantification was undertaken following the median absolute deviation from the median (MAD) approach described in ISO 22117 Microbiology of the food chain – Specific requirements and guidance for proficiency testing by interlaboratory comparison (ISO, 2019). The MAD approach is recommended for assessment of PT data where less than 50 participants return quantitative results and/or for new proficiency assessments. Where laboratories submitted quantitative results determined using both their own quantification standards, and those provided by the FAO RC, only the results using their own standards were considered for performance scoring; however, where laboratories submitted quantitative results using the FAO RC standards only, these were considered. Where laboratories reported positive results at levels below their limit of quantification (LOQ), or positive; not quantifiable results due to quality control issues (e.g. unacceptable extraction efficiencies), these results were not subject to performance scoring for quantification; where laboratories reported positive results at levels below LOQ determined using their own quantification standards, but quantifiable positive results using the FAO RC standards, results using the FAO RC standards were used for performance scoring. Laboratories were given a score for each virus for which they reported at least one quantitative result.

For each sample / target virus combination where the intended result was positive, a statistically robust acceptability range was determined by calculation of the median absolute deviation (MAD) of each participant's result from the median of all participants' results. This figure was then multiplied by a constant (1.4826) to obtain a robust estimate of the standard deviation (σMAD; Table 3). For each individual result, its absolute deviation from the participants' median was compared with the calculated σMAD to determine its acceptability and score as follows:-

- Difference between result and participants' median <2 σMAD = satisfactory (2 points)
- Difference between result and participants' median >2 σMAD and <3 σMAD = questionable (1 point)
- Difference between result and participants' median >3 σMAD = unsatisfactory (0 points)
- Result reported as negative = unsatisfactory (0 points)

The differences between individual participants' results and the participants' median, expressed in terms of σMAD are shown in Appendix 4, and the graphs in Appendix 5 include lines showing the boundaries of the satisfactory and questionable ranges for each sample / target matrix combination.

For each sample / target virus combination where the intended result was negative, its acceptability and score was determined as follows:-

- Result reported as negative = satisfactory (2 points)
- Result reported as positive = unsatisfactory (0 points)

Table 3 - Dataset characteristics for quantitative results

Characteristic	Shellfish sample 2	Shellfish sample 3			Shellfish sample 4	
	GII	GI	GII	HAV	GI	HAV
MEDIAN	3.519	3.299	3.132	2.701	2.817	3.392
MAD	0.273	0.335	0.418	0.458	0.407	0.587
σMAD	0.405	0.496	0.620	0.678	0.604	0.871

Values in $\log_{10}$ copies/g

For each laboratory an overall score (usually out of 8) is provided for each target virus, taking into account the results of all 4 samples (Table 4).

Table 4 - Performance scoring

Lab ID	Presence / Absence			Quantification[a]		
	NoV		HAV	NoV		HAV
	GI	GII		GI	GII	
2	8 out of 8	8 out of 8	8 out of 8	NE	NE	NE
3	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8	NE
10	8 out of 8	8 out of 8	8 out of 8	6 out of 6	8 out of 8	6 out of 6
20	8 out of 8	8 out of 8	8 out of 8	NE	NE	NE
24 [a]	8 out of 8	6 out of 8	8 out of 8	8 out of 8	6 out of 8	8 out of 8
39	8 out of 8	8 out of 8	8 out of 8	8 out of 8	7 out of 8	8 out of 8
47	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8
48	8 out of 8	8 out of 8	8 out of 8	8 out of 8	7 out of 8	8 out of 8
57	8 out of 8	8 out of 8	8 out of 8	6 out of 6	6 out of 6	6 out of 6
96	8 out of 8	8 out of 8	8 out of 8	7 out of 8	8 out of 8	8 out of 8
143	8 out of 8	8 out of 8	8 out of 8	7 out of 8	8 out of 8	8 out of 8
237	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8
364	NE	NE	8 out of 8	NE	NE	NE
413	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8	8 out of 8
498	6 out of 8	8 out of 8	8 out of 8	6 out of 8	8 out of 8	8 out of 8
552	8 out of 8	8 out of 8	NE	7 out of 8	6 out of 8	NE

Key: [a] = Samples arrived at ambient temperature which could impact final results; **NE** = Target virus not examined or not quantified; Labs that scored less than full marks for any criteria are highlighted in yellow.

4. Discussion

4.1. General comments

The transport of PT 104 samples was undertaken by Ihrglobal (Courier company) using DHL on the 21st July 2025. During this 1st distribution, the FAORC was informed that a number of European boxes had been stopped at airport customs in Belgium and Germany. The FAORC requested Ihrglobal to return all boxes stuck at customs to the FAORC and to identify an alternative way to ship the outstanding participants material to ensure similar delays do not occur.

On the 7th October Ihrglobal arranged for the remaining 9 boxes to be collected using Fedex with all except 1 arriving at their destinations within 7 days.

The courier was requested to identify the cause of all delays during the shipment of PT 104, which it was confirmed to be due to staff shortages at customs, incorrect paperwork supplied or communication issues between the courier and the receiving laboratory. It was also noted that Labs 24 and 98 reported no dry ice remaining in their sample boxes on arrival and Lab 498 encountered significant transport delays of around 20 days.

The FAORC recommends those participants experiencing issues at customs to review the documentation requested by customs and/or brokers.

On arrival participants were requested to use their routine method to analyse the samples. This information is shown in Appendix 6, while brief details of the types of materials used as quantification standards are included as Appendix 7.

For the sample analysis, Lab 364 only tested for HAV and Lab 552 tested only for Norovirus. Lab 242 experienced issues during the testing of the samples and did not report any results.

4.2. Presence / absence determination

For NoV, 13 out of 15 labs that tested for NoV (87%) obtained the intended presence / absence result (as determined by the FAO RC) for all samples and both genogroups. A total of 2 false negative results (1 for GI, 1 for GII) were reported by laboratories 24 and 498, resulting in an overall sensitivity for NoV of 97%. The overall specificity and accuracy for norovirus results were 100% and 98% respectively.

For HAV, all 15 labs that tested for HAV (100%) obtained the intended presence / absence result (as determined by the FAO RC) for all samples. No false positives or negatives were reported. Overall sensitivity, specificity and accuracy levels for HAV were 100%.

4.3. Quantification

A total of 13 laboratories (81%) reported quantitative data for at least one sample / virus combination and were subject to performance scoring; 6 laboratories (46%) received maximum scores for all target viruses for which they were assessed.

Laboratories 96, 143 and 552 reported one quantitative result for GI in the questionable range; laboratories 39 and 48 reported one quantitative result each for GII in the questionable range and laboratory 552 reported one quantitative result for GII in the unsatisfactory range. Laboratories 24 and 498 received a zero score for a false negative result for GII and GI respectively.

The FAO RC recommends any laboratory with an unsatisfactory result for either presence / absence or quantification, please refer to the trouble shooting guide available on the FAO RC website Troubleshooting guidance for virus PT (cefas.co.uk)

5. References

Codd AA, Richardson IR, Andrews N. 1998. Lenticules for the control of quantitative methods in food microbiology. J Appl Microbiol. 85(5):913–7.

Anon. 2017. 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.

Anon. 2019. ISO 22117:2019 Microbiology of the food chain – Specific requirements and guidance for proficiency testing by interlaboratory comparison.

6. Appendices

6.1. Appendix 1 – FAORC Results

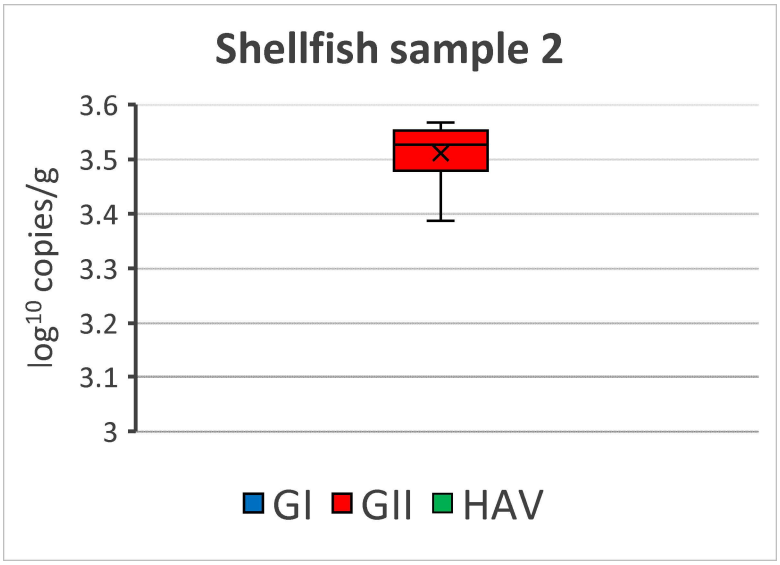

Figure 1. Box and Whisker plot showing $\log_{10}$ copies per gram of norovirus genogroup II for shellfish sample 2.

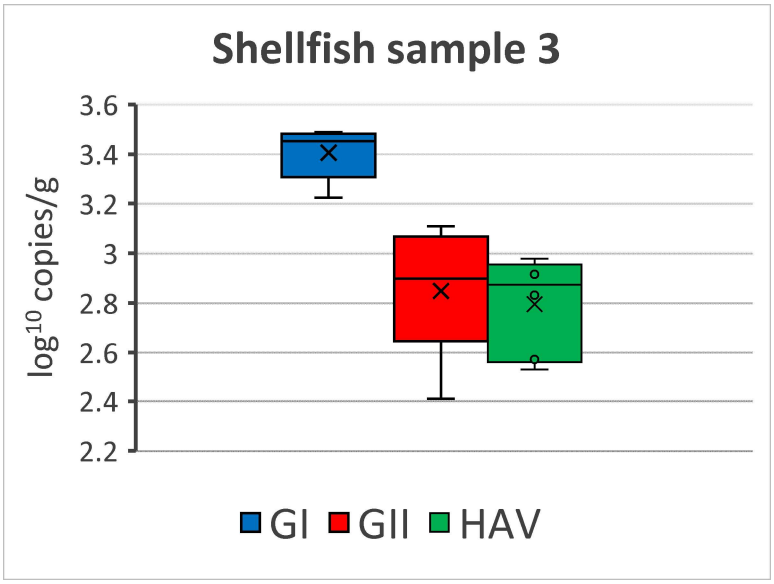

Figure 2. Box and Whisker plot showing $\log_{10}$ copies per gram of norovirus genogroup I, norovirus genogroup II, and hepatitis A virus for shellfish sample 3.

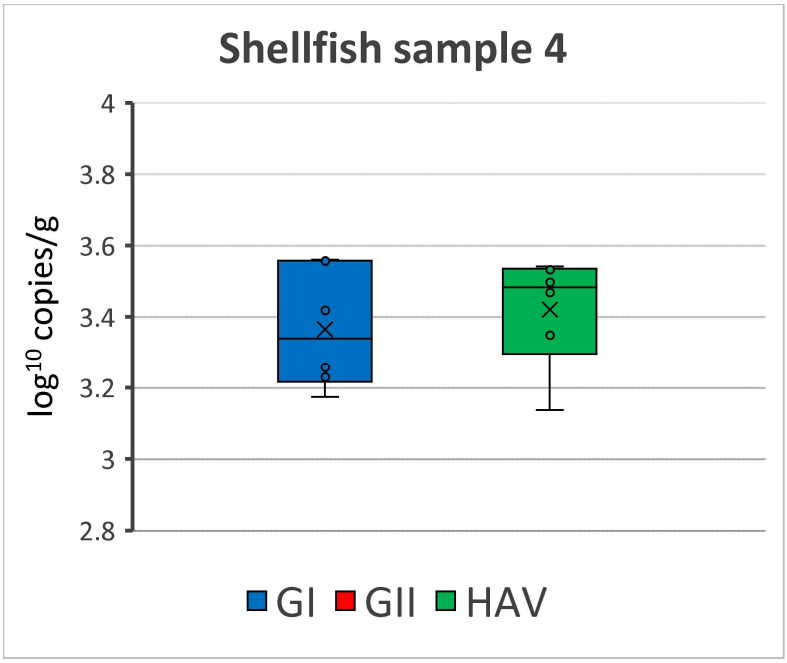

Figure 3. Box and Whisker plot showing log_{10} copies per gram of norovirus genogroup I and hepatitis A virus for shellfish sample 4.

6.2. Appendix 2 - Participants' presence / absence results and C_t values

Lab ID	Shellfish sample 1						Shellfish sample 2						Shellfish sample 3						Shellfish sample 4					
	GI		GII		HAV		GI		GII		HAV		GI		GII		HAV		GI		GII		HAV	
	-	Ct	-	Ct	-	Ct	-	Ct	+	Ct	-	Ct	-	Ct	+	Ct	+	Ct	+	Ct	-	Ct	+	Ct
2	-		-		-		-		+	33.51	-		+	33.74	+	35.40	+	33.77	+	35.38	-		+	31.23
3	-		-		-		-		+	35.99, 36.42	-		+	34.03, 34.22	+	34.43, 34.54	+	36.86	+	36.76, 36.96	-		+	36.71
10	-		-		-		-		+	34.71	-		+	35.23	+	35.87	+	39.62	+	38.95	-		+	39.92
20	-		-		-		-		+	34.02	-		+	30.60	+	35.09	+	36.77	+	33.32	-		+	33.33
24	-		-		-		-		+	33.70	-		+	37.30	-		+	37.30	+	38.60	-		+	34.50
39	-		-		-		-		+	32.42	-		+	32.34	+	36.65	+	38.34	+	35.62	-		+	32.45
47	-		-		-		-		+	30.08	-		+	34.84	+	31.82	+	39.00	+	35.39	-		+	36.40
48	-		-		-		-		+	38.49, 37.23	-		+	36.29, 36.35	+	37.99, 38.99	+	38.18, 39.85	+	39.94	-		+	37.58, 36.51
57	-		-		-		-		+	33.55	-		+	32.52	+	34.90	+	35.44	+	33.54	-		+	33.03
96	-		-		-		-		+	32.58	-		+	32.26	+	34.51	+	33.34	+	34.26	-		+	31.01
143	-		-		-		-		+	34.81, 34.42	-		+	35.02, 35.53	+	37.67, 38.47	+	37.89, 39.71	+	37.89, 39.71	-		+	38.32, 38.73
237	-		-		-		-		+	30.44	-		+	31.81	+	32.02	+	33.36	+	33.25	-		+	31.60
364	NE		NE		-		NE		NE		-		NE		NE		+	36.76	NE		NE		+	36.49
413	-		-		-		-		+	32.68	-		+	33.49	+	35.45	+	37.06	+	35.07	-		+	34.79
498	-		-		-		-		+	33.25	-		+	34.81	+	35.89	+	38.26	-		-		+	35.12
552	-		-		NE		-		+	33.16	NE		+	35.02	+	35.81	NE		+	36.88	-		NE	

Key: - = negative; **+** = positive; **NE** = Not examined; **Yellow shading** denotes false negative results.

6.3. Appendix 3 – Participants' reported quantities for positive results

Reported quantities for each target given in genome copies per gram for positive sample/target virus combinations.

Lab ID	Shellfish sample 2		Shellfish sample 3						Shellfish sample 4			
	GII		GI		GII		HAV		GI		HAV	
	A	B	A	B	A	B	A	B	A	B	A	B
3	1.20E+04	NE	4.30E+03	NE	3.55E+03	NE	NE	NE	7.15E+02	NE	NE	NE
10	2.81E+03	NE	1.81E+03	NE	1.36E+03	NE	4.36E+02	NE	<LOQ	NE	<LOQ	NE
24	4.00E+03	4.10E+03	1.35E+03	NE	ND	ND	NE	5.78E+02	2.27E+02	NE	NE	1.40E+03
39	NE	1.08E+03	NE	3.36E+03	NE	6.70E+01	NE	5.00E+01	NE	3.35E+02	NE	2.27E+03
47	9.17E+03	4.68E+03	1.52E+03	6.72E+02	2.73E+03	1.44E+03	1.51E+03	<LOQ	9.03E+02	3.99E+02	9.67E+03	4.39E+03
48	NE	3.80E+02	NE	4.50E+02	NE	2.39E+02	NE	1.83E+02	NE	6.00E+01	NE	5.97E+02
57	3.30E+03	4.85E+03	4.45E+03	5.50E+03	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.10E+04	9.00E+03
96	3.12E+03	6.46E+03	3.53E+04	1.07E+04	8.80E+02	1.93E+03	3.12E+03	2.47E+03	8.79E+03	2.75E+03	1.47E+04	1.09E+04
143	1.39E+04	1.62E+04	2.20E+04	3.57E+04	2.56E+03	2.89E+03	3.66E+02	4.42E+02	2.52E+03	1.35E+03	2.67E+03	7.50E+02
237	3.89E+03	2.97E+03	4.51E+03	2.11E+03	1.37E+03	1.06E+03	7.42E+03	5.42E+02	1.75E+03	9.18E+02	2.65E+04	1.87E+03
413	3.69E+03	3.78E+03	1.99E+03	3.57E+03	5.73E+03	6.42E+03	6.33E+02	5.46E+02	6.03E+02	1.12E+03	6.47E+02	5.74E+02
498	NE	1.76E+03	NE	1.74E+03	NE	2.89E+02	NE	1.08E+02	NE	ND	NE	9.74E+02
552	1.51E+02	9.14E+02	1.08E+02	9.71E+02	<LOQ	1.24E+02	NE	NE	<LOQ	2.68E+02	NE	NE

Key: A = results obtained with lab's own quantification standards; **B** = results obtained with FAO Reference Centre quantification standards; **NE** = sample/target virus/quantification standard combination not tested (shaded grey); **ND** = target virus not detected (shaded red); **<LOQ** = positive results at below the laboratory LOQ reported (shaded blue).

6.4. Appendix 4 - Comparison of results and median

Differences between participants' results and the participants' median, expressed in terms of σMAD, for positive sample/target virus combinations.

Lab ID	Shellfish sample 2	Shellfish sample 3			Shellfish sample 4	
	GII	GI	GII	HAV	GI	HAV
3	1.39	0.67	0.67	NE	0.06	NE
10	-0.17	-0.08	0.00	-0.09	NS	NS
24	0.21	-0.34	ND	0.09	-0.76	-0.28
39	-1.20	0.47	-2.11	-1.48	-0.48	-0.04
47	1.10	-0.24	0.49	0.70	0.23	0.68
48	-2.32	-1.30	-1.22	-0.65	-1.72	-0.71
57	0.00	0.70	NS	NS	NS	0.75
96	-0.06	2.52	-0.30	1.17	1.87	0.89
143	1.54	2.10	0.44	-0.20	0.97	0.04
237	0.18	0.72	0.01	1.72	0.70	1.18
413	0.12	0.00	1.01	0.15	-0.06	-0.67
498	-0.67	-0.12	-1.08	-0.98	ND	-0.46
552	-3.31	-2.55	-1.68	NE	-0.64	NE

Key: NE = Quantitative results for sample/target virus combination not reported (grey shading); **NS** = sample/target virus quantification not scored due to positive, <LOQ results (shaded blue); **orange shading** = questionable results (magnitude of difference between result and participants' median >2 σMAD and <3 σMAD); **red shading** = unsatisfactory results (magnitude of difference between result and participants' median >3 σMAD) or false negative results (ND).

6.5. Appendix 5 - Participants' and reference quantities by sample

Note: Where quantities were reported using both the laboratory's own quantification standards and those provided by the FAO Reference Centre, only those using the lab's own standards are considered for performance scoring.

Shellfish sample 2 – GII

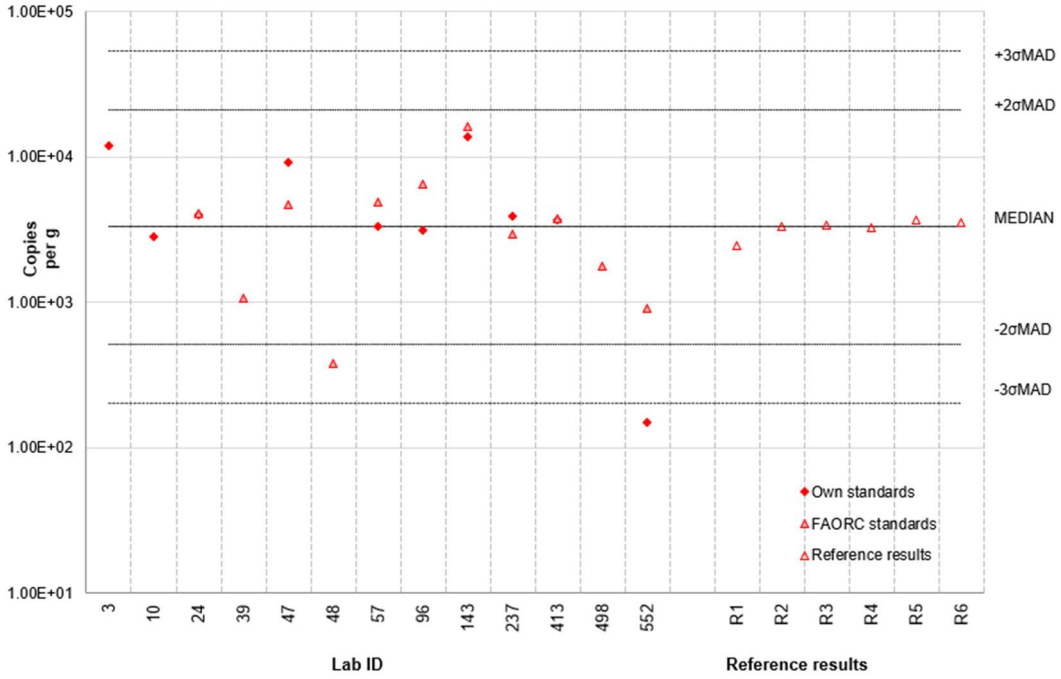

Figure 3. Scatter graph showing $\log_{10}$ copies per gram of norovirus genogroup II for shellfish sample 2.

Shellfish sample 3 – GI

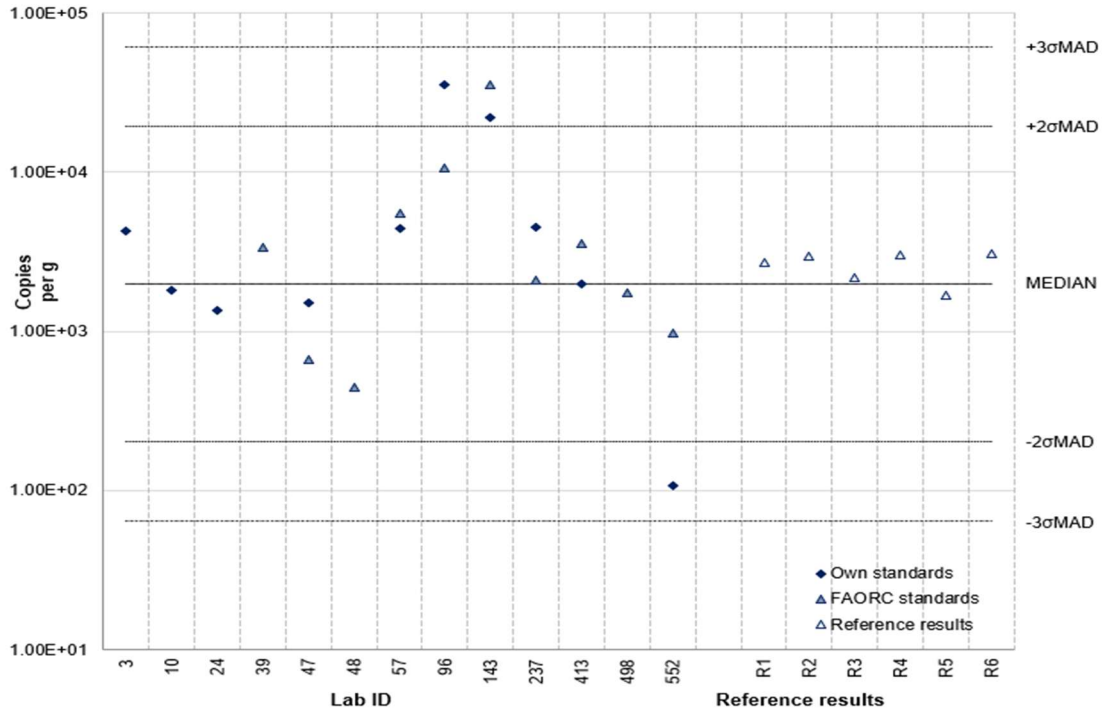

Figure 4. Scatter graph showing $\log_{10}$ copies per gram of norovirus genogroup I for shellfish sample 3.

Shellfish sample 3 – GII

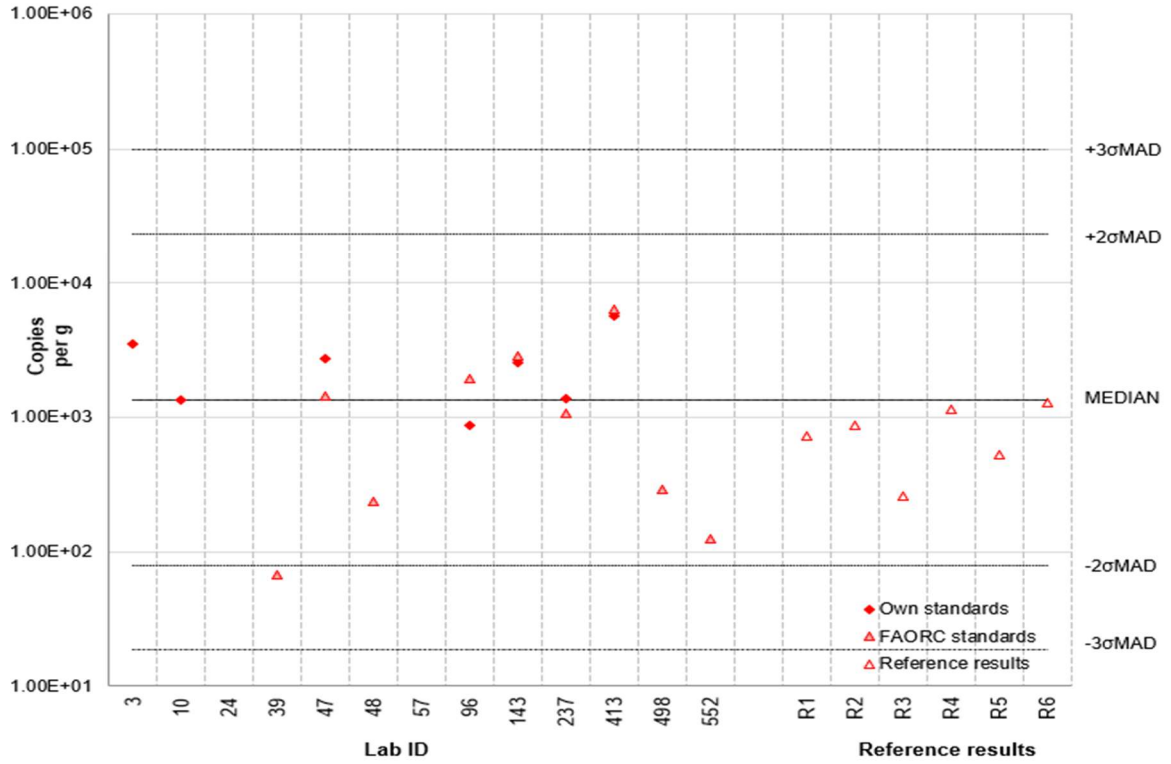

Figure 5. Scatter graph showing $\log_{10}$ copies per gram of norovirus genogroup II for shellfish sample 3.

Shellfish sample 3 – HAV

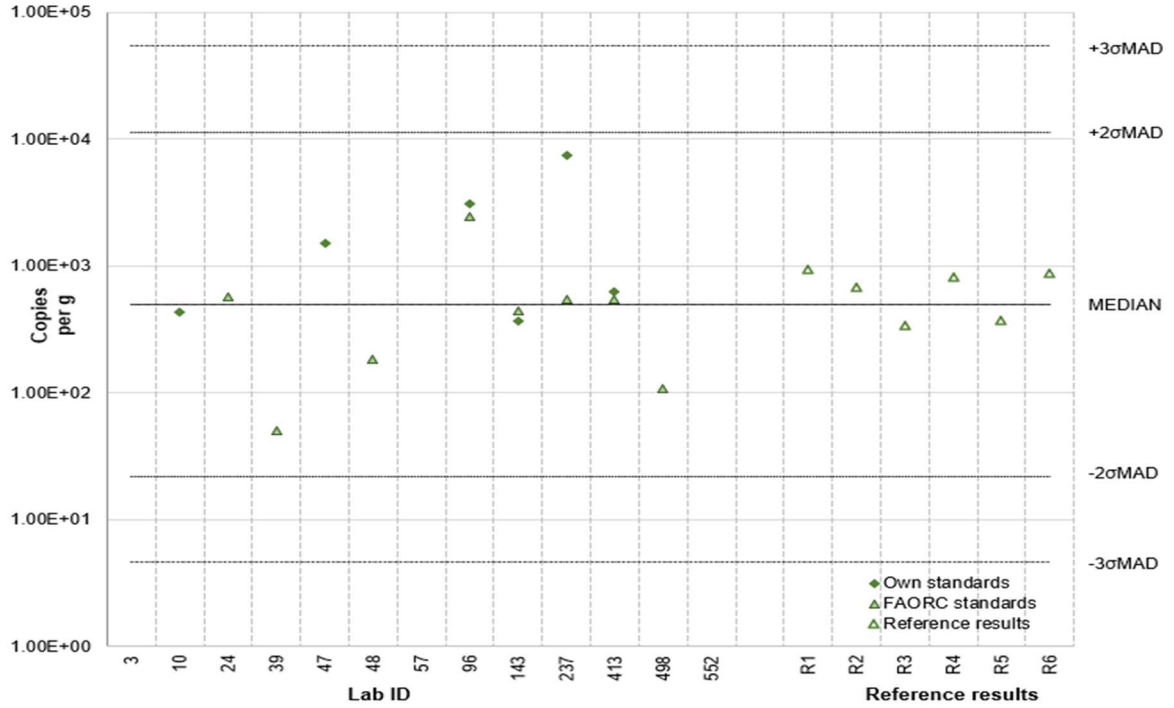

Figure 6. Scatter graph showing $\log_{10}$ copies per gram of hepatitis A virus for shellfish sample 3.

Shellfish sample 4 – GI

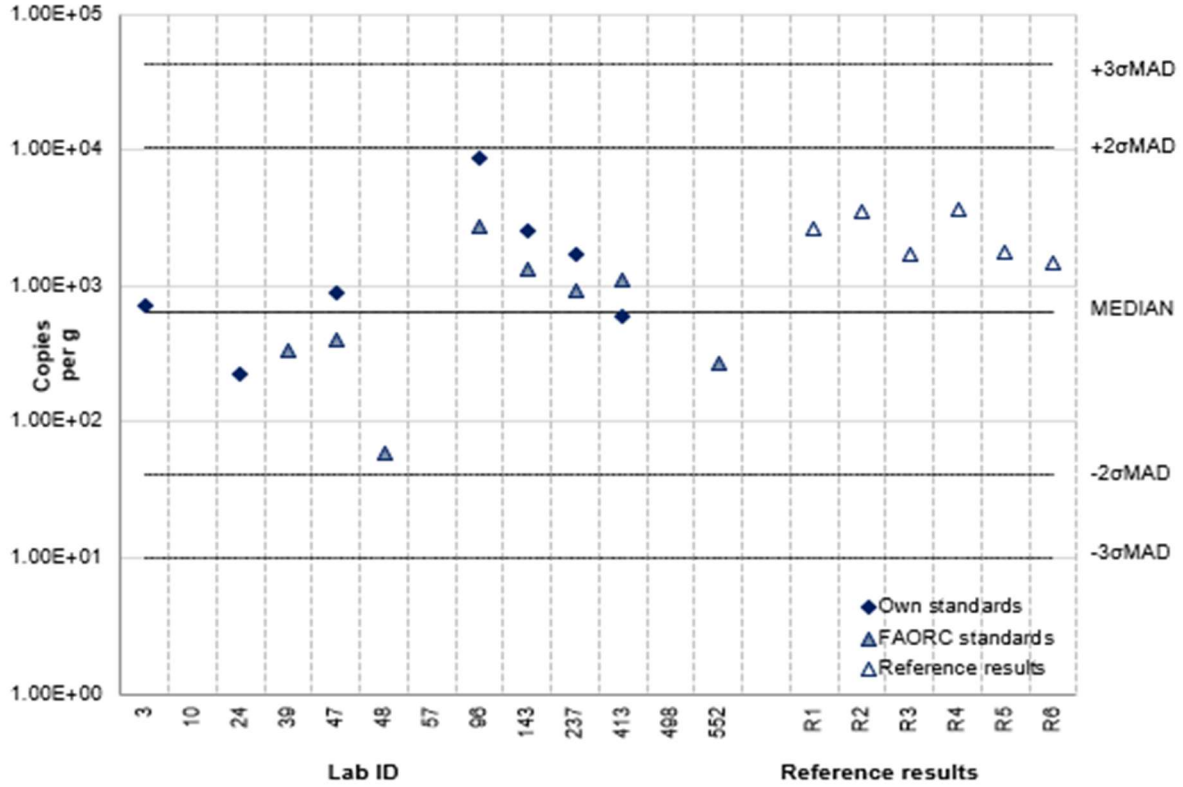

Figure 7. Scatter graph showing $\log_{10}$ copies per gram of norovirus genogroup I for shellfish sample 4.

Shellfish sample 4 – HAV

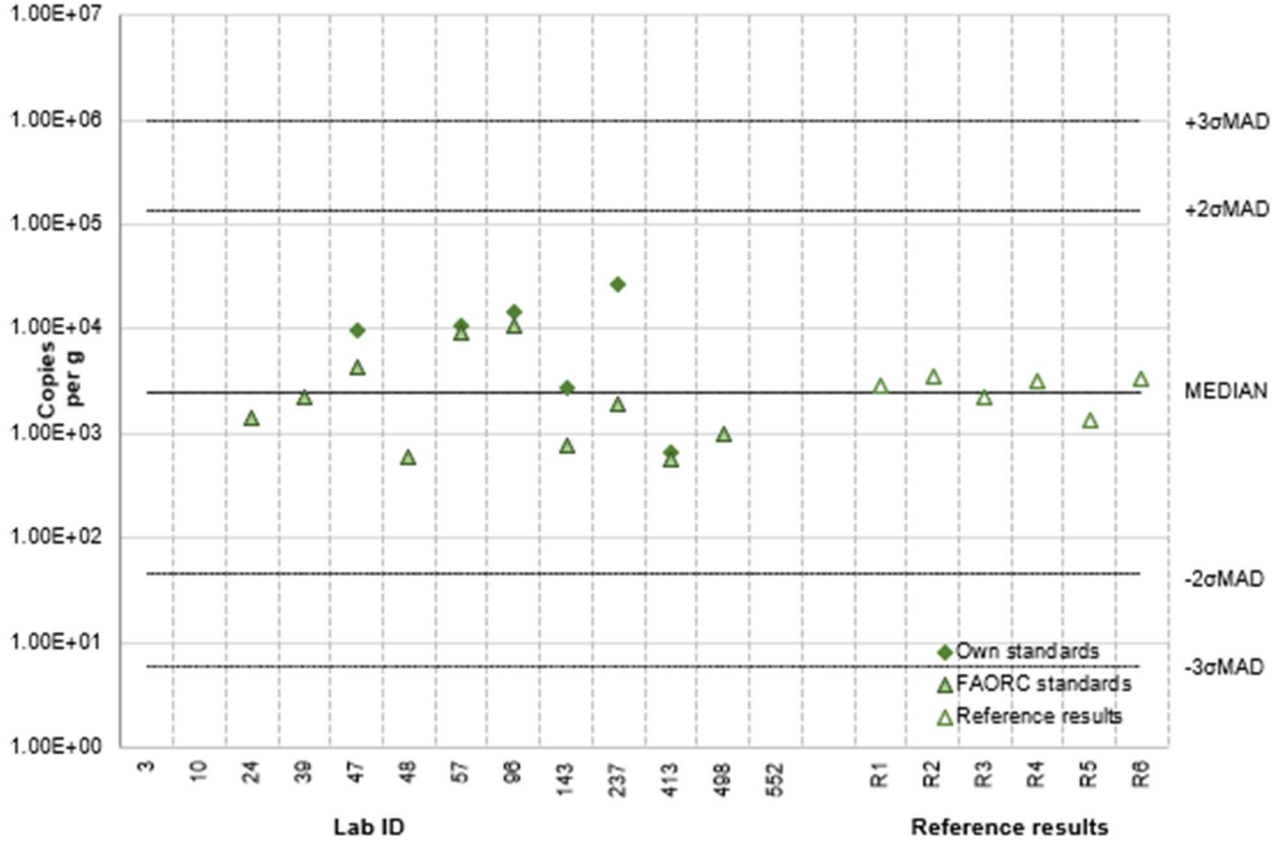

Figure 8. Scatter graph showing $\log_{10}$ copies per gram of hepatitis A virus for shellfish sample 4

6.6. Appendix 6 - Methods used by participants

LAB ID	Virus extraction	RNA extraction	RT-PCR method	RT-PCR reagents	Primers		
					GI	GII	HAV
2	A	B	G	J	O-1	O	R
3	A	B	G	K	O-1	O	O
10	A	B	G	K	O-1	O	O
20	A	B	G	L	P	P	P
24	A	C	H	M	Q	O	O
39	A	B	G	K	O-2	O	O
47	A	B	G	K	O-1	O	O
48	A	D	G	K	O-1	O	O
57	A	E	G	K	O-2	O	O
96	A	B	G	J	O-2	O	O
143	A	B	G	L	P	P	P
237	A	B	G	K	O-2	O	O
364	A	B	G	K			O
413	A	E	G	K	O-1	O	O
498	A	D	G	K	O-2	O	O
552	A	F	G	N	O-1	O	

Method elements as described in the main body and informative annexes of ISO 15216-1 are shaded grey.

Key to method codes

Virus extraction methods

A Proteinase K digestion

RNA extraction methods

B NucliSens Magnetic extraction reagents (BioMerieux)

C Roche High Pure Viral Nucleic Acid Kit

D Hygiena foodproof Magnetic Preparation kit VI

E Maxwell RSC PureFood GMO and Authentication Kit

F Invitek RTP Pathogen kit

RT-PCR methods

G Real-time RT-PCR - one-step

H Real-time RT-PCR - two-step

RT-PCR reagents

J Fast Virus 1-step MasterMix (ThermoFisher Scientific)

K RNA Ultrasense (Invitrogen)

L Ceeram Tools

M RT: Invitrogen Superscript III; PCR: Invitrogen Platinum® qPCR SuperMix-UDG

N Invitek Norovirus detection kit

Primers/probes

O ISO 15216-1; 1) with TM9 probe for NoV GI; 2) with NVGG1p probe for NoV GI
P Ceeram Tools (sequences as O-2)
Q Wolf *et al*, 2010
R OPFLP-07

6.7. Appendix 7 - Laboratory quantification standards

Details of each laboratory's own quantification standards

LAB ID	
3	Linearised ISO 15216-1 plasmid DNA, quantified using fluorimetry
10	PCR product amplified from ISO 15216-1 plasmid, quantified using A260 spectrophotometry
24	Linearised plasmid DNA
47	Commercially produced linear dsDNA, quantified using digital PCR
57	Commercially produced linear dsDNA, quantified by supplier
96	Linearised ISO 15216-1 plasmid DNA, quantified using A260 spectrophotometry and fluorimetry
143	Standards provided in Ceeram Tools kit
237	Commercially produced linear dsDNA, quantified by supplier
413	Commercially produced linear dsDNA, quantified using fluorimetry
552	Standards provided in Invitek kit

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