European Union Reference Laboratory (EURL) Proficiency Testing Scheme

Norovirus (Genogroup I and II) and hepatitis A virus proficiency testing 2013

EURL proficiency testing reference number: PT 46

Sample number: Shellfish sample 1, 2, 3 and 4. LENTICULE 1 and 2.

Contents **Page number**

Distribution date:	**15th October 2012**
Report date:	**9th April 2013**
Report compiled by:	**Louise Stockley** **James Lowther**
Authorisation by:	**Rachel Hartnell**

Samples
Material dispatched consisted of naturally contaminated and bioaccumulated Pacific oysters (*Crassostrea gigas*) and bioaccumulated common mussels (*Mytilus edulis*) (Samples 1, 2, 3 and 4), laboratory constructed LENTICULES™ (Vials 1 and 2) and dsDNA control material for quantification. The reference results for each shellfish and LENTICULE™ sample are shown in Table 2.

Sample preparation
Shellfish Sample 1
Approximately 500 Pacific oysters (*Crassostrea gigas*) were placed in trays and re-immersed in 500 litres of re-circulating natural seawater at 16±1°C. The shellfish were left for 30 hours to acclimatise before 50ml of shellfish food containing known levels of genogroup I and II (GI and GII) norovirus from human faeces and hepatitis A virus (HAV) cell culture supernatant (Table 1) was added to the tank. After approximately 17 hours the shellfish were removed from the tank and rinsed in fresh water. The oysters were then shucked and randomly sorted into samples of 10 animals and sealed in plastic bags. Bags were held at <-15°C until required for quality control testing, dispatch or reference analysis.

Shellfish Sample 2
Sample 2 comprised shucked, frozen Pacific oysters (*Crassostrea gigas*), originating from Korea, that had been involved in a norovirus outbreak that occurred in New Zealand on the 17th June 2006 with a total of 115 cases being identified. Prior testing of the sample indicated the shellfish contained both norovirus genogroup I and genogroup II. The oysters were randomly sorted into samples of 10 animals and sealed in plastic bags. Bags were held at <-15°C until required for quality control testing, dispatch or reference analysis.

Shellfish Sample 3
Approximately 500 Pacific oysters (*Crassostrea gigas*) from a UK commercial harvesting area were collected following commercial purification. Testing of the sample indicated the shellfish were negative for HAV and GI and GII norovirus. The oysters were shucked, then randomly sorted into samples of 10 animals and sealed in plastic bags. Bags were held at <-15°C until required for quality control testing, dispatch or reference analysis.

Shellfish Sample 4
Approximately 500 common mussels (*Mytilus edulis*) were placed in trays and re-immersed in 500 litres of re-circulating natural seawater at 16±1°C. The shellfish were left for 30 hours to acclimatise before 50ml of shellfish food containing known levels of HAV cell culture supernatant (Table 1) was added to the tank. After approximately 17 hours the shellfish were removed from the tank and rinsed in fresh water. The mussels were then randomly sorted into samples of 10 animals and sealed in plastic bags. Bags were held at <-15°C until required for quality control testing, dispatch or reference analysis.

LENTICULES™ 1 and 2
Two batches of laboratory constructed LENTICULES™ were prepared following the method of Codd *et al* (1998) with minor modifications. The mix prepared for LENTICULE™ 1 included known levels of GI norovirus. The mix prepared for LENTICULE™ 2 included known levels of GII norovirus and HAV. Table 1 shows details of the stock viruses used in the preparation of the LENTICULES™.

Table 1: Origin and strain/genotype of viruses used for shellfish bioaccumulation and preparation of LENTICULES™

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

Sample distribution
Samples were dispatched on dry ice in accordance with IATA packing instructions 650 for UN3373 'Diagnostic Specimens' on 15th October 2012 to 42 participating laboratories. All participants were requested to examine the samples using their routine method. Those laboratories using quantitative real-time PCR were requested to calculate

the quantity of target virus in each sample using both their own standard material and using the dsDNA control material provided with this PT distribution (10^4 copies/µl for each target virus).

Results

Reference results

Reference analyses were performed by the EURL 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 triplicate 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 I.

Table 2: Reference results for PT 46 proficiency testing material

Sample	Norovirus		HAV
	GI	GII	
Shellfish sample 1 [a]	+ (1.38×10^1 - 3.44×10^3)	+ (5.89×10^2 - 8.54×10^4)	+ (1.76×10^2 - 4.98×10^4)
Shellfish sample 2 [a]	+ (2.12×10^2 - 6.21×10^2)	+ (1.68×10^3 - 5.47×10^3)	-
Shellfish sample 3 [a]	-	-	-
Shellfish sample 4 [a]	-	-	+ (3.06×10^4 - 9.24×10^4)
LENTICULE™ 1 [b]	+ (9.23×10^3 - 3.55×10^4)	-	-
LENTICULE™ 2 [b]	-	+ (3.96×10^3 - 5.75×10^3)	+ (1.15×10^5 - 7.84×10^5)

[a] Copies/g; [b] Copies/ LENTICULE. Ranges based on a 95% confidence limit determined as 2 geometric standard deviations above and below the geometric mean.

Participants' results

Performance assessment was assessed as percentage relative accuracy, specificity and sensitivity for each determinant according to the calculations described in Appendix II. As this proficiency testing distribution included both shellfish matrices and LENTICULE™ discs, an overall performance assessment was performed to assess each laboratory's performance for all samples (Table 3) as well as assessing the performance on shellfish matrices (Table 4) and LENTICULE™ discs only (Table 5).

Note: Participants' results were expressed as percentage concordance with intended results generated by the EURL. In this assessment presence/absence data was used and no consideration of quantitative measurements (Ct values) were made.

Table 3: Participants' results for all dispatched material

Lab ID No.	GI AC	GI SP	GI SE	GII AC	GII SP	GII SE	HAV AC	HAV SP	HAV SE
2	100	100	100	100	100	100	100	100	100
3 *	100	100	100	100	100	100	100	100	100
7 *	100	100	100	100	100	100	100	100	100
10 *	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100
17 *	100	100	100	100	100	100	100	100	100
19 *	100	100	100	100	100	100	100	100	100
21 *	83	100	67	100	100	100	83	100	67
24	100	100	100	100	100	100	100	100	100
25 *	100	100	100	100	100	100	100	100	100
27 *	100	100	100	100	100	100	100	100	100
32 *	100	100	100	100	100	100	100	100	100
33 *	83	67	100	100	100	100	100	100	100
35 *	83	100	67	100	100	100	100	100	100
39 *	100	100	100	100	100	100	100	100	100
41 *	100	100	100	100	100	100	100	100	100
43*	NR	NR	NR	NR	NR	NR	NR	NR	NR
47 *	100	100	100	100	100	100	100	100	100
48	NE	NE	NE	100	100	100	100	100	100
49	83	100	67	100	100	100	100	100	100
53	67	67	67	67	67	67	100	100	100
57	100	100	100	100	100	100	100	100	100
72	100	100	100	83	67	100	NE	NE	NE
83 *	83	100	67	83	100	67	100	100	100
94	100	100	100	100	100	100	100	100	100
95	100	100	100	100	100	100	100	100	100
98	100	100	100	100	100	100	100	100	100
110	67	100	33	33	67	0	83	100	67
113	83	100	67	100	100	100	NE	NE	NE
122	100	100	100	100	100	100	100	100	100
146	NR	NR	NR	NR	NR	NR	NR	NR	NR
147 *	83	100	67	100	100	100	100	100	100
152	100	100	100	100	100	100	100	100	100
158	83	100	67	100	100	100	100	100	100
176	100	100	100	100	100	100	100	100	100
177	100	100	100	100	100	100	100	100	100
186	50	100	0	50	100	0	67	100	33
190	83	100	67	100	100	100	100	100	100
199	100	100	100	100	100	100	100	100	100
203	83	100	67	50	67	33	83	100	67
207	83	100	67	100	100	100	100	100	100
239	83	100	67	100	100	100	100	100	100

* - Designated NRL, NE= Not examined, NR = Results not returned at date of report. AC - Relative accuracy, SP – Relative specificity, SE – Relative sensitivity

Note 22/40 laboratories which returned results tested all matrix/determinand combinations and scored 100% overall accuracy. 2/40 laboratories scored 100% overall accuracy but did not test all matrix/determinand combinations.

Table 4: Participants' results for all shellfish material (Samples 1, 2, 3 and 4)

Lab ID No.	GI			GII			HAV		
	AC	SP	SE	AC	SP	SE	AC	SP	SE
2	NE	NE	NE	NE	NE	NE	NE	NE	NE
3 *	100	100	100	100	100	100	100	100	100
7 *	100	100	100	100	100	100	100	100	100
10 *	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100
17 *	100	100	100	100	100	100	100	100	100
19 *	100	100	100	100	100	100	100	100	100
21 *	75	100	50	100	100	100	75	100	50
24	100	100	100	100	100	100	100	100	100
25 *	100	100	100	100	100	100	100	100	100
27 *	100	100	100	100	100	100	100	100	100
32 *	100	100	100	100	100	100	100	100	100
33 *	100	100	100	100	100	100	100	100	100
35 *	75	100	50	100	100	100	100	100	100
39 *	100	100	100	100	100	100	100	100	100
41 *	100	100	100	100	100	100	100	100	100
43*	NR	NR	NR	NR	NR	NR	NR	NR	NR
47 *	100	100	100	100	100	100	100	100	100
48	NE	NE	NE	100	100	100	100	100	100
49	75	100	50	100	100	100	100	100	100
53	100	100	100	100	100	100	100	100	100
57	100	100	100	100	100	100	100	100	100
72	100	100	100	75	50	100	NE	NE	NE
83 *	100	100	100	75	100	50	100	100	100
94	100	100	100	100	100	100	100	100	100
95	100	100	100	100	100	100	100	100	100
98	100	100	100	100	100	100	100	100	100
110	50	100	0	50	100	0	75	100	50
113	100	100	100	100	100	100	NE	NE	NE
122	100	100	100	100	100	100	100	100	100
146	NR	NR	NR	NR	NR	NR	NR	NR	NR
147 *	75	100	50	100	100	100	100	100	100
152	100	100	100	100	100	100	100	100	100
158	75	100	50	100	100	100	100	100	100
176	100	100	100	100	100	100	100	100	100
177	100	100	100	100	100	100	100	100	100
186	50	100	0	50	100	0	50	100	0
190	75	100	50	100	100	100	100	100	100
199	100	100	100	100	100	100	100	100	100
203	75	100	50	75	100	50	75	100	50
207	75	100	50	100	100	100	100	100	100
239	75	100	50	100	100	100	100	100	100

* - Designated NRL, NE= Not examined, NR = Results not returned at date of report. AC - Relative accuracy, SP – Relative specificity, SE – Relative sensitivity

Note 24/39 laboratories which returned results for shellfish samples tested all determinands and scored 100% overall accuracy. 2/39 laboratories scored 100% overall accuracy for shellfish samples but did not test all determinands.

Table 5: Participants' results for all LENTICULES™ (L1 – L2)

Lab ID No.	GI			GII			HAV		
	AC	SP	SE	AC	SP	SE	AC	SP	SE
2	100	100	100	100	100	100	100	100	100
3 *	100	100	100	100	100	100	100	100	100
7 *	100	100	100	100	100	100	100	100	100
10 *	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100
17 *	100	100	100	100	100	100	100	100	100
19 *	100	100	100	100	100	100	100	100	100
21 *	100	100	100	100	100	100	100	100	100
24	100	100	100	100	100	100	100	100	100
25 *	100	100	100	100	100	100	100	100	100
27 *	100	100	100	100	100	100	100	100	100
32 *	100	100	100	100	100	100	100	100	100
33 *	50	0	100	100	100	100	100	100	100
35 *	100	100	100	100	100	100	100	100	100
39 *	100	100	100	100	100	100	100	100	100
41 *	100	100	100	100	100	100	100	100	100
43*	NR	NR	NR	NR	NR	NR	NR	NR	NR
47 *	100	100	100	100	100	100	100	100	100
48	NE	NE	NE	100	100	100	100	100	100
49	100	100	100	100	100	100	100	100	100
53	0	0	0	0	0	0	100	100	100
57	100	100	100	100	100	100	100	100	100
72	100	100	100	100	100	100	NE	NE	NE
83 *	50	100	0	100	100	100	100	100	100
94	100	100	100	100	100	100	100	100	100
95	100	100	100	100	100	100	100	100	100
98	100	100	100	100	100	100	100	100	100
110	100	100	100	0	0	0	100	100	100
113	50	100	0	100	100	100	NE	NE	NE
122	100	100	100	100	100	100	100	100	100
146	NR	NR	NR	NR	NR	NR	NR	NR	NR
147 *	100	100	100	100	100	100	100	100	100
152	100	100	100	100	100	100	100	100	100
158	100	100	100	100	100	100	100	100	100
176	100	100	100	100	100	100	100	100	100
177	100	100	100	100	100	100	100	100	100
186	50	100	0	50	100	0	100	100	100
190	100	100	100	100	100	100	100	100	100
199	100	100	100	100	100	100	100	100	100
203	100	100	100	0	0	0	100	100	100
207	100	100	100	100	100	100	100	100	100
239	100	100	100	100	100	100	100	100	100

[a] - Designated NRL, NT= Not tested, NR =0 Not returned, AC – Relative accuracy, SP – Relative specificity, SE – Relative sensitivity

Note 31/40 laboratories which returned results for LENTICULE™ samples tested all determinands and scored 100% overall accuracy. 2/40 laboratories scored 100% overall accuracy for LENTICULE™ samples but did not test all determinands.

Conclusion and discussion

General comments

Fourty-two laboratories (16 NRLs and 26 other laboratories) received samples. Laboratory 2 did not examine any shellfish samples, Laboratory 48 did not examine for GI and laboratories 72 and 113 did not examine for HAV. Laboratories 43 and 146 did not return results. Results reported to the EURL are shown in Appendices III, IV and V.

Discussion

24/40 (60%) of the laboratories which returned results obtained the intended results (as determined by EURL reference designations) for all the sample/determinand combinations which they tested, an increase of 22% from last year. The overall accuracies across all laboratories were 92%, 94% and 98% for GI, GII and HAV respectively. The false positive reporting rates for GI, GII and HAV were 2%, 3% and 0% respectively. The false negative reporting rates for GI, GII and HAV were 15%, 8% and 1% respectively.

The overall accuracies for LENTICULES were 92%, 91% and 100% for GI, GII and HAV respectively and for shellfish samples they were 91%, 96% and 97% for GI, GII and HAV respectively. The false positive reporting rates for GI, GII and HAV were 5%, 7% and 0% for LENTICULES and 0%, 1% and 0% for shellfish samples. The false negative reporting rates for GI, GII and HAV were 10%, 10% and 0% for LENTICULES and 17%, 8% and 7% for shellfish samples respectively.

Thirty-seven laboratories (93%) returned data expressed as C_t values for at least one sample/determinand combination (Appendices III and IV). Twenty-eight laboratories (70%) returned quantitative data using their own routine method and/or using the standard materials provided expressed as detectable copies per g or copies per LENTICULES™ for at least one sample/determinand combination (Appendix V). Quantitative results from individual labs alongside reference results are shown in Appendix VI. Reference results were not corrected using extraction efficiency data.

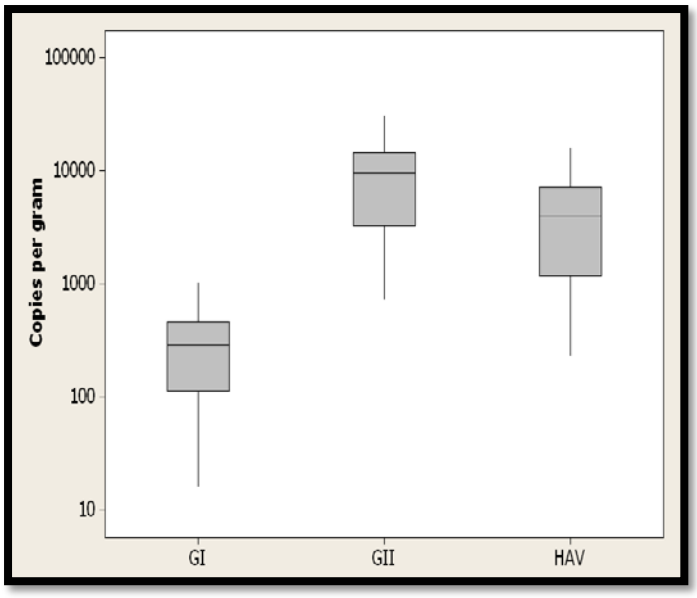
Methods used by participants to analyse the shellfish matrix, with the labs listed according to their overall accuracy score for the shellfish samples, are shown in Appendix VII.

References

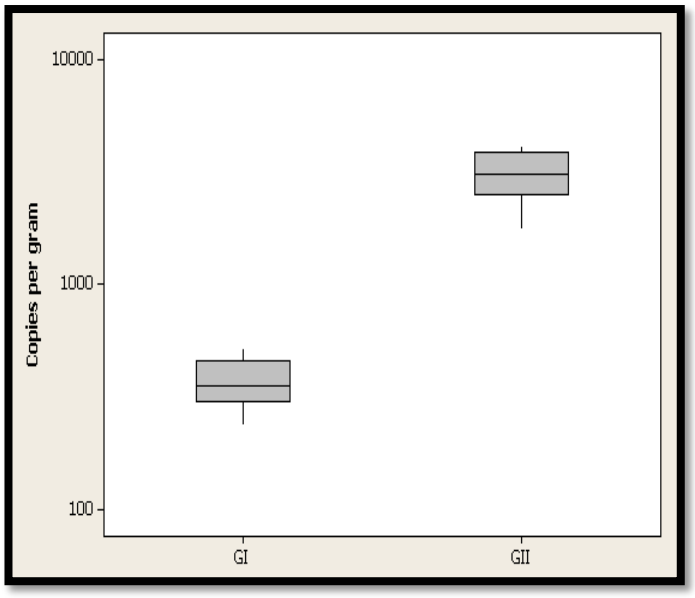
Codd AA, Richardson IR, Andrews N. 1998. Lenticules for the control of quantitative methods in food microbiology. J Appl Microbiol. **85(5)**:913–7.

Appendix I: EURL reference results displayed as box and whisker plots of detectable genome copies per gram or 25µl LENTICULE™.

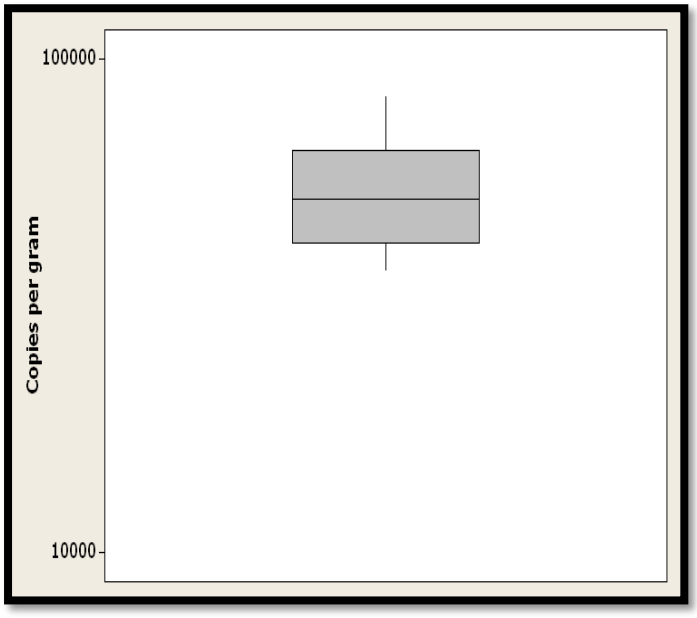
Shellfish sample 1



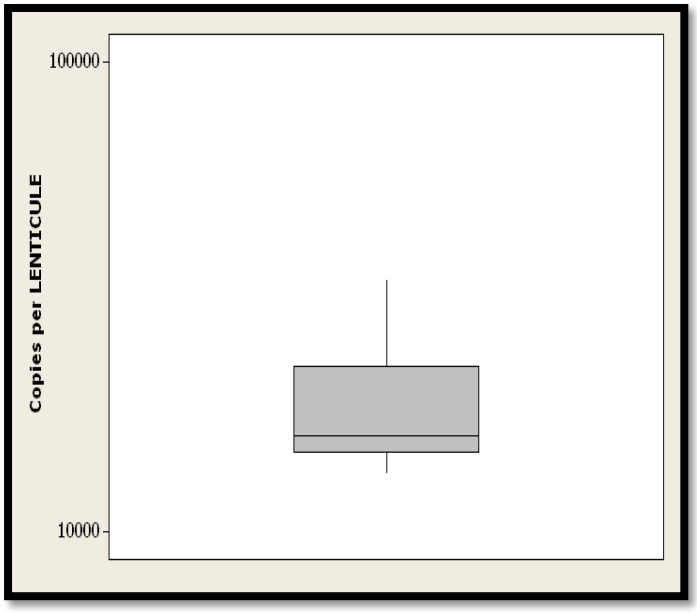
Shellfish sample 2



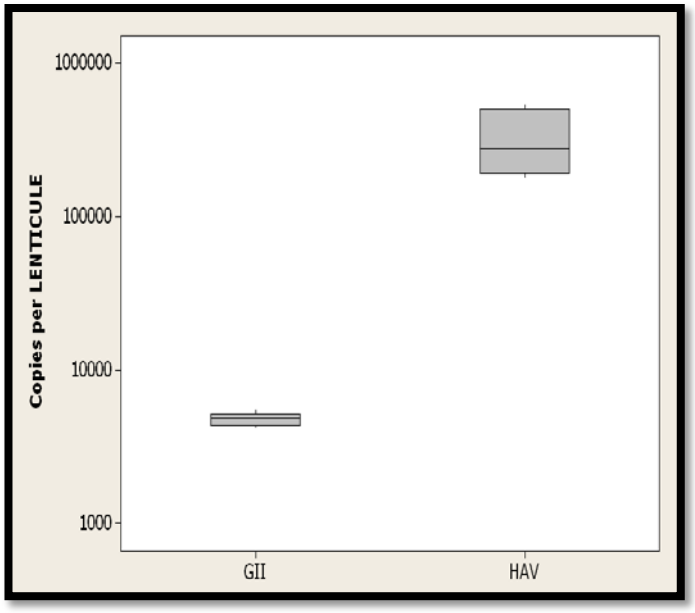
Shellfish sample 4



LENTICULE 1



LENTICULE 2



Appendix II:

Percentage relative sensitivity: Relative sensitivity (SE) = $\frac{TP}{(TP+FN)} \times 100\%$

Percentage relative specificity: Relative specificity (SP) = $\frac{TN}{(TN+FP)} \times 100\%$

Percentage relative accuracy: Relative accuracy (AC) = $\frac{TP+TN}{N} \times 100\%$

Where TP = true positives
FN = false negatives
FP = false positives
TN = true negatives
N = total number of tests

Note: Participants' results were expressed as percentage concordance with intended results generated by the EURL. In this assessment presence/absence data was used and no consideration of quantitative measurements (C_t values) was made.

Appendix III: Participants' presence/absence results and C_t values for shellfish samples

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	
No.	+	CT	+	CT	+	CT	+	CT	+	CT	-	CT	-	CT	-	CT	-	CT	-	CT	-	CT	+	CT
2	NE		NE		NE		NE		NE		NE		NE		NE		NE		NE		NE		NE	
3	+	37.00	+	32.28	+	31.88	+	36.65	+	36.07	-		-		-		-		-		-		+	33.8
7	+		+		+		+		+		-		-		-		-		-		-		+	
10	+	35.49	+	32.08	+	32.61	+	35.92	+	34.21	-		-		-		-		-		-		+	33.53
11	+	38.08	+	34.5	+	29.24	+	40.45	+	38.27	-		-		-		-		-		-		+	28.53
17	+	35.97	+	30.06	+	33.73	+	33.54	+	31.15	-		-		-		-		-		-		+	31.42
19	+	32.81	+	32.96	+	31.18	+	35.91	+	36.52	-		-		-		-		-		-		+	31.37
21	-		+	34.40	-		+	32.70	+	33.80	-		-		-		-		-		-		+	39.16
24	+	32.30	+	28.90	+	28.60	+	35.10	+	34.40	-		-		-		-		-		-		+	27.10
25	+	39.39	+	33.26	+	36.40	+	36.07	+	32.57	-		-		-		-		-		-		+	32.34
27	+	37.29	+	33.93	+	32.08	+	39.67	+	38.92	-		-		-		-		-		-		+	32.18
32	+	41.90	+	32.70	+	35.60	+	34.50	+	33.20	-		-		-		-		-		-		+	33.40
33	+		+		+		+		+		-		-		-		-		-		-		+	
35	+	38.99	+	32.95	+		-		+	37.01	-		-		-		-		-		-		+	
39	+	41.50	+	38.60	+	37.10	+	39.00	+	39.50	-		-		-		-		-		-		+	35.70
41	+	36.26	+	29.80	+	30.57	+	33.78	+	31.11	-		-		-		-		-		-		+	31.08
43	NR		NR		NR		NR		NR		NR		NR		NR		NR		NR		NR		NR	
47	+	39.40	+	30.10	+	34.20	+	34.20	+	33.50	-		-		-		-		-		-		+	31.50
48	NE		+	35.64	+	38.96	NE		+	38.38	-		NE		-		-		NE		-		+	33.63
49	-		+	32.73	+	28.34	+	38.53	+	35.41	-		-		-		-		-		-		+	29.97
53	+	35.67	+	32.87	+	32.51	+	38.98	+	33.60	-		-		-		-		-		-		+	31.12
57	+	39.09	+	34.95	+	32.64	+	36.97	+	37.20	-		-		-		-		-		-		+	31.64
72	+	35.51	+	31.37	NE		+	36.91	+	33.49	NE		-		-		NE		-		+		NE	
83	+	37.70	+	36.70	+	34.70	+	38.20	-		-		-		-		-		-		-		+	35.80
94	+	37.79	+	37.93	+	30.90	+	37.10	+	35.09	-		-		-		-		-		-		+	32.14
95	+	42.63	+	39.17	+		+	40.63	+	39.14	-		-		-		-		-		-		+	
98	+	38.90	+	29.38	+	29.13	+	35.31	+	33.88	-		-		-		-		-		-		+	35.86
110	-		-		-		-		-		-		-		-		-		-		-		+	39.00
113	+	40.00	+	40.00	NE		+	40.00	+	38.23	NE		-		-		NE		-		-		NE	
122	+	35.11	+	29.61	+	32.95	+	34.42	+	35.06	-		-		-		-		-		-		+	28.76
146	NR		NR		NR		NR		NR		NR		NR		NR		NR		NR		NR		NR	
147	-		+	39.29	+	37.97	+	38.32	+	38.22	-		-		-		-		-		-		+	35..02
152	+	38.00	+	34.00	+	32.00	+	38.00	+	34.00	-		-		-		-		-		-		+	32.00
158	-		+	32.23	+	31.43	+	40.05	+	34.38	-		-		-		-		-		-		+	30.91
176	+	37.07	+	32.55	+	30.81	+	33.58	+	32.95	-		-		-		-		-		-		+	31.45
177	+	37.05	+	31.35	+	32.95	+	35.08	+	35.60	-		-		-		-		-		-		+	32.19
186	-		-		-		-		-		-		-		-		-		-		-		-	
190	-		+	35.30	+	37.60	+	35.50	+	35.20	-		-		-		-		-		-		+	32.50
199	+	38.00	+	38.55	+	36.90	+	35.00	+	37.15	-		-		-		-		-		-		+	32.24
203	-		-		-		+	36.03	+		-		-		-		-		-		-		+	35.56
207	-		+	30.00	+	33.00	+	36.00	+	36.00	-		-		-		-		-		-		+	33.00
239	+	36.98	+	30.33	+	34.70	-		+	37.05	-		-		-		-		-		-		+	33.50

NE= not examined, NR= results not returned at time of report, Yellow denotes false positives, Red denotes false negatives

Appendix IV: Participants' presence/absence results and C_t values for LENTICULE™ samples

Lab ID No.	LENTICULE 1 GI +	CT	GII -	CT	HAV -	CT	LENTICULE 2 GI -	CT	GII +	CT	HAV +	CT
2	+		-		-		-		+		+	
3	+	34.5	-		-		-		+	32.63	+	27.97
7	+		-		-		-		+		+	
10	+	31.62	-		-		-		+	32.19	+	27.97
11	+	36.03	-		-		-		+	34.24	+	25.29
17	+	32.28	-		-		-		+	31.03	+	26.69
19	+	28.84	-		-		-		+	32.05	+	24.19
21	+	33.16	-		-		-		+	32.82	+	30.60
24	+	28.90	-		-		-		+	31.30	+	23.90
25	+	33.80	-		-		-		+	30.71	+	25.89
27	+	33.00	-		-		-		+	32.90	+	27.17
32	+	35.40	-		-		-		+	32.20	+	28.20
33	+		-		-		+		+		+	
35	+	35.82	-		-		-		+	38.23	+	
39	+	39.70	-		-		-		+	39.00	+	30.40
41	+	35.07	-		-		-		+	32.57	+	28.87
43	NR		NR		NR		NR		NR		NR	
47	+	33.50	-		-		-		+	31.10	+	26.10
48	NE		-		-		NE		+	32.63	+	25.76
49	+	32.63	-		-		-		+	33.47	+	25.75
53	-		+		-		+		-		+	31.57
57	+	37.80	-		-		-		+	36.15	+	26.45
72	+	30.59	-		NE		-		+	31.38	NE	
83	-		-		-		-		+	35.20	+	28.20
94	+	37.52	-		-		-		+	32.11	+	26.26
95	+	37.24	-		-		-		+	38.36	+	
98	+	32.98	-		-		-		+	43.26	+	24.52
110	+	36.00	+		-		-		-		+	30.00
113	-	40.00	-		NE		-		+	40.00	NE	
122	+	30.25	-		-		-		+	30.51	+	24.55
146	NR		NR		NR		NR		NR		NR	
147	+	39.20	-		-		-		+	36.00	+	30.69
152	+	30.00	-		-		-		+	31.00	+	26.00
158	+	38.75	-		-		-		+	36.04	+	26.51
176	+	34.19	-		-		-		+	32.70	+	23.84
177	+	35.84	-		-		-		+	31.73	+	27.14
186	-		-		-		-		-		+	35.35
190	+	32.50	-		-		-		+	30.10	+	25.30
199	+	33.45	-		-		-		+		+	
203	+	39.74	+		-		-		-		+	29.68
207	+	33.00	-		-		-		+	31.00	+	25.00
239	+	36.98	-		-		-		+	32.57	+	

NE= not examined, NR= results not returned at time of report, Yellow denotes false positives, Red denotes false negatives

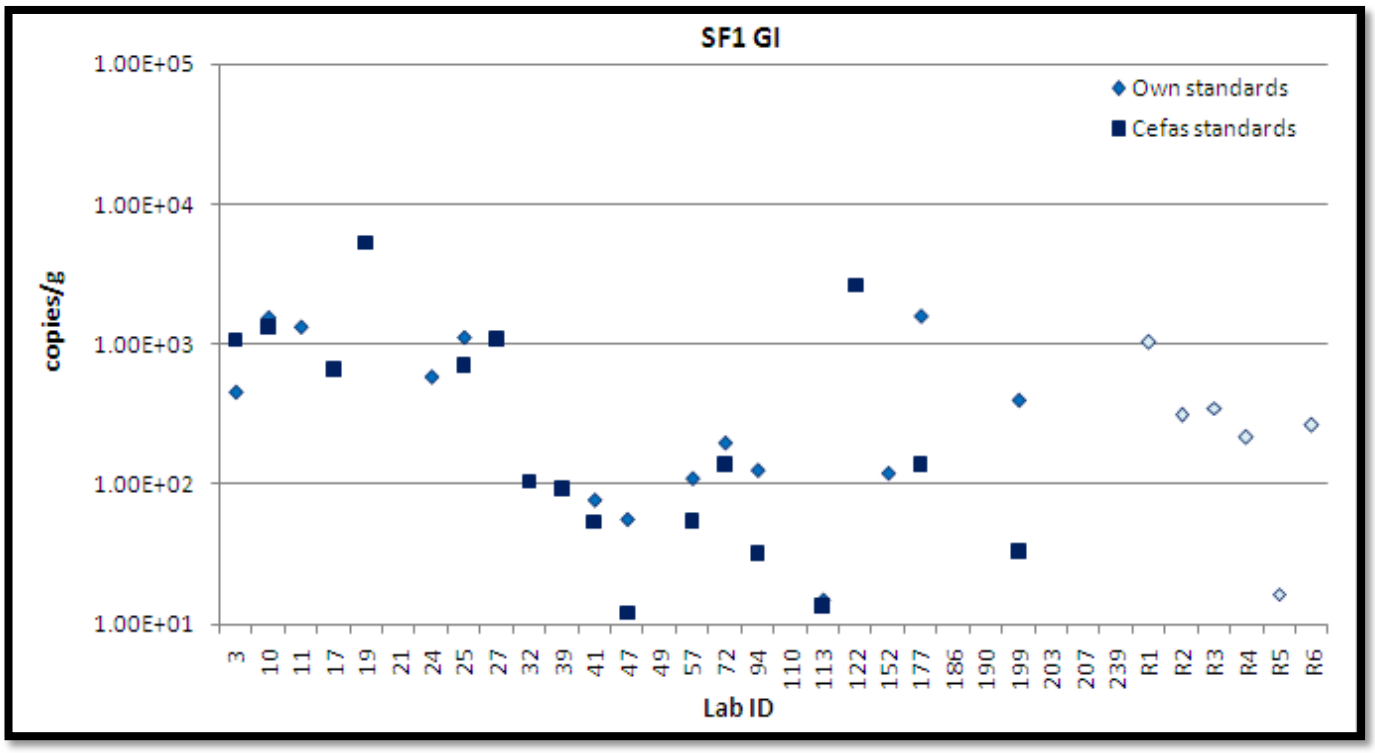
Appendix V: Participants reported quantities for each target (copies/g (shellfish) or copies/LENTICULE)

Lab ID No.	Shellfish sample1						Shellfish sample2				Shellfish sample4		LENTICULE 1		LENTICULE 2			
	GI		GII		HAV		GI		GII		HAV		GI		GII		HAV	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
3 *	4.57E+02	1.07E+03	1.19E+04	2.15E+04	3.82E+04	3.69E+04	7.54E+02	1.76E+03	1.16E+03	2.16E+03	1.42E+04	1.12E+04	1.80E+03	4.15E+03	7.79E+03	1.42E+04	3.74E+05	5.50E+05
10*	1.56E+03	1.32E+03	1.59E+04	1.48E+04	2.49E+04	6.10E+04	1.45E+03	1.25E+03	4.89E+03	5.09E+03	1.98E+04	4.76E+04	2.29E+04	1.65E+04	1.49E+04	1.40E+04	3.88E+05	1.05E+06
11	1.34E+03		4.09E+04		1.06E+06		2.90E+02		3.75E+03		1.61E+06		5.45E+03		4.64E+04		1.36E+07	
17*		6.60E+02		1.80E+04		6.40E+03		1.60E+03		9.10E+03		3.10E+04		3.20E+03		6.40E+03		6.50E+05
19*		5.32E+03		4.21E+03		2.84E+04		4.87E+02		2.07E+02		2.57E+04		1.86E+04		2.31E+03		1.12E+06
21			5.10E+01	5.10E+01			9.80E+01	1.42E+02	2.98E+02	2.83E+02			6.50E+02	7.50E+02	1.00E+03	8.00E+02		
24	5.88E+02		1.72E+04	2.68E+04	8.35E+04	2.26E+05	8.83E+01		4.16E+02	2.42E+02	2.36E+05	6.46E+05	3.00E+03		1.64E+03	1.71E+03	9.85E+05	3.05E+06
25*	1.13E+03	7.05E+02	8.11E+02	2.17E+03		2.10E+03	1.08E+04	4.57E+03	1.29E+03	3.50E+03		2.16E+04	2.43E+04	8.07E+03	2.46E+03	7.00E+03		3.93E+05
27*		1.10E+03		1.00E+04		1.40E+04		2.00E+03		2.30E+03		1.30E+04		5.40E+03		1.20E+04		5.70E+05
32*		1.04E+02		1.78E+04		1.36E+04		9.31E+03		2.87E+04		4.67E+04		4.66E+02		5.42E+03		3.70E+05
39*		9.20E+01		4.90E+02				4.90E+02		3.50E+02				7.70E+01		1.20E+02		
41*	7.70E+01	5.40E+01	7.70E+03	7.30E+03	4.50E+03	6.80E+03	4.50E+02	3.00E+02	3.04E+03	7.80E+03	3.10E+03	4.90E+03	3.60E+03	2.50E+03	2.20E+04	2.40E+04	3.10E+05	4.10E+05
47*	5.60E+01	1.20E+01	2.40E+03	6.60E+03	1.80E+03	6.20E+02	1.90E+03	5.20E+02	2.40E+02	7.40E+02	1.30E+04	3.80E+03	7.90E+03	1.50E+03	2.50E+03	6.10E+03	6.70E+05	1.40E+05
49			3.09E+02	7.02E+02	4.96E+04		3.60E+01	5.00E+01	4.70E+01	9.60E+01	1.62E+04		1.73E+03	2.84E+03	1.82E+02	4.00E+02	2.90E+05	
57	1.10E+02	5.50E+01	2.50E+03	4.70E+03	7.90E+03	1.10E+04	5.40E+02	2.90E+02	7.30E+02	1.30E+03	2.00E+04	2.70E+04	7.10E+02	3.70E+02	3.40E+03	6.10E+03	1.60E+06	2.00E+06
72	1.98E+02	1.38E+02	1.59E+02	6.22E+02			8.40E+01	5.40E+01	4.20E+01	1.76E+02			7.77E+04	6.89E+04	3.11E+03	1.22E+04		
94	1.26E+02	3.20E+01	8.88E+02	3.26E+02	1.68E+04	7.25E+03	2.04E+03	5.08E+02	5.14E+02	2.07E+02	6.95E+03	3.06E+03	1.53E+03	3.83E+02	6.52E+03	2.91E+03	9.18E+05	3.66E+05
110											1.76E+05	4.80E+02	1.29E+04	1.10E+02			3.60E+07	1.62E+05
113	1.48E+01	1.36E+01	2.42E+01	3.31E+01			1.36E+01	1.26E+01	2.78E+01	3.51E+01	3.89E+01	5.18E+01	6.39E+01	5.89E+01	1.04E+02	1.43E+02		
122		2.65E+03		1.97E+04		4.59E+04		3.30E+03		1.18E+03		2.24E+05		2.62E+04		1.06E+04		6.72E+05
152	1.20E+02		3.70E+03		5.90E+04		1.50E+02		2.70E+03		9.50E+04		6.10E+04		2.70E+04		1.40E+07	
177	1.60E+03	1.40E+02	3.10E+05	1.30E+04	3.90E+04	3.40E+03	7.10E+03	5.90E+02	1.20E+04	4.80E+02	6.80E+04	8.50E+03	4.00E+03	3.40E+02	6.00E+04	9.50E+03	2.80E+06	3.10E+05
186																		4.92E+01
190			5.60E+03	1.60E+02	6.30E+03	2.80E+01	8.80E+04	1.30E+02	5.50E+03	1.60E+02	2.40E+05	2.00E+03	1.90E+05	6.30E+02	5.50E+04	1.70E+03	3.30E+06	5.40E+04
199	4.00E+02	3.30E+01	9.00E+01	1.90E+01	3.60E+03	1.20E+02	3.70E+03	2.80E+02	2.00E+02	4.80E+01	4.20E+04	1.70E+03	1.20E+04	8.00E+02	6.30E+03	1.70E+03	7.20E+06	2.50E+05
203							1.14E+03	6.34E+03	4.85E+07	6.16E+08	1.69E+05	2.31E+04	1.82E+02	2.59E+02			3.62E+06	6.65E+05
207				7.20E+03		1.98E+03		3.78E+03		9.00E+02		1.17E+03		9.00E+02		6.00E+02		2.60E+05
239				4.18E+04		2.80E+02				4.20E+01		7.46E+02				1.51E+04		3.39E+05

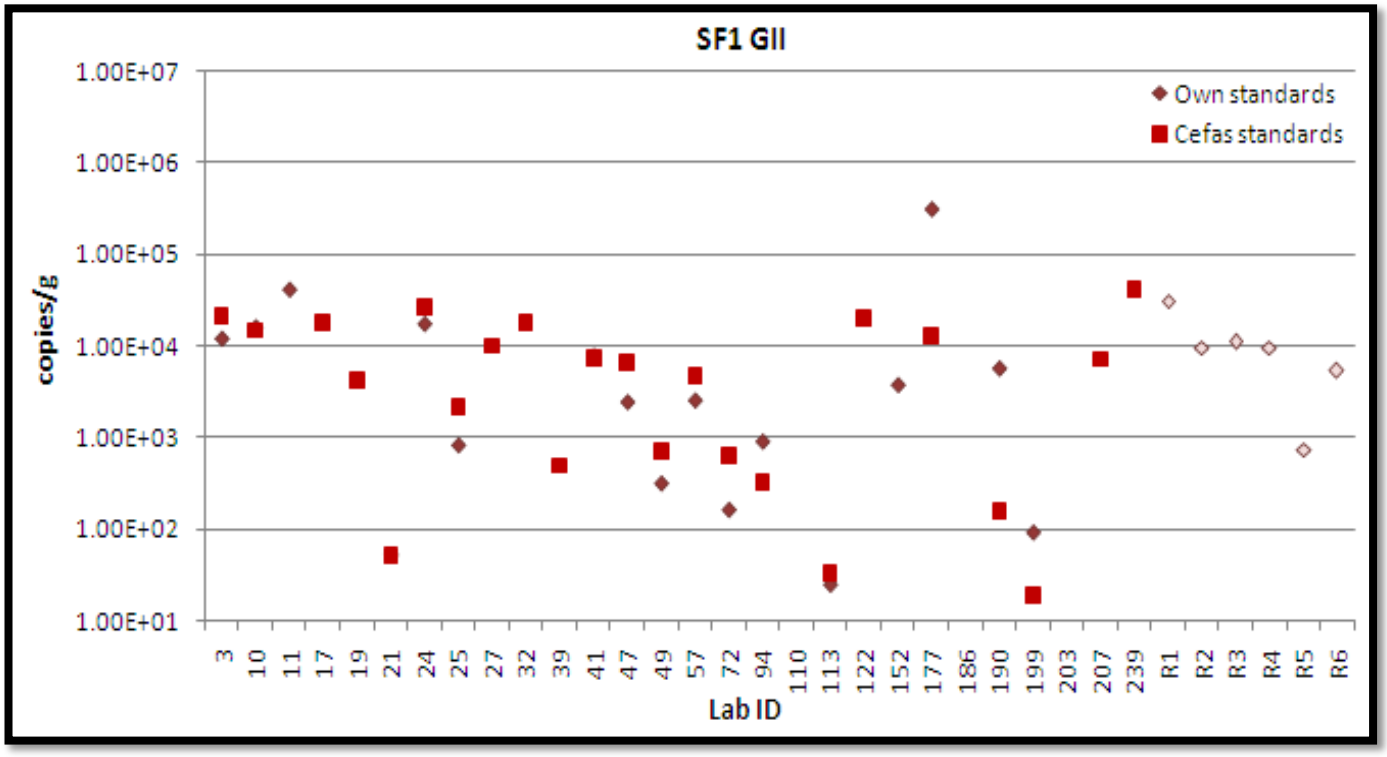
* Designated NRL, A - Quantity determined using routine method, B - Quantity determined using standard material provided. Labs which did not report any quantities not included.

Appendix VI: Participants' and reference quantities for each sample.

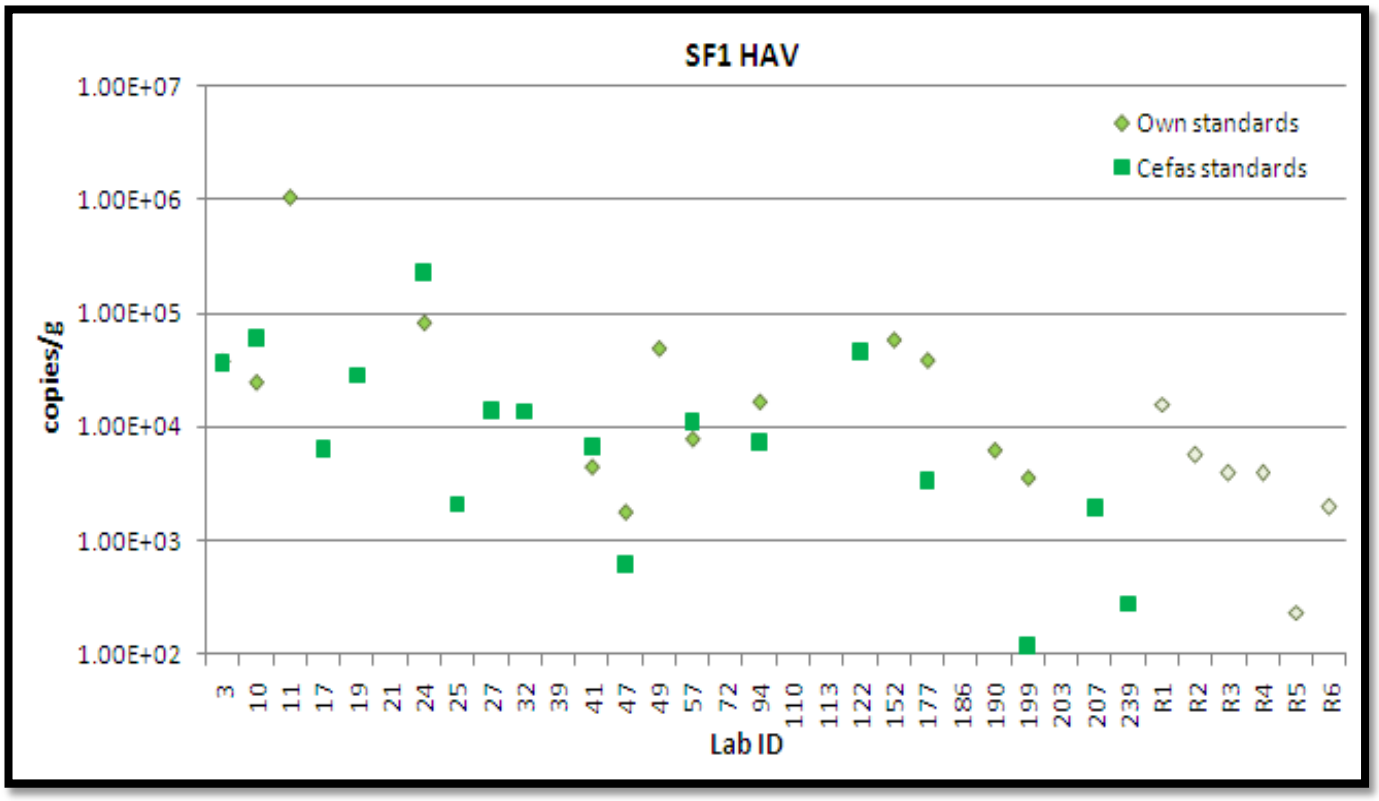
Shellfish sample 1 - GI



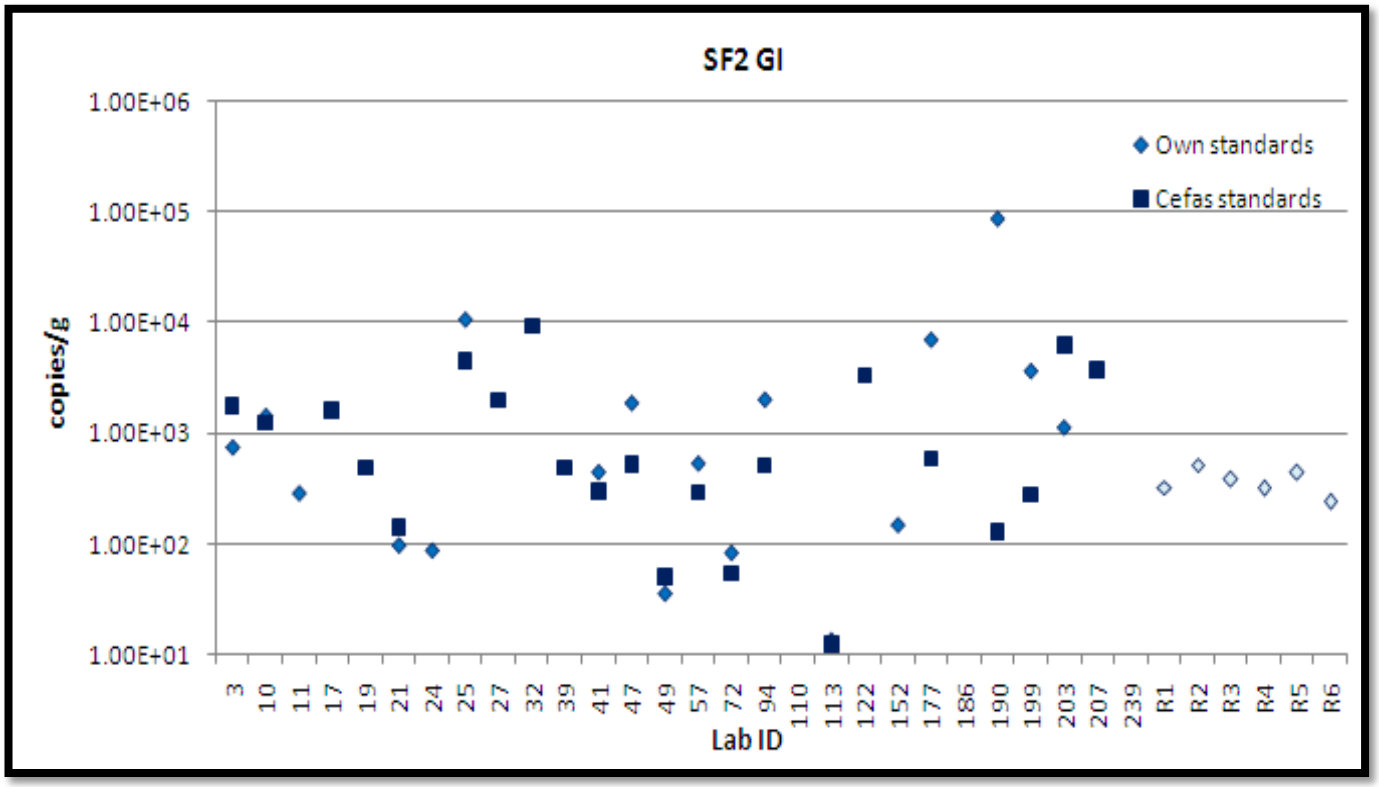
Shellfish sample 1 – GII



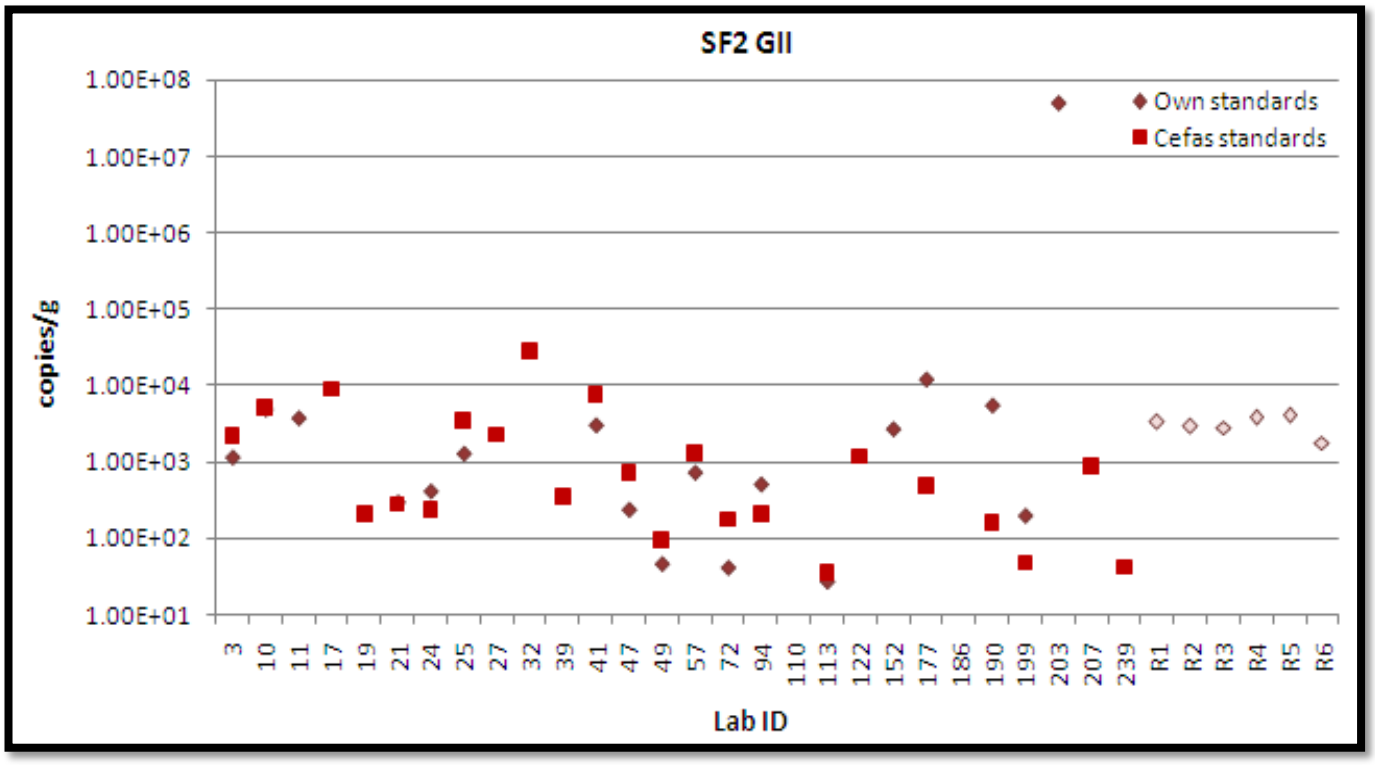
Shellfish sample 1 – HAV



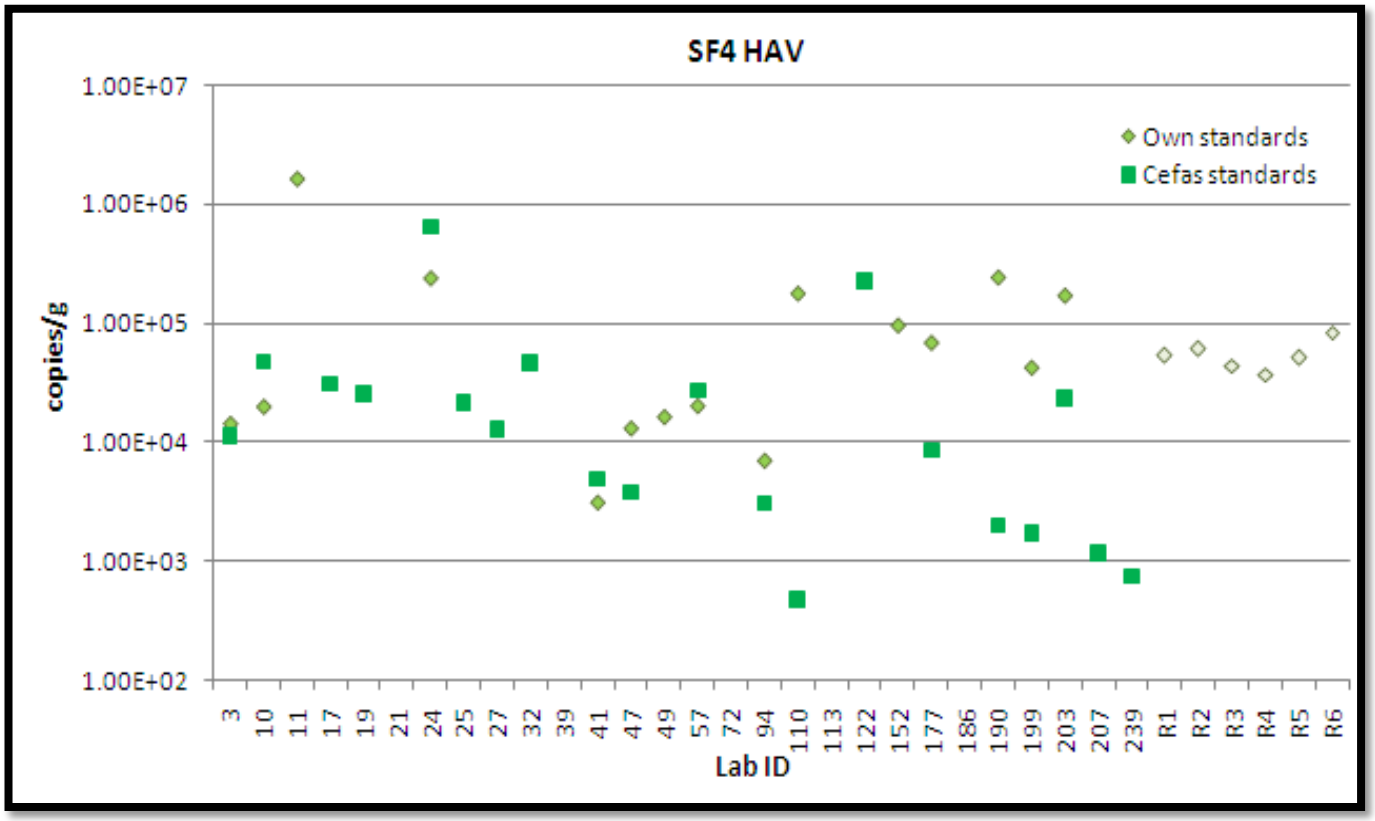
Shellfish Sample 2 – GI



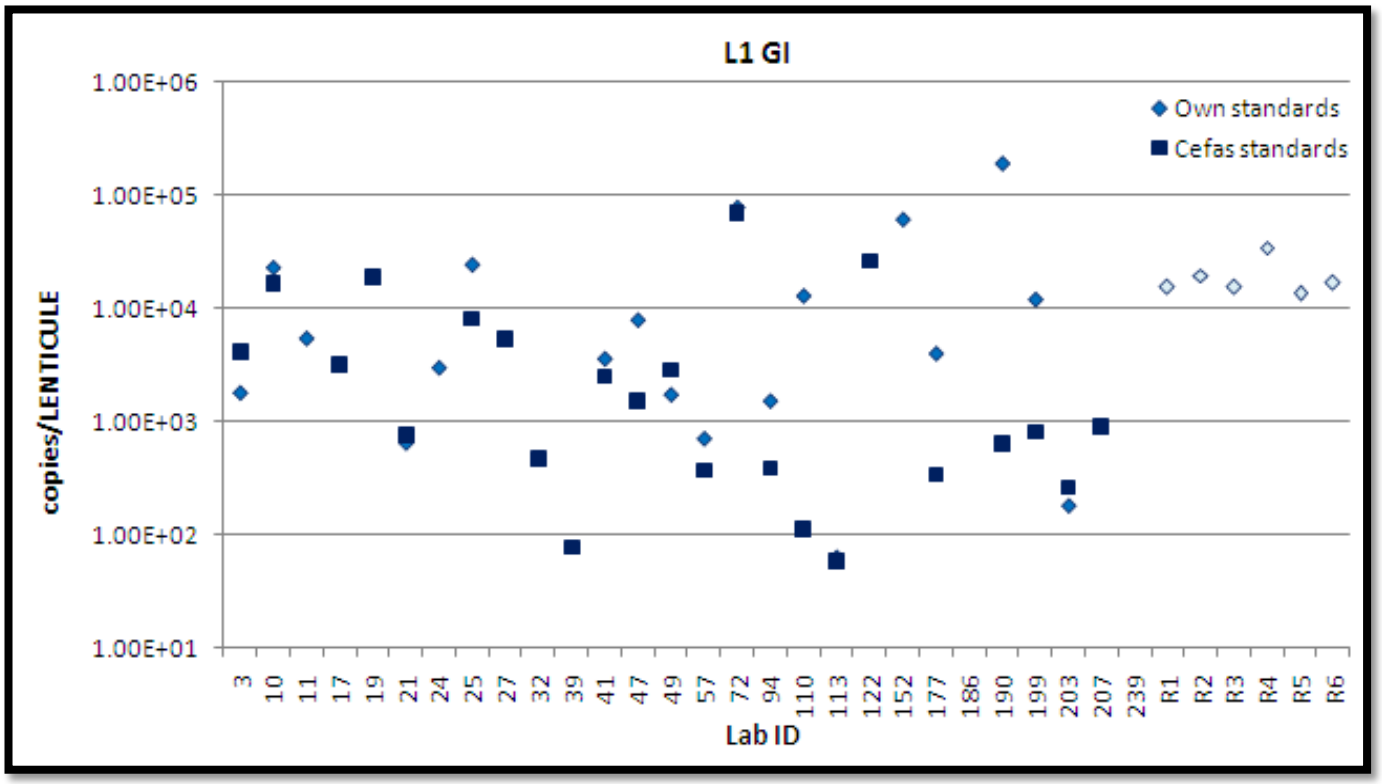
Shellfish Sample 2 – GII



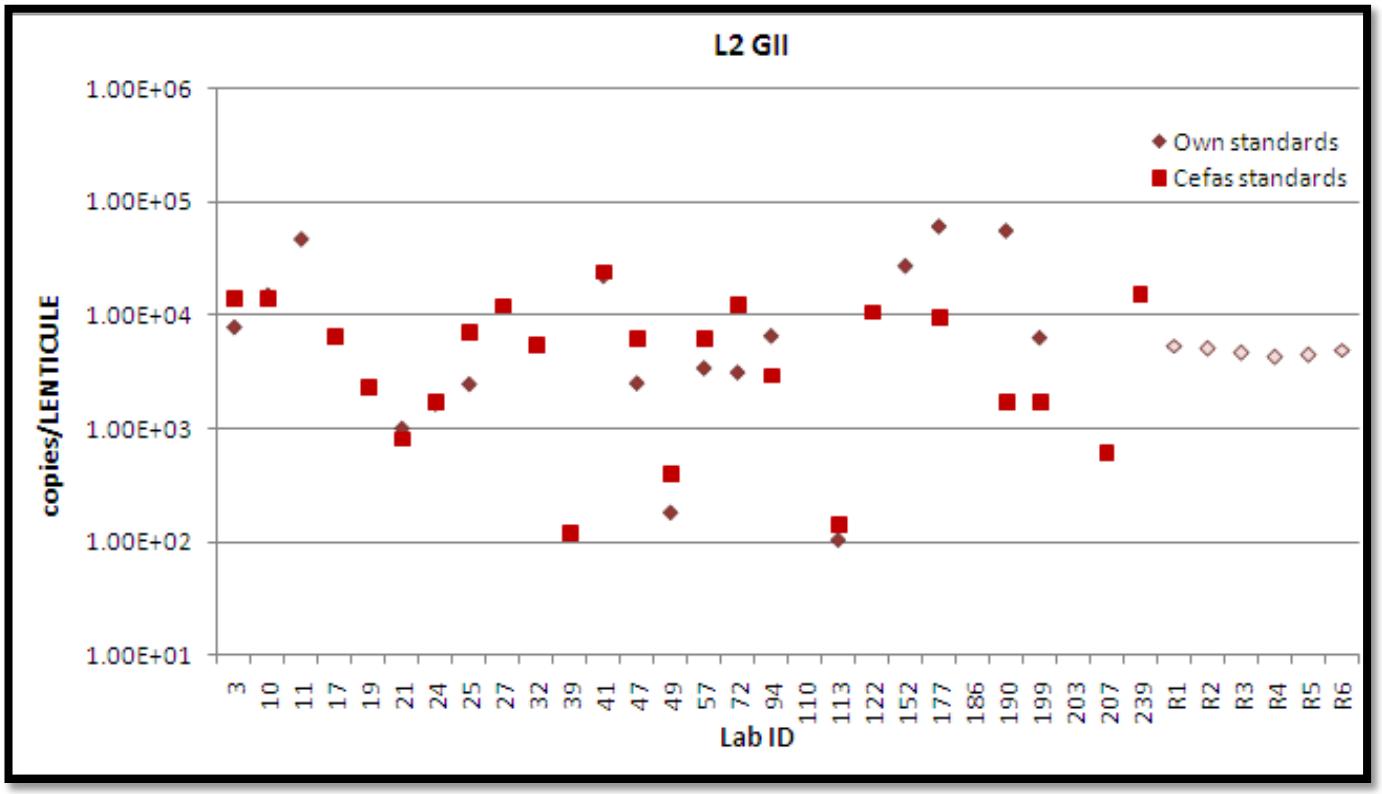
Shellfish Sample 4 - HAV



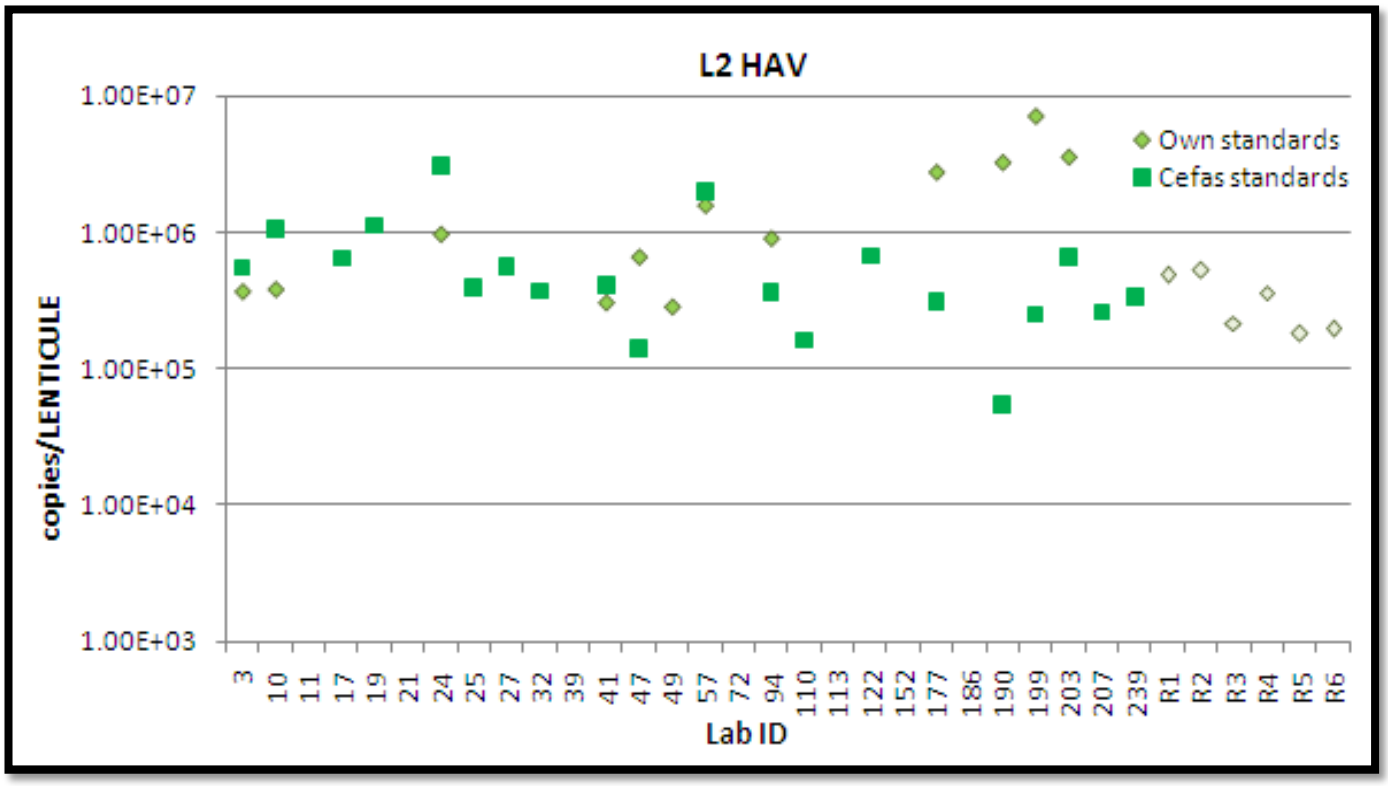
LENTICULE 1 - GI



LENTICULE 2 - GII



LENTICULE 2 – HAV



Appendix VII: Results and methods used for shellfish samples. (For key to method codes see page 21)

LAB ID	ACC SF[a]	SF1			SF2			SF3			SF4			Virus extraction	RNA extraction	RT-PCR method	RT-PCR reagents	Primers		
		GI	GII	HAV	GI	GII	HAV	GI	GII	HAV	GI	GII	HAV					GI	GII	HAV
3	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	CC	DD	DD
7	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N[b]	P[e]	DD	DD	DD[h]
10	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	CC	DD	DD
11	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	F	N	Q	EE	DD	DD
17	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
19	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
24	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	G	O	R	FF	EE	DD
25	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
27	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	F	N	P	CC	DD	DD
32	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
33	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	B	E	N	P	DD	DD	DD
39	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	B	F	N	S	EE	EE	HH
41	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
47	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	T	DD[g]	DD[g]	DD
48	100.00%	NE	+	+	NE	+	-	NE	-	-	NE	-	+	A	E	N	P	-	DD	DD
53	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	F	N	T	DD	DD	DD
57	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
94	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	C	E	N	U	GG	GG	GG
95	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	F	O[c]	V	EE	EE	II
98	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	U	GG	GG	GG
113	100.00%	+	+	NE	+	+	NE	-	-	NE	-	-	NE	C	H	N	Q	EE[g]	EE[g]	-
122	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
152	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	C	F	N	W	EE	EE	JJ
176	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	U	GG	GG	GG
177	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	A	E	N	P	DD	DD	DD
199	100.00%	+	+	+	+	+	-	-	-	-	-	-	+	C	E	N	U	GG	GG	GG

LAB ID	ACC SF[a]	SF1			SF2			SF3			SF4			Virus extraction	RNA extraction	RT-PCR method	RT-PCR reagents	Primers		
		GI	GII	HAV	GI	GII	HAV	GI	GII	HAV	GI	GII	HAV					GI	GII	HAV
35	91.67%	+	+	+	-	+	-	-	-	-	-	-	+	A	E	N[d]	Q[f]	EE	DD	KK
49	91.67%	-	+	+	+	+	-	-	-	-	-	-	+	D	I	N	X	CC	DD	LL
83	91.67%	+	+	+	+	-	-	-	-	-	-	-	+	A	E	N	Y	CC	DD	DD
147	91.67%	-	+	+	+	+	-	-	-	-	-	-	+	B	J	N	U	GG	GG	GG
158	91.67%	-	+	+	+	+	-	-	-	-	-	-	+	A	E	N	U	GG	GG	GG
190	91.67%	-	+	+	+	+	-	-	-	-	-	-	+	A	E	N	U	GG	GG	GG
207	91.67%	-	+	+	+	+	-	-	-	-	-	-	+	A	K	N	S	DD	DD	DD
239	91.67%	+	+	+	-	+	-	-	-	-	-	-	+	A	L	N	U	GG	GG	GG
72	87.50%	+	+	NE	+	+	NE	-	-	NE	-	+	NE	A	E	N	Z	CC	DD	-
21	83.33%	-	+	-	+	+	-	-	-	-	-	-	+	A	E	N	P	DD[g]	DD[g]	HH
203	75.00%	-	-	-	+	+	-	-	-	-	-	-	+	A	K	N	AA	CC	DD	DD
110	58.33%	-	-	-	-	-	-	-	-	-	-	-	+	C	E	N	U	GG	GG	GG
186	50.00%	-	-	-	-	-	-	-	-	-	-	-	-	A	M	O	BB	DD	DD	DD

Red = false negative results; yellow = false positive results; dark grey = CEN TAG4 (or very similar) method; light grey = CEN TAG4 method with modifications

a - ACC SF = accuracy for shellfish samples – labs listed according to scores
b - Real-time one-step used for NoV, conventional two-step for HAV
c - Real-time two-step used for NoV, conventional two-step for HAV
d - Real-time one-step used for NoV, real-time two-step for HAV
e - Ultrasense used for NoV :- MuLV (Applied Biosystems) & Ampli Taq Gold (Applied Biosystems) used for HAV
f - Qiagen OneStep RT-PCR kit used for NoV:- enzyme mix from Qiagen OneStep RT-PCR kit used for HAV
g - NoV analysis carried out as multiplex
h - CEN primers used in conventional PCR

Key to method codes

Virus extraction methods

A	Pro K (CEN or minor modifications)
B	Pro K with significant modifications
C	Glycine buffer elution/ organic extraction
D	Sonication with glycine buffer/ PEG precipitation

RNA extraction methods

E	NucliSens Magnetic extraction reagents (BioMerieux)
F	QIAamp/Rneasy kits (Qiagen)
G	Roche High Pure Viral Nucleic Acid Kit
H	GITC lysis then ethanol precipitation
I	Trizol/Dynabeads
J	Maxwell® 16 LEV simplyRNA
K	Nucleo spin (Macherey-Nagel)
L	MagMax Viral RNA isolation kit
M	Trizol/ chloroform/ purelink RNA mini kit (Life Tech)

RT-PCR methods

N	Real-time one-step
O	Real-time two-step

RT-PCR reagents

P	RNA Ultrasense (Invitrogen)
Q	OneStep RT-PCR kit (Qiagen)
R	Superscript III (RT) & Platinum® qPCR SuperMix-UDG (Invitrogen)
S	SuperScript® III PlatinumOne-Step qRT-PCR System (Invitrogen)
T	Quantitect (Qiagen)
U	ceeram Tools
V	ABI High Capacity cDNA RT Kit ; ABI Taqman Universal Mastermix
W	One-step real-time RT-PCR kit (Ambion)
X	TaqMan® Fast Virus 1-Step Master Mix
Y	Agilent, Brilliant II QRT-PCR Master Mix Kit
Z	Life Technologies (Applied Biosystems) RNA to Ct One step kit
AA	Platinum quantitative RT-PCR Thermoscript One-step system (Invitrogen)
BB	Thermoscript RT (Invitrogen); Lightcycler TaqMan mastermix (Roche)

Primers/probes

CC	Primers; CEN. Probe; Hohne and Schreier, 2006
DD	CEN bench protocols
EE	Kageyama et al, 2003
FF	Wolf et al, 2010
GG	Ceeram Tools (sequences as CEN)
HH	In-house sequences
II	FDA/BAM Chapter 26 “Detection and Quantification of Hepatitis A virus in Shellfish by the Polymerase Chain Reaction”
JJ	Pasqual et al., 2010
KK	Schwab et al. 1995
LL	Guevremont et al. 2006, Houde et al. 2007

About us

Cefas is a multi-disciplinary scientific research and consultancy centre providing a comprehensive range of services in fisheries management, environmental monitoring and assessment, and aquaculture to a large number of clients worldwide.

We have more than 500 staff based in 2 laboratories, our own ocean-going research vessel, and over 100 years of fisheries experience.

We have a long and successful track record in delivering high-quality services to clients in a confidential and impartial manner.
(www.cefas.defra.gov.uk)

Cefas Technology Limited (CTL) is a wholly owned subsidiary of Cefas specialising in the application of Cefas technology to specific customer needs in a cost-effective and focussed manner.

CTL systems and services are developed by teams that are experienced in fisheries, environmental management and aquaculture, and in working closely with clients to ensure that their needs are fully met.
(www.cefastechnology.co.uk)

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Customer focus

With our unique facilities and our breadth of expertise in environmental and fisheries management, we can rapidly put together a multi-disciplinary team of experienced specialists, fully supported by our comprehensive in-house resources.

Our existing customers are drawn from a broad spectrum with wide ranging interests. Clients include:

- international and UK government departments
- the European Commission
- the World Bank
- Food and Agriculture Organisation of the United Nations (FAO)
- oil, water, chemical, pharmaceutical, agro-chemical, aggregate and marine industries
- non-governmental and environmental organisations
- regulators and enforcement agencies
- local authorities and other public bodies

We also work successfully in partnership with other organisations, operate in international consortia and have several joint ventures commercialising our intellectual property

Head office
Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK

Tel +44 (0) 1502 56 2244
Fax +44 (0) 1502 51 3865
Web www.cefas.defra.gov.uk

Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601

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