FAO Reference Centre for Bivalve Mollusc Sanitation

Norovirus (Genogroup I and II) and Hepatitis A virus Proficiency Testing (PT 94)

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Contents

1. Preparation of sample material

1.1. Sample and virus strain origin

Materials dispatched consisted of whole Pacific oysters (*C. 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 glands base material

A single batch of approximately 750 Pacific oysters (*C. gigas*) was collected from a UK commercial harvesting area in April 2023. The shellfish were 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.

Note: this base material tested negative for NoV GI on initial testing and was used to prepare both NoV GI negative and positive samples. However, later testing indicated that the base material was apparently naturally contaminated with very low levels of NoV GI (enough to produce a mixture of mostly negative but some low level positive test results). For this reason, participant results for NoV GI for samples prepared using this material and intended to be NoV GI negative have not been subjected to performance assessment.

1.3. Sample preparation

1.3.1. Sample 1

Pacific oysters (*C. gigas*) collected from a UK commercial harvesting area in September 2021 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 (see Table 2 for 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.

1.3.3. Sample 3

Blended digestive gland base material (see above) was mixed with NoV GII and HAV to obtain the desired target levels (see Table 2 for 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.

1.3.4. Sample 4

Blended digestive gland base material (see above) was mixed with NoV GI, NoV GII and HAV to obtain the desired target levels (see Table 2 for 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.

2. Sample distribution

Samples were dispatched on dry ice in accordance with IATA packing instructions for UN3373 'Diagnostic Specimens' on 17th July 2023 to 18 participating laboratories. Due to complications at customs, one laboratory did not receive the samples. On arrival, participants were given a month to analyse the test samples using their routine method. For laboratory 57, delivery of the package was significantly delayed due to customs processes (the sample was held for 24 days) resulting in the samples arriving at the laboratory in an unsuitable condition. The laboratory continued to process the samples and reported results. Results as reported are included in this report but due to the delays laboratory 57 has not been subject to performance scoring and their quantitative results were not included in calculation of the sample characteristics.

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 94 Proficiency testing material

Sample	NoV		HAV
	GI	GII	
Sample 1 (Whole animal)	-	-	-
Sample 2 (Digestive gland)	- *	+ (9.10×10^3 – 1.49×10^4)	-
Sample 3 (Digestive gland)	- *	+ (7.72×10^3 – 1.26×10^4)	+ (4.83×10^3 – 1.83×10^4)
Sample 4 (Digestive gland)	+ (1.87×10^4 – 3.63×10^4)	+ (7.16×10^3 – 1.75×10^4)	+ (3.89×10^3 – 1.10×10^4)

Quantities in copies/g, ranges based on a 95% confidence limit determined as 2 geometric standard deviations above and below the geometric mean. * = Intended results for NoV GI for samples 2 and 3 were negative as indicated. However, due to low level contamination of the base material used to prepare the samples (see above), participant results for NoV GI for these samples have not been subject to performance scoring.

3.2. Participants' results

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.

3.3. Performance scoring

3.3.1. Presence / absence

Performance scoring was undertaken on each participant's presence/absence results (not including NoV GI results for samples 2 and 3 – see above). 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. Any results reported as invalid due to quality control issues (e.g. unacceptable extraction efficiencies) were not subject to performance scoring for presence/absence. For each laboratory an overall score is provided for each target virus, taking into account the results of all samples (Table 4).

3.3.2. 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/TS 22117 Microbiology of food and animal feeding stuffs – 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, 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. Laboratories were given a score for each virus for which they reported at least one quantifiable positive 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 (σ_{MAD}; Table 3). For each individual result, its absolute deviation from the participants' median was compared with the calculated σ_{MAD} to determine its acceptability and score as follows:-

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

The differences between individual participants' results and the participants' median, expressed in terms of σ_{MAD} 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	Sample 2	Sample 3		Sample 4		
	GII	GII	HAV	GI	GII	HAV
MEDIAN	3.772	3.608	3.555	4.185	3.673	3.631
MAD	0.230	0.412	0.258	0.279	0.247	0.265
σMAD	0.341	0.610	0.382	0.414	0.366	0.392

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		
	NoV		HAV	NoV		HAV
	GI	GII		GI	GII	
2	4 out of 4	8 out of 8	8 out of 8	NE	NE	NE
3	4 out of 4	8 out of 8	8 out of 8	4 out of 4	8 out of 8	NE
10	4 out of 4	8 out of 8	8 out of 8	4 out of 4	8 out of 8	8 out of 8
20	4 out of 4	8 out of 8	8 out of 8	NE	NE	NE
24[a]	4 out of 4	6 out of 8	4 out of 8	NS	NS	NS
47	4 out of 4	8 out of 8	8 out of 8	4 out of 4	8 out of 8	8 out of 8
48	4 out of 4	8 out of 8	8 out of 8	4 out of 4	6 out of 8	8 out of 8
53	NE	NE	8 out of 8	NE	NE	NE
57[b]	NS	NS	NS	NS	NS	NS
96	4 out of 4	8 out of 8	8 out of 8	4 out of 4	8 out of 8	8 out of 8
143	4 out of 4	8 out of 8	NE	4 out of 4	8 out of 8	NE
158	4 out of 4	8 out of 8	8 out of 8	NE	NE	NE
177	NE	NE	8 out of 8	NE	NE	NE
190[c]	4 out of 4	8 out of 8	6 out of 6	4 out of 4	NS	NS
214	4 out of 4	6 out of 8	8 out of 8	NE	NE	NE
237	4 out of 4	8 out of 8	8 out of 8	4 out of 4	8 out of 8	7 out of 8
462	4 out of 4	8 out of 8	6 out of 8	3 out of 4	7 out of 8	6 out of 8

Key: NE = Target virus not examined or not quantified; NS; Laboratory reported results but was not scored for this criteria. Labs that scored less than full marks for any criteria are highlighted in yellow; a = Laboratory reported use of incorrect method for some samples after release of intended results. Reported quantitative results were not subject to performance scoring; b = Laboratory samples arrived in unsuitable condition due to delays at customs. Reported results were not subject to performance scoring; c = Laboratory reported high number of invalid, positive; <limit of quantification and positive; not quantifiable results. Performance scoring was only carried out for a subset of reported results.

4. Discussion

Seventeen laboratories received samples. Laboratories 53 and 113 only analysed the samples for HAV. Laboratory 143 only analysed the sample for NoV GI and GII. Methods used by participants to analyse the test samples are shown in Appendix 6, while brief details of the types of materials used as quantification standards are included as Appendix 7.

4.1. Material dispatch

Thirteen laboratories received material within 4 days of dispatch. A number of participants experienced delays at customs; the causes included incorrect paperwork supplied or communication issues with receiving laboratory. The FAO RC recommends those laboratories who experience problems to review the documentation requested by customs and/or brokers

prior to dispatch in future PT schemes, ensure the documents are available and that the contact details provided are correct and suitable for the week of dispatch.

4.2. Presence / absence determination

Note: Laboratory 57 results as reported are included in this report but have been excluded from all performance scoring assessments.

For NoV, a total of 2 false negative results (both for GII) were reported by laboratories 24 and 214, resulting in an overall sensitivity for NoV of 96%. Laboratories 10 and 158 reported positive results for NoV GI for samples 2 and 3 respectively. These samples were intended upon preparation to be negative for NoV GI, however testing indicated that the base material used to prepare these samples was contaminated with very low levels of NoV GI (see above) and these sample target/virus combinations were excluded from performance assessment. The results reported by these labs are not considered as false positives therefore. No other false positive results for NoV were reported (overall specificity of 100%). The overall accuracy for NoV results was 98%. Overall, 12 out of 14 labs (86%) that were subject to performance scoring for presence/absence of NoV GI and GII received maximum scores. In addition, laboratory 57 reported correct results for NoV for all samples.

For HAV, laboratory 24 reported two false negative results. Laboratory 57 reported a negative HAV result for one sample that was intended as positive however this is not considered a false negative due to the unsuitable condition of the samples on arrival (see above). A single false positive result for HAV was reported by laboratory 462. Overall sensitivity, specificity and accuracy levels for HAV were 93%, 97% and 95% respectively.

Across all target viruses four false negative results were reported. The majority of these were reported by laboratory 24, which reported use of an incorrect method for the affected samples after the release of the intended results. A single false positive result was reported. Thirteen laboratories out of 16 (81%) that were subject to performance scoring for presence/absence for one or more target viruses received maximum scores. Overall sensitivity, specificity and accuracy levels for all target viruses combined were 95%, 98% and 97% respectively.

4.3. Quantification

Note: Quantitative results as reported by laboratories 24 (where the laboratory reported use of an incorrect method for some samples after the release of the intended results) and 57 (where samples arrived in unsuitable condition) are included in this report but have been excluded from performance scoring for quantification and calculation of the sample characteristics.

A total of 11 laboratories (65%) reported quantitative data for at least one sample/virus combination. Of these, 9 laboratories were subject to performance scoring for at least one target virus (see above) of which 6 (67%) received maximum scores for all target viruses for which they were assessed. Three laboratories reported one or more quantitative results in the questionable range. One of these laboratories additionally received a zero score for a false positive result, however across the entire PT no reported quantities for positive sample/target virus combinations were considered to be in the unsatisfactory range. Where a small number of laboratories report quantitative results this will tend to result in relatively wide ranges for satisfactory and questionable results in some sample/virus combinations which may partly explain the low number of unsatisfactory and questionable results.

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

5. References

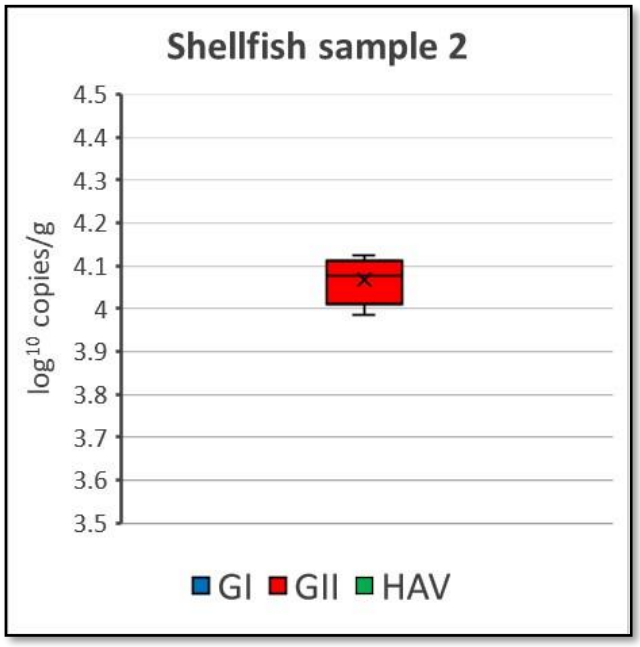
Codd AA, Richardson IR, Andrews N. 1998. Lenticules for the control of quantitative methods in food microbiology. J Appl Microbiol. 85(5):913–7.

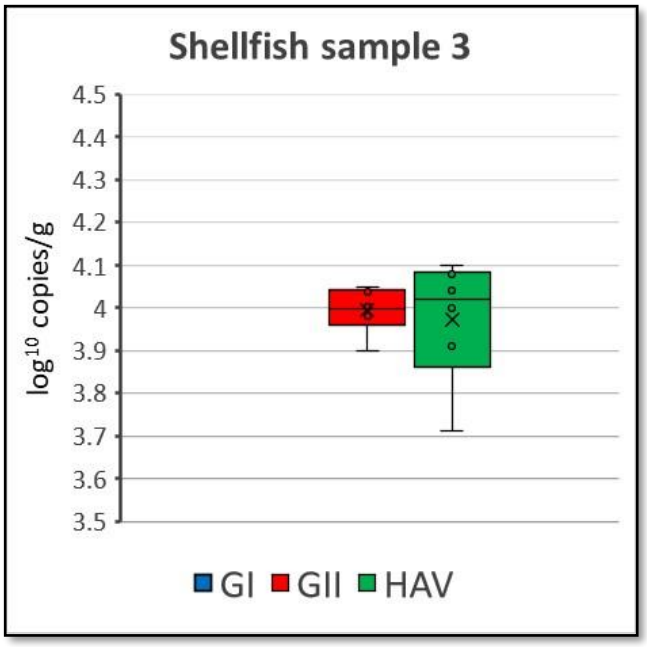
Anon 2017. ISO 15216-1:2017 Microbiology of the food chain -- Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR -- Part 1: Method for quantification.

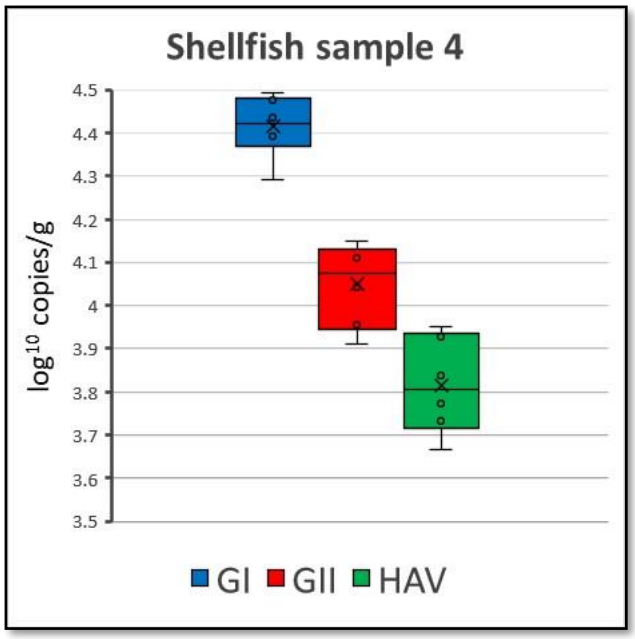
Anon 2019. ISO 22117:2019 Microbiology of the food chain – Specific requirements and guidance for proficiency testing by interlaboratory comparison.

6. Appendices

Appendix 1 - FAO Reference Centre results displayed as box and whisker plots of log_{10} detectable genome copies per gram digestive tissues.







Appendix 1 - 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	-		-		-		-		+	32.20	-		-		+	34.39	+	31.89	+	31.26	+	32.64	+	30.94
3	-		-		-		-		+	32.72/ 32.98	-		-		+	35.99/ 35.40	+	37.58/ 37.49	+	31.67/ 31.66	+	32.95/ 32.89	+	34.05/ 34.19
10	-		-		-		+	44.77	+	34.17	-		-		+	35.22	+	34.02	+	33.26	+	34.65	+	34.21
20	-		-		-		-		+	34.15	-		-		+	34.42	+	31.59	+	27.65	+	32.7	+	33.8
24	-		-		-		-		+	30.95	-		-		-		-		+	28.54	+	30.51	-	
47	-		-		-		-		+	32.47	-		-		+	33.46	+	38.01	+	30.43	+	29.17	+	34.46
48	-		-		-		-		+	34.34/ 34.78	-		-		+	38.82/ 36.86	+	36.78/ 38.73	+	32.59/ 32.92	+	35.75/ 35.91	+	33.93/ 33.81
53	NE		NE		-		NE		NE		-		NE		NE		+	34.81	NE		NE		+	33.30
57*	-		-		-		-		+	38.38	-		-		+	38.23	-		+	34.73	+	38.59	+	38.57
96	-		-		-		-		+	31.92	-		-		+	33.48	+	33.59	+	30.55	+	31.94	+	30.88
143	-		-		NE		-		+	36.30	NE		-		+	36.17	NE		+	30.63	+	36.35	NE	
158	-		-		-		-		+	34.99	-		+	43.19	+	36.86	+	33.55	+	28.68	+	37.11	+	33.24
177	NE		NE		-		NE		NE		-		NE		NE		+	35.98	NE		NE		+	35.27
190	-		-		-		invalid		+	34.77	invalid		invalid		+	35.85	+	38.87	+	30.53	+	34.99	+	38.98
214	-		-		-		-		-		-		-		+	40.00	+	39.46	+	35.24	+	37.95	+	36.27
237	-		-		-		-		+	33.00	-		-		+	33.03	+	33.91	+	32.72	+	33.48	+	31.39
462	-		-		+	36.14	-		+	35.11	-		-		+	36.95	+	34.17	+	33.56	+	34.12	+	34.43

Key: NE = sample/target virus combination not examined; invalid = lab reported results as invalid due to quality control issues; * results for laboratory 57 are included however this lab was not subject to performance scoring due to the unsuitable condition of the samples on arrival at the lab. Yellow shading denotes false negative results, pale yellow shading denotes a negative result for an intended positive sample/target virus combination for laboratory 57 that is not considered a false negative due to that lab's exclusion from performance assessment. Red shading denotes false positive results, pink shading denotes positive results in sample/target virus combinations originally intended as negative but where testing indicated that the base material was contaminated with very low levels of target virus. These sample/target virus combinations were excluded from performance assessment and these results are not considered as false positives.

Appendix 2 – Participants' reported quantities for each target (copies/g) for positive sample/target virus combinations

Lab ID	Shellfish sample 2		Shellfish sample 3				Shellfish sample 4					
	GII		GII		HAV		GI		GII		HAV	
	A	B	A	B	A	A	A	B	A	B	A	B
3	5.12 x10^3	1.08 x10^4	7.63 x10^3	1.78 x10^4	NE	NE	2.23 x10^4	3.72 x10^4	4.87 x10^3	1.03 x10^4	NE	NE
10	6.84 x10^3	8.07 x10^3	3.07 x10^3	3.73 x10^3	5.81 x10^3	5.76 x10^3	1.02 x10^4	1.11 x10^4	4.56 x10^3	5.46 x10^3	5.34 x10^3	5.31 x10^3
24[c]	5.30 x10^3	4.62 x10^3	ND	ND	ND	ND	2.65 x10^4	NE	7.12 x10^3	6.32 x10^3	ND	ND
47	1.11 x10^4	9.58 x10^3	5.36 x10^3	4.70 x10^3	4.03 x10^3	2.13 x10^3	1.53 x10^4	1.09 x10^4	9.38 x10^3	5.43 x10^3	3.42 x10^3	1.80 x10^3
48	NE	8.49 x10^2	NE	4.62 x10^2	NE	3.19 x10^3	NE	3.72x10^3	NE	3.92 x10^2	NE	2.53 x10^3
57[d]	2.68 x10^2	1.07 x10^3	2.92 x10^2	1.20x10^3	ND	ND	4.60x10^3	1.00 x10^4	2.20 x10^3	9.50 x10^3	7.70 x10^3	1.30 x10^3
96	3.85 x10^3	7.27 x10^3	1.42 x10^3	2.67x10^3	1.34 x10^3	1.34 x10^3	2.91x10^4	1.39 x10^4	3.68 x10^3	6.95 x10^3	8.56 x10^3	8.17 x10^3
143	8.64 x10^3	4.00 x10^3	9.45 x10^3	4.35x10^3	NE	NE	3.45x10^4	2.10 x10^4	8.32 x10^3	3.83 x10^3	NE	NE
190	NQ	NQ	NQ	NQ	NQ	NQ	1.41x10^4	9.37 x10^3	<5.20 x10^2	6.42 x10^2	<5.20 x10^2	<2.60 x10^2
237	1.25 x10^4	4.86 x10^3	1.36 x10^4	5.28x10^3	7.25 x10^3	5.40 x10^2	9.30x10^4	8.47 x10^3	8.32 x10^3	3.19 x10^3	3.47 x10^4	2.57 x10^3
462	1.07 x10^3	NE	2.70 x10^2	NE	1.23 x10^3	NE	1.92x10^3	7.21 x10^3	1.77 x10^3	NE	1.77 x10^3	1.96 x10^3

Key: ND = Not detected (shaded yellow); NE = sample/target virus/quantification standard combination not tested (shaded grey); NQ; results reported as positive; not quantifiable (shaded blue); A = results obtained with lab's own quantification standards; B = results obtained with FAO Reference Centre quantification standards; c = laboratory reported use of incorrect method for some samples after release of intended results - reported quantitative results were not subject to performance scoring; d = laboratory samples arrived in unsuitable condition due to delays at customs - reported results were not subject to performance scoring. Results reported as positive; <limit of quantification are included for information and are presented as reported by the relevant participant; these results were not subjected to performance scoring for quantification.

Appendix 3 - Differences between participants' results and the participants' median, expressed in terms of $\sigma_{MAD,}$ for positive sample/target virus combinations

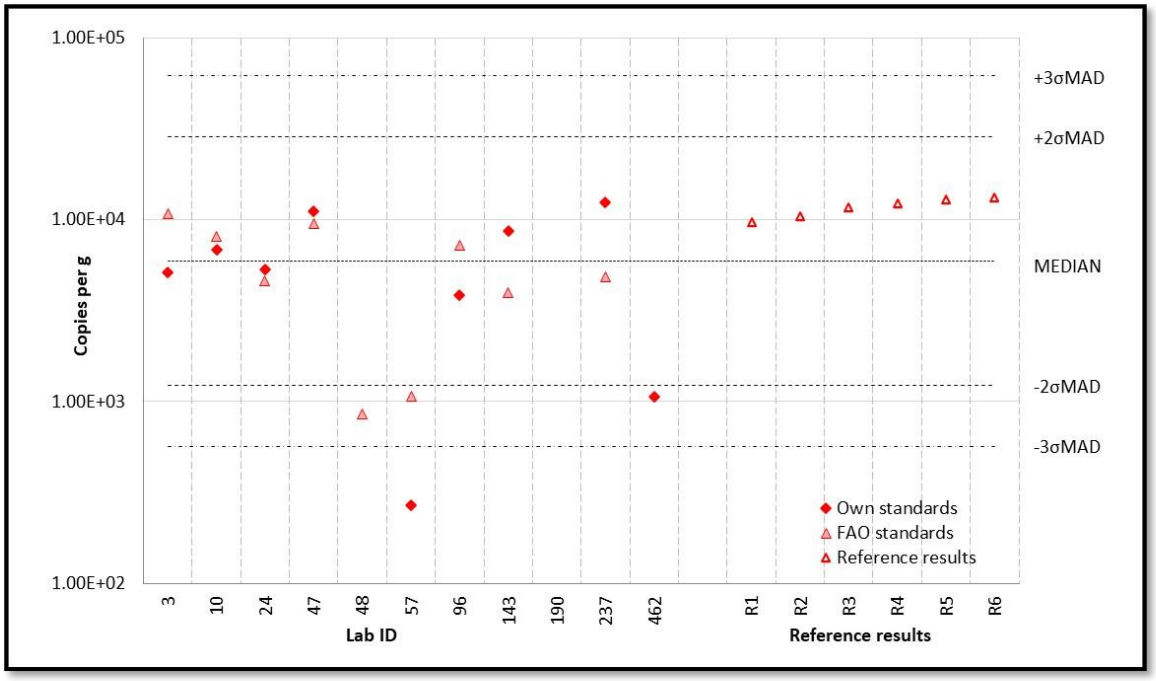
Lab ID	Shellfish sample 2			Shellfish sample 4		
	GI	GII	HAV	GI	GII	HAV
3	-0.18	0.45	NE	0.40	0.04	NE
10	0.18	-0.20	0.55	-0.43	-0.04	0.25
47	0.80	0.20	0.13	0.00	0.82	-0.25
48	-2.47	-1.55	-0.13	-1.48	-2.95	-0.58
96	-0.55	-0.75	-1.12	0.67	-0.29	0.77
143	0.48	0.60	NE	0.85	0.67	NE
190	NS	NS	NS	-0.09	NS	NS
237	0.95	0.86	0.80	1.89	0.67	2.32
462	-2.18	-1.93	-1.21	-2.18	-1.16	-0.97

Key: NE = Quantitative results for sample/target virus combination not reported; NS; Quantitative results for sample/target virus combination not assessed as they were reported as positive; <limit of quantification or positive; not quantifiable; orange shading = questionable results (magnitude of difference between result and participants' median >2 σ_{MAD} and <3 σ_{MAD}). Note: no unsatisfactory results (magnitude of difference between result and participants' median >3 σ_{MAD}) were reported for positive sample/target virus combinations in this PT scheme.

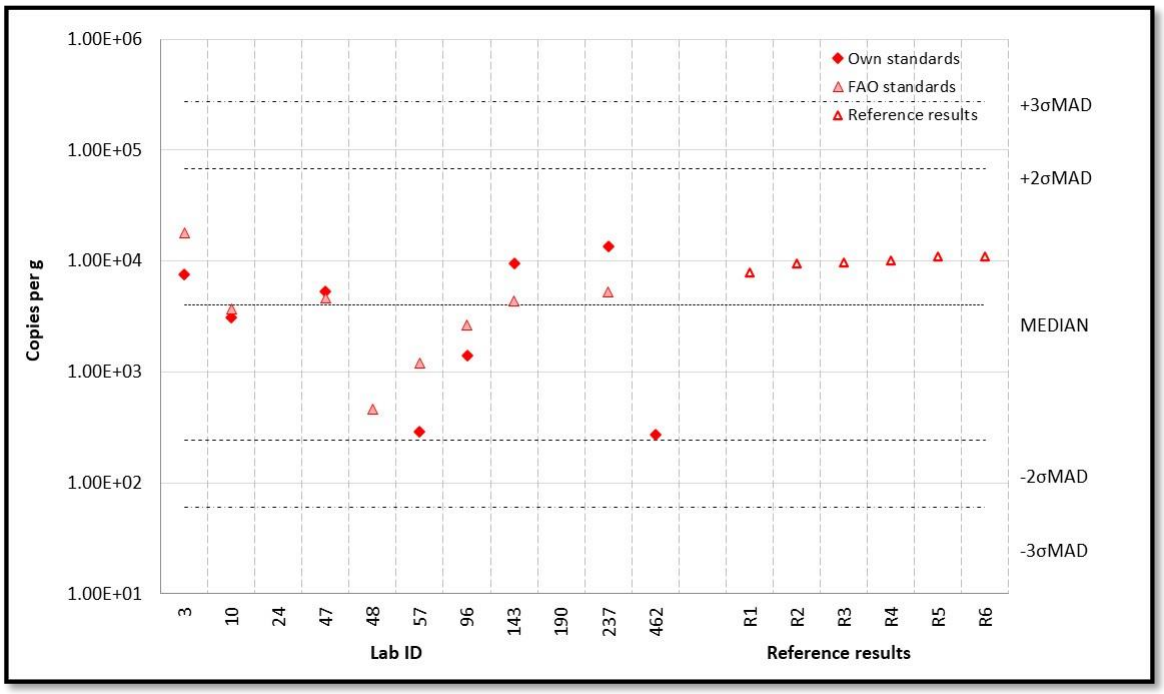
Appendix 4 - Participants' and reference quantities for each sample.

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. Quantitative results for laboratories 24 and 57 are included on the graphs but were not subject to performance scoring or used to calculate sample characteristics.

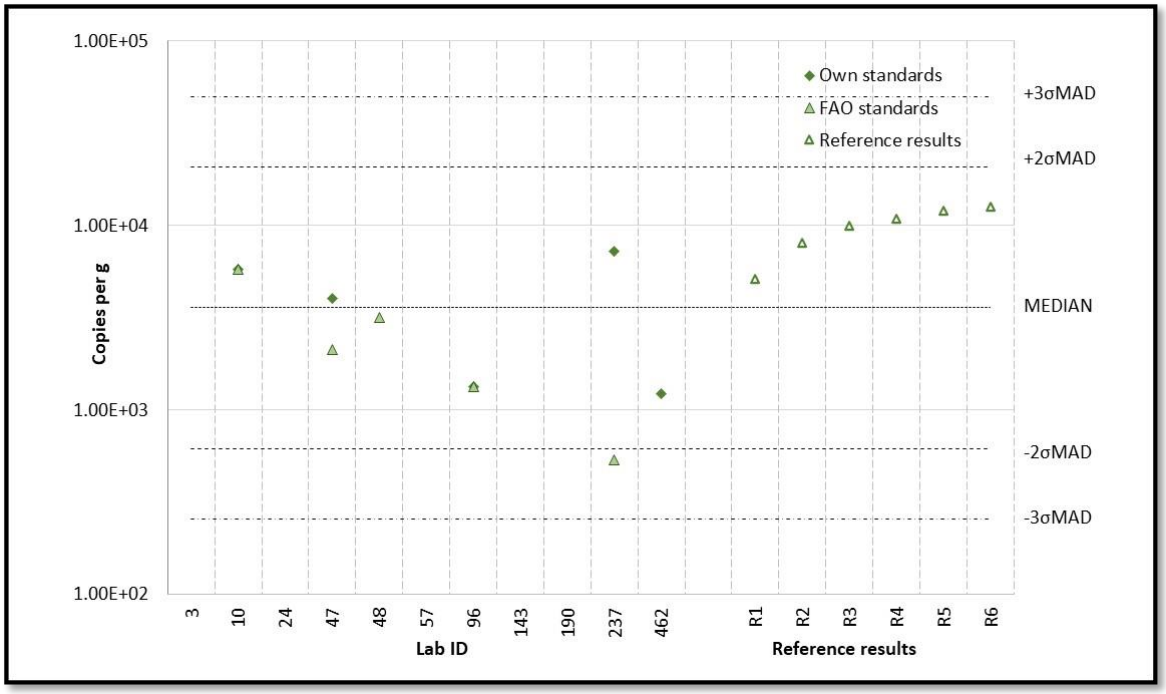
Shellfish sample 2 – GII



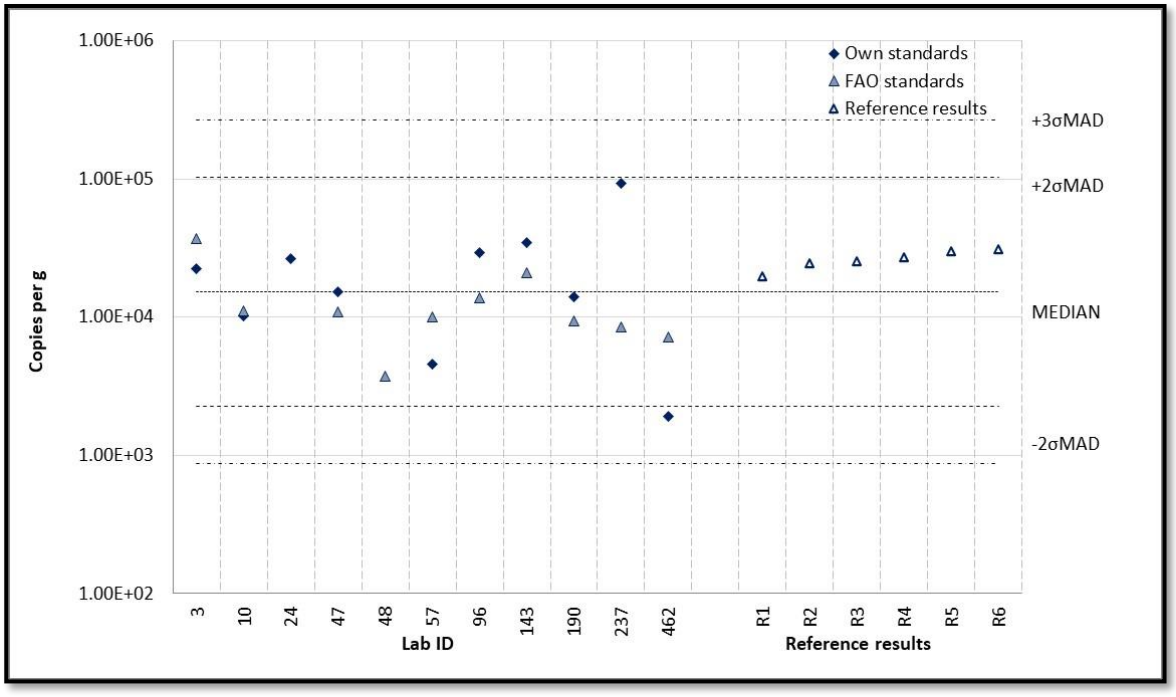
Shellfish sample 3 – GII



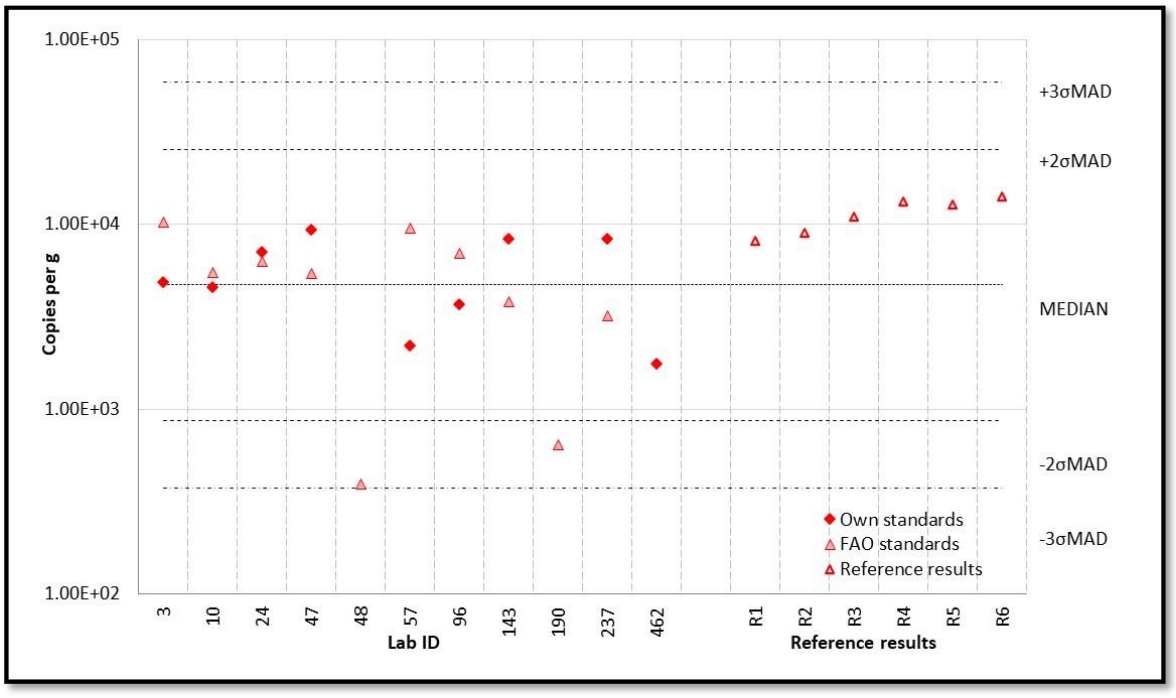
Shellfish sample 3 – HAV



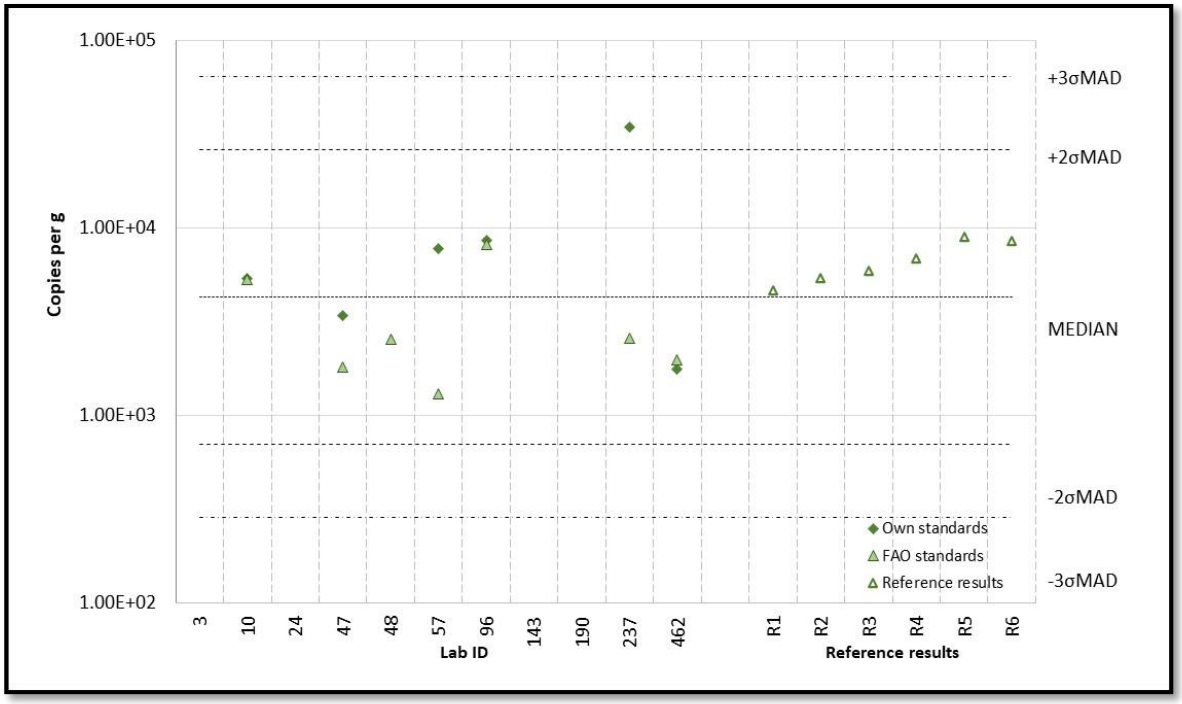
Shellfish sample 4 – GI



Shellfish sample 4 – GII



Shellfish sample 4 – HAV



Appendix 5 - Methods used by participants.

LAB ID	Virus extraction	RNA extraction	RT-PCR method	RT-PCR reagents	Primers		
					GI	GII	HAV
2	A	B	F	F	N-1	N	R
3	A	B	F	J	N-1	N	N
10	A	B	F	J	N-1	N	N
20	A	B	F	K	O	O	O
24	A	C	G	L	P	Q	N
47	A	B	F	J	N-1	N	N
48	A	D	F	J	N-1	N	N
53	A	E	F	M	NE	NE	N
57	A	B	F	J	N-2	N	N
96	A	B	F	J	N-2	N	N
143	A	B	F	K	N-2	N	NE
158	A	B	F	K	O	O	O
177	A	B	F	J	NE	NE	N
190	A	B	F	K	N-2	N	N
214	A	D	F	K	N-2	N	N
237	A	B	F	J	N-2	N	N
462	A	B	F	J	N-1	N	N

Key: NE = target virus not examined. For key to method codes see page 23.

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	Biotecon Foodproof Preparation Kits
E	NucliSens Magnetic extraction reagents (BioMerieux) & Roche High Pure Viral Nucleic Acid Kit
RT-PCR methods	
F	Real-time (quantitative) PCR - one-step
G	Real-time (quantitative) PCR - two-step
RT-PCR reagents	
F	Norovirus GI and HAV: TaqMan® Fast Virus 1-Step Master Mix (Applied Biosystems). Norovirus GII: RNA Ultrasense (Invitrogen)
J	RNA Ultrasense (Invitrogen)
K	Ceeram Tools
L	RT: Invitrogen Superscript III ; PCR: Invitrogen Platinum® qPCR
M	RNA Ultrasense (Invitrogen) & Ceeram Tools
Primers/probes	
N	ISO 15216-1; 1) with TM9 probe for NoV GI; 2) with NVGG1p probe for NoV GI
O	Ceeram Tools (sequences as N-2)
P	Wolf *et al*, 2010
Q	Kageyama *et al*, 2003
R	OPFLP-07

Appendix 6 - Details of 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	Linearised plasmid DNA
57	cDNA from a commercial supplier
96	Linearised ISO 15216-1 plasmid DNA, quantified using A260 spectrophotometry and fluorimetry
143	Standards provided in Ceeram Tools kit
190	Standards provided in Ceeram Tools kit
237	Commercially produced linear dsDNA (quantified by supplier)
462	PCR product amplified from ISO 15216-1 plasmid, quantified using A260 spectrophotometry

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