

# **European Union Reference Laboratory (EU-RL) Proficiency Testing scheme**

## **Norovirus and hepatitis A virus proficiency testing 2011**

**EURL ring trial reference number: RT 43**

**Sample distributions: Shellfish samples 1 and 2, LENTICULES™ 1 and 2.**

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<b>Report compiled by:</b>	<b>Louise Stockley James Lowther</b>
<b>Authorisation by:</b>	<b>Rachel Hartnell</b>

## Samples

### Sample preparation

Material dispatched comprised of naturally contaminated and bioaccumulated Pacific oysters (*Crassostrea gigas*) (Samples 1 and 2), laboratory constructed LENTICULES™ (Vial 1 and 2) and standard curve material (plasmid solutions for each target virus at concentrations of  $10^4$ ,  $10^3$ ,  $10^2$ ,  $10^1$  and  $10^0$  copies/ $\mu$ l) to estimate the levels of virus in the samples. The reference results for each sample are included as Appendix I.

### Sample 1

Sample 1 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 17<sup>th</sup> June 2006 with a total of 115 cases being identified. Prior testing of the sample had 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  $<-15^\circ\text{C}$  until required for quality control testing, dispatch or reference analysis.

### Sample 2

A batch of approximately 500 Pacific oysters (*Crassostrea gigas*) were placed in trays and re-immersed in 500 litres of re-circulating natural seawater at  $16\pm 1^\circ\text{C}$  and 36.5ppt. 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) and HAV cell culture supernatant (see table 1) was added to the tank. After approximately 17 hours the shellfish were removed from the tank and rinsed in fresh water. The oyster sample was then shucked and randomly sorted into samples of 10 animals and sealed in plastic bags. Bags were held at  $<-15^\circ\text{C}$  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. Table 1 shows details of the stock viruses used in the preparation of the LENTICULES™.

**Table 1: The origin and designation of viruses used for shellfish bioaccumulation and LENTICULES™**

Description	Source	Sequence type
Hepatitis A	Cell culture supernatant	Strain HM175/43c
Norovirus genogroup I	Faecal material	GI.4 capsid type
Norovirus genogroup II	Faecal material	GI.4 capsid type

### Sample distribution

Samples were dispatched on dry ice in accordance with IATA packing instructions 650 for UN3373 'Diagnostic Specimens' on 12<sup>th</sup> December 2011 to 26 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 materials and using the standard materials provided with this PT distribution.

## Results

### Reference results

Reference analyses were performed by the EURL on samples stored at  $<-15^\circ\text{C}$ . Six randomly selected samples from each sample type were extracted in duplicate and RT-PCR (TaqMan™) was carried out using triplicate PCR reactions for each RNA 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 RT 43 proficiency testing material**

Sample	Norovirus GI	GI	HAV
RT 43 – Shellfish sample 1	+ ( $4.3 \times 10^2$ )	+ ( $2.2 \times 10^3$ )	-
RT 43 – Shellfish sample 2	+ ( $3.2 \times 10^3$ )	+ ( $4.6 \times 10^2$ )	+ ( $1.0 \times 10^4$ )
RT 43 – LENTICULE™ 1	-	-	-
RT 43 – LENTICULE™ 2	+ ( $4.8 \times 10^3$ )	+ ( $4.2 \times 10^3$ )	+ ( $3.0 \times 10^5$ )

Average quantities in copies/g or copies/lenticule shown in brackets

### 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 ring trial distribution included both shellfish matrices and LENTICULE™ discs, an overall performance assessment was performed to assess each laboratory's performance (Table 3) as well as assessing the performance on shellfish matrices (Table 4) and LENTICULE™ discs (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			GII			HAV		
	AC	SP	SE	AC	SP	SE	AC	SP	SE
2	50	100	33	75	100	67	100	100	100
3 <sup>a</sup>	50	100	33	100	100	100	NT	NT	NT
7 <sup>a</sup>	100	100	100	25	100	0	100	100	100
10 <sup>a</sup>	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100
17 <sup>a</sup>	100	100	100	100	100	100	100	100	100
19 <sup>a</sup>	75	100	67	100	100	100	100	100	100
24	100	100	100	100	100	100	100	100	100
25 <sup>a</sup>	100	100	100	100	100	100	100	100	100
27 <sup>a</sup>	100	100	100	100	100	100	100	100	100
32 <sup>a</sup>	75	100	67	75	0	100	100	100	100
35 <sup>a</sup>	NR	NR	NR	NR	NR	NR	NR	NR	NR
39 <sup>a</sup>	75	100	67	75	100	67	100	100	100
41 <sup>a</sup>	100	100	100	100	100	100	100	100	100
43 <sup>a</sup>	50	100	33	50	100	33	75	100	50
47 <sup>a</sup>	100	100	100	100	100	100	100	100	100
48	50	100	33	50	100	33	100	100	100
94	100	100	100	100	100	100	100	100	100
95	75	100	67	50	100	33	100	100	100
98	100	100	100	75	100	67	100	100	100
113	25	100	0	50	100	33	NT	NT	NT
147 <sup>a</sup>	50	100	33	25	0	33	100	100	100
178	25	100	0	50	0	67	100	100	100
179	100	100	100	100	100	100	100	100	100
186	25	100	0	25	100	0	50	50	50
190	100	100	100	100	100	100	100	100	100

<sup>a</sup> - Designated NRL, NT= Not tested, NR = Not returned, AC - Relative accuracy, SP – Relative specificity, SE – Relative sensitivity

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

Lab ID No.	GI		GII		HAV		
	AC	SE	AC	SE	AC	SP	SE
2	0	0	100	100	100	100	100
3 <sup>a</sup>	50	50	100	100	NT	NT	NT
7 <sup>a</sup>	100	100	0	0	100	100	100
10 <sup>a</sup>	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100
17 <sup>a</sup>	100	100	100	100	100	100	100
19 <sup>a</sup>	50	50	100	100	100	100	100
24	100	100	100	100	100	100	100
25 <sup>a</sup>	100	100	100	100	100	100	100
27 <sup>a</sup>	100	100	100	100	100	100	100
32 <sup>a</sup>	50	50	100	100	100	100	100
35 <sup>a</sup>	NR	NR	NR	NR	NR	NR	NR
39 <sup>a</sup>	50	50	50	50	100	100	100
41 <sup>a</sup>	100	100	100	100	100	100	100
43 <sup>a</sup>	0	0	0	0	50	100	0
47 <sup>a</sup>	100	100	100	100	100	100	100
48	0	0	0	0	100	100	100
94	100	100	100	100	100	100	100
95	50	50	0	0	100	100	100
98	100	100	50	50	100	100	100
113	0	0	50	50	NT	NT	NT
147 <sup>a</sup>	0	0	50	50	100	100	100
178	0	0	100	100	100	100	100
179	100	100	100	100	100	100	100
186	0	0	0	0	50	0	100
190	100	100	100	100	100	100	100

<sup>a</sup> - Designated NRL, NT= Not tested, NR = Not returned, AC - Relative accuracy, SP – Relative specificity, SE – Relative sensitivity

**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	50	100	0	100	100	100
3 <sup>a</sup>	50	100	0	100	100	100	NT	NT	NT
7 <sup>a</sup>	100	100	100	50	100	0	100	100	100
10 <sup>a</sup>	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100
17 <sup>a</sup>	100	100	100	100	100	100	100	100	100
19 <sup>a</sup>	100	100	100	100	100	100	100	100	100
24	100	100	100	100	100	100	100	100	100
25 <sup>a</sup>	100	100	100	100	100	100	100	100	100
27 <sup>a</sup>	100	100	100	100	100	100	100	100	100
32 <sup>a</sup>	100	100	100	50	0	100	100	100	100
35 <sup>a</sup>	NR	NR	NR	NR	NR	NR	NR	NR	NR
39 <sup>a</sup>	100	100	100	100	100	100	100	100	100
41 <sup>a</sup>	100	100	100	100	100	100	100	100	100
43 <sup>a</sup>	100	100	100	100	100	100	100	100	100
47 <sup>a</sup>	100	100	100	100	100	100	100	100	100
48	100	100	100	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
113	50	100	0	50	100	0	NT	NT	NT
147 <sup>a</sup>	100	100	100	0	0	0	100	100	100
178	50	100	0	0	0	0	100	100	100
179	100	100	100	100	100	100	100	100	100
186	50	100	0	50	100	0	50	100	0
190	100	100	100	100	100	100	100	100	100

<sup>a</sup> - Designated NRL, NT= Not tested, NR = Not returned, AC - Relative accuracy, SP – Relative specificity, SE – Relative sensitivity

## Conclusion and discussion

### General comments

Twenty-six laboratories (14 NRLs and 12 other laboratories) received samples. Laboratories 3 and 113 did not examine for HAV. Laboratory 35 did not return results. Results reported to the EURL are shown in Appendices I and V.

### Discussion

Ten (38%) of the participating laboratories obtained intended results (as determined by EURL reference designations) for all the different materials sent and determinands tested. The overall accuracies across all laboratories were 77%, 77% and 97% for GI, GII and HAV respectively. The false positive reporting rates for GI, GII and HAV were 0%, 12% and 2% respectively. The false negative reporting rates for GI, GII and HAV were 31%, 27% and 4% respectively.

Comparing the overall accuracies of LENTICULES against shellfish matrices, the accuracies for LENTICULES were 86%, 76% and 91% for GI, GII and HAV respectively and for shellfish matrices they were 62%, 72% and 96% for GI, GII and HAV respectively. The false positive reporting rates for GI, GII and HAV were 8%, 20% and 9% for LENTICULES and 4% for HAV in shellfish matrices. The false negative reporting rates for GI, GII and HAV were 20%, 28% and 9% for LENTICULES and 38%, 28% and 4% for shellfish matrices respectively.

Twenty-three laboratories (92%) returned data expressed as  $C_t$  values (Appendix III). Seventeen laboratories (68%) 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™ (Appendix IV). Quantitative results from individual labs alongside reference results are shown in Appendix V. Reference results were not corrected using extraction efficiency data; where labs provided both corrected and uncorrected quantities, the latter are shown in Appendices IV and V.

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 VI.

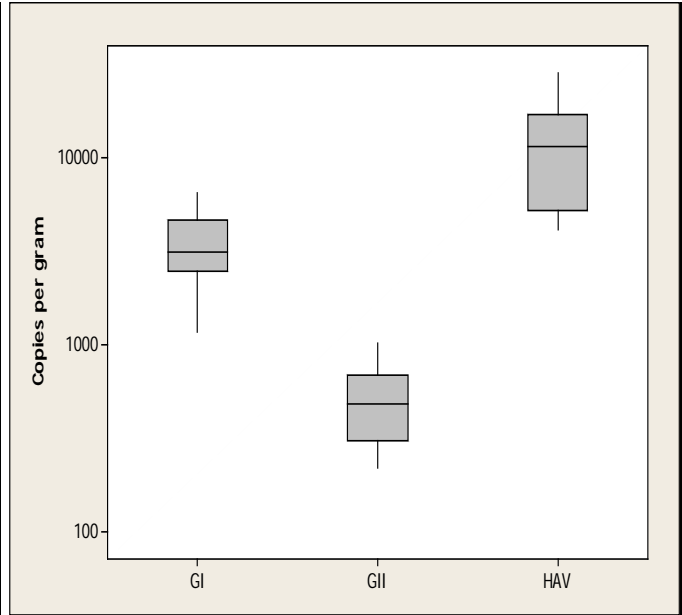
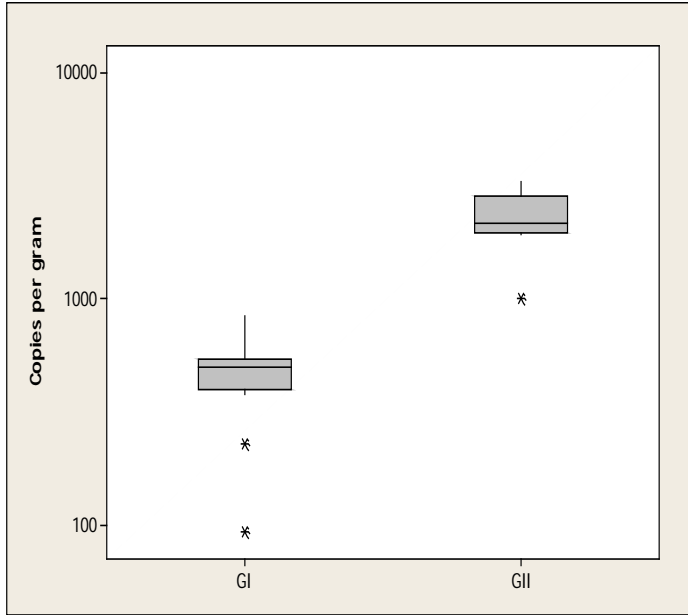
**References**

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

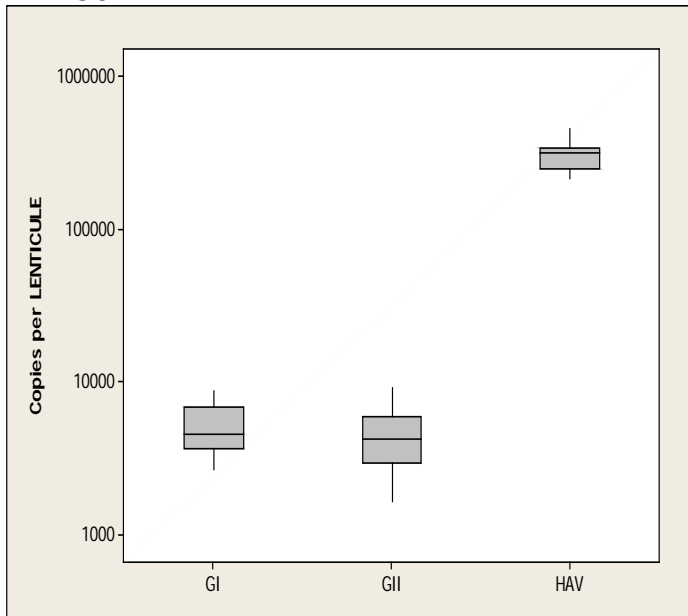
**Appendix I:** EURL reference results displayed as box and whisker plots of detectable genome copies per gram or 25µl LENTICULE™.

Shellfish sample 1

Shellfish sample 2



LENTUCLE 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<sub>t</sub> values for all samples

Lab ID No.	Shellfish sample 1						Shellfish sample 2						Lenticule 1						Lenticule 2					
	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	-		+		-		-		+		+		-		-		-		W+	-		+		
3 <sup>a</sup>	+	36.4	+	34.02	NT		-		+		34.59	NT		-		-		NT	-		+		37.42	NT
7 <sup>a</sup>	+		-		-		+		-		+		-		-		-		+	-		+		
10 <sup>a</sup>	+	36.48	+	33.97	-		+	33.91	+	34.05	+	30.1	-		-		-		+	35.51	+	36.69	+	30.57
11	+	40.21	+	33.51	-		+	35.03	+	33.92	+	30.58	-		-		-		+	38.55	+	36.2	+	31.65
17 <sup>a</sup>	+	35.52	+	31.75	-	45	+	33.61	+	30.55	+	29.57	-	45	-	45	-	45	+	36.68	+	34.24	+	29.2
19 <sup>a</sup>	-		+	37.86	-		+	35.26	+	37.26	+	32.71	-		-		-		+	34.62	+	36.62	+	20.23
24	+	35.6	+	32.9	-		+	30.3	+	31.7	+	27.3	-		-		-		+	32.8	+	36.3	+	28.7
25 <sup>a</sup>	+	33.73	+	31.77	-		+	31.68	+	31.14	+	28.66	-		-		-		+	36.51	+	35.91	+	28.26
27 <sup>a</sup>	+	37.73	+	38.1	-		+	35.05	+	34	+	31.39	-		-		-		+	37.09	+	37.22	+	29.37
32 <sup>a</sup>	+	34.05	+	38.17	-		-		+	37.44	+	30.08	-		+	45.6	-		+	41.19	+	43.37	+	28.66
35 <sup>a</sup>	NR		NR		NR		NR		NR		NR		NR		NR		NR		NR		NR		NR	
39 <sup>a</sup>	-		-		-		+	41.08	+	40.38	+	32.8	-		-		-		+	38.45	+	39.89	+	35.35
41 <sup>a</sup>	+	34.46	+	33.52	-		+	34.69	+	37.06	+	28.02	-		-		-		+	34.96	+	34.49	+	29.08
43 <sup>a</sup>	-		-		-		-		-		-		-		-		-		+	47.02	+	46.79	+	
47 <sup>a</sup>	+	35.6	+	33.5	-	-	+	35.8	+	32.2	+	30.6	-	-	-	-	-	-	+	38.8	+	34.9	+	29.7
48	-		-		-		-		-		+	30.17	-		-		-		+	37.13	+	35.02	+	25.92
94	+	33.35	+	32.95	-	-	+	33.84	+	37.36	+	28.31	-	-	-	-	-	-	+	40.38	+	36.04	+	29.77
95	-		-		-		+	42.42	-		+		-		-		-		+	39.65	+	40.64	+	
98	+	36.58	+	35.47	-		+	45.12	-		+	36.54	-		-		-		+	38.71	+	35.23	+	28.42
113	-		-		NT		-		+	33.48	NT		-		-		NT		-		-		NT	
147 <sup>a</sup>	-		+	47.1	-		-		-		+	36.15	-		+	46.75	-		+	46	-		+	34.23
178	-		+		-		-		+		+	33.72	-		+		-		-		-		+	36.56
179	+	40.96	+	42.49	-		+	35.86	+	39.07	+	33.91	-	-	-	-	-	-	+	39.27 37.67	+	42.28	+	31.7
186	-		-		+	35.45	-		-		+	35.03	-		-		-		-		-		-	
190	+	37.51	+	35.19	-	X	+	38.41	+	36.41	+	30.97	-	X	-	X	-	X	+	38.31	+	35.91	+	29.08

NR – Results not returned, NT – Not tested, W+ - Weak positive, Yellow denotes false negatives, Red denotes false positives

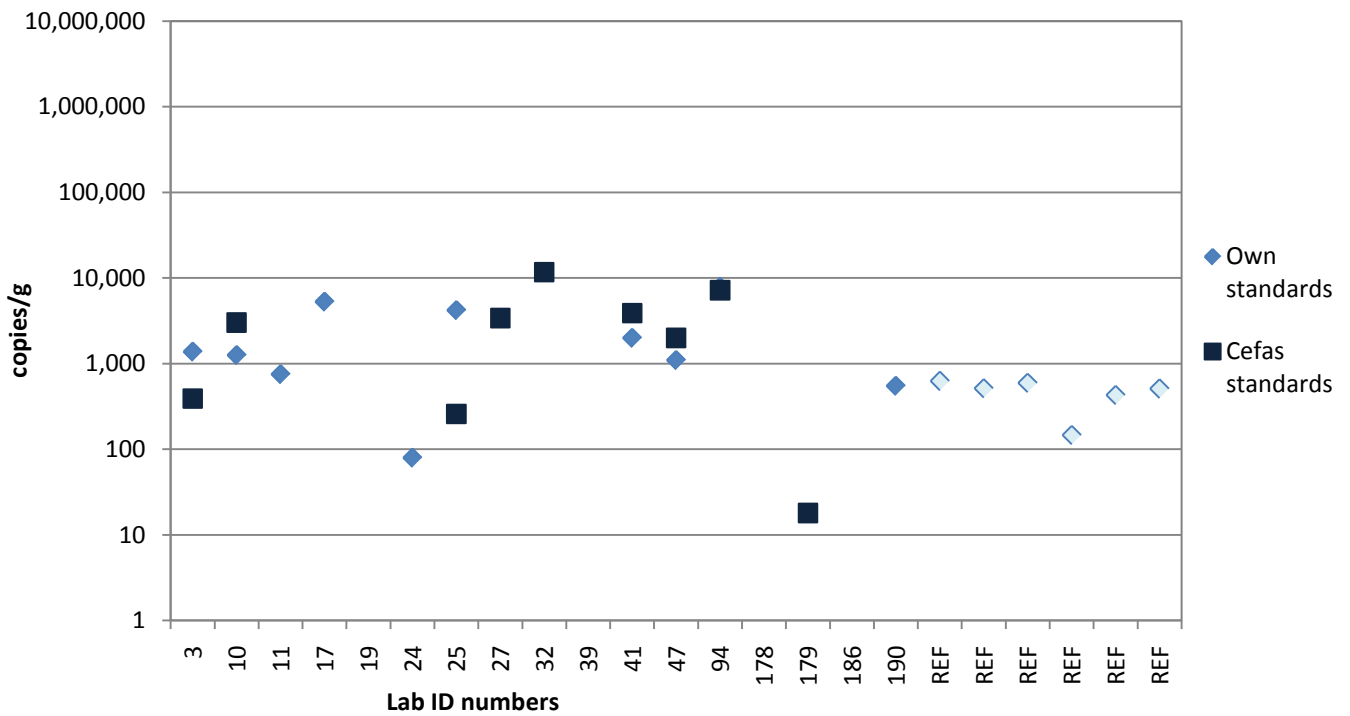
**Appendix IV:** Participants reported quantities for each target (copies/g (shellfish) or copies/LENTICULE)

Lab ID No.	Shellfish sample 1				Shellfish sample 2						LENTICULE 2					
	GI A	B	GII A	B	GI A	B	GII A	B	HAV A	B	GI A	B	GII A	B	HAV A	B
3	1.38x10 <sup>3</sup>	3.91x10 <sup>2</sup>	4.88x10 <sup>3</sup>	1.48x10 <sup>3</sup>	ND	ND	3.19x10 <sup>3</sup>	6.80x10 <sup>2</sup>	x	x	ND	4.90x10 <sup>1</sup>	4.74x10 <sup>2</sup>	2.04x10 <sup>2</sup>	x	x
10	1.26x10 <sup>3</sup>	3.02x10 <sup>3</sup>	1.89x10 <sup>3</sup>	1.29x10 <sup>4</sup>	6.77x10 <sup>3</sup>	1.71x10 <sup>4</sup>	1.90x10 <sup>3</sup>	1.30x10 <sup>4</sup>	7.95x10 <sup>4</sup>	7.90x10 <sup>6</sup>	1.86x10 <sup>3</sup>	4.54x10 <sup>3</sup>	2.57x10 <sup>2</sup>	1.89x10 <sup>3</sup>	5.24x10 <sup>4</sup>	5.22x10 <sup>6</sup>
11	7.52x10 <sup>2</sup>	x	2.18x10 <sup>4</sup>	x	3.35x10 <sup>4</sup>	x	1.98x10 <sup>4</sup>	x	1.11x10 <sup>4</sup>	x	2.37x10 <sup>3</sup>	x	2.85x10 <sup>3</sup>	x	4.30x10 <sup>3</sup>	x
17	5.30x10 <sup>3</sup>	x	2.80x10 <sup>3</sup>	x	1.70x10 <sup>4</sup>	x	6.90x10 <sup>3</sup>	x	5.70x10 <sup>4</sup>	x	4.30x10 <sup>3</sup>	x	7.50x10 <sup>2</sup>	x	1.40x10 <sup>4</sup>	x
19	x	ND	x	4.96x10 <sup>2</sup>	x	1.30x10 <sup>3</sup>	x	7.10x10 <sup>2</sup>	x	3.40x10 <sup>4</sup>	x	1.10x10 <sup>3</sup>	x	5.20x10 <sup>2</sup>	x	1.40x10 <sup>6</sup>
24	7.94x10 <sup>1</sup>	x	1.26x10 <sup>3</sup>	x	2.51x10 <sup>3</sup>	x	2.51x10 <sup>3</sup>	x	7.94x10 <sup>3</sup>	x	5.01x10 <sup>2</sup>	x	1.58x10 <sup>2</sup>	x	3.98x10 <sup>3</sup>	x
25	4.22x10 <sup>3</sup>	2.59x10 <sup>2</sup>	4.05x10 <sup>2</sup>	5.71x10 <sup>2</sup>	1.38x10 <sup>4</sup>	1.00x10 <sup>3</sup>	6.02x10 <sup>2</sup>	8.45x10 <sup>2</sup>	x	7.68x10 <sup>4</sup>	4.39x10 <sup>3</sup>	2.16x10 <sup>2</sup>	2.64x10 <sup>2</sup>	3.81x10 <sup>2</sup>	x	5.44x10 <sup>5</sup>
27	x	3.41x10 <sup>3</sup>	x	3.98x10 <sup>3</sup>	x	1.41x10 <sup>4</sup>	x	3.20x10 <sup>4</sup>	x	1.06x10 <sup>6</sup>	x	5.22x10 <sup>3</sup>	x	5.72x10 <sup>3</sup>	x	5.28x10 <sup>6</sup>
32	x	1.17x10 <sup>4</sup>	5.28x10 <sup>2</sup>	4.34x10 <sup>3</sup>	x	ND	9.70x10 <sup>2</sup>	7.33x10 <sup>3</sup>	x	9.65x10 <sup>5</sup>	x	1.09x10 <sup>2</sup>	6.10x10 <sup>1</sup>	2.04x10 <sup>3</sup>	x	1.56x10 <sup>6</sup>
39	x	ND	x	ND	x	4.90x10 <sup>2</sup>	x	5.10x10 <sup>2</sup>	x	x	x	3.10x10 <sup>2</sup>	x	6.80x10 <sup>1</sup>	x	x
41	2.00x10 <sup>3</sup>	3.90x10 <sup>3</sup>	5.70x10 <sup>2</sup>	8.20x10 <sup>3</sup>	4.40x10 <sup>3</sup>	8.50x10 <sup>3</sup>	1.10x10 <sup>2</sup>	9.30x10 <sup>2</sup>	3.30x10 <sup>6</sup>	1.70x10 <sup>7</sup>	8.50x10 <sup>2</sup>	2.50x10 <sup>3</sup>	1.30x10 <sup>3</sup>	1.80x10 <sup>3</sup>	3.50x10 <sup>4</sup>	3.30x10 <sup>6</sup>
47	1.10x10 <sup>3</sup>	2.00x10 <sup>3</sup>	5.50x10 <sup>2</sup>	2.10x10 <sup>3</sup>	8.80x10 <sup>2</sup>	1.70x10 <sup>3</sup>	1.30x10 <sup>3</sup>	6.40x10 <sup>3</sup>	x	2.70x10 <sup>5</sup>	2.50x10 <sup>2</sup>	5.00x10 <sup>2</sup>	4.60x10 <sup>2</sup>	1.30x10 <sup>3</sup>	x	9.00x10 <sup>5</sup>
94	7.90x10 <sup>3</sup>	7.20x10 <sup>3</sup>	8.10x10 <sup>3</sup>	5.10x10 <sup>3</sup>	5.80x10 <sup>3</sup>	5.10x10 <sup>3</sup>	5.40x10 <sup>2</sup>	5.10x10 <sup>2</sup>	8.90x10 <sup>4</sup>	x	9.10x10 <sup>2</sup>	6.40x10 <sup>2</sup>	7.60x10 <sup>4</sup>	1.10x10 <sup>4</sup>	3.60x10 <sup>5</sup>	x
178	ND	ND	ND	ND	ND	ND	ND	ND	4.09x10 <sup>5</sup>	x	ND	ND	ND	ND	4.78x10 <sup>5</sup>	x
179	x	1.80x10 <sup>1</sup>	x	9.10x10 <sup>3</sup>	x	5.70x10 <sup>3</sup>	x	1.90x10 <sup>3</sup>	x	1.70x10 <sup>5</sup>	x	5.00x10 <sup>2</sup>	x	1.00x10 <sup>4</sup>	x	4.50x10 <sup>5</sup>
186	ND	ND	ND	ND	ND	ND	ND	ND	x	1.54x10 <sup>3</sup>	ND	ND	ND	ND	ND	ND
190	5.50x10 <sup>2</sup>	x	1.70x10 <sup>3</sup>	x	2.90x10 <sup>2</sup>	x	6.70x10 <sup>2</sup>	x	2.90x10 <sup>5</sup>	x	1.20x10 <sup>2</sup>	x	3.90x10 <sup>2</sup>	x	4.30x10 <sup>5</sup>	x

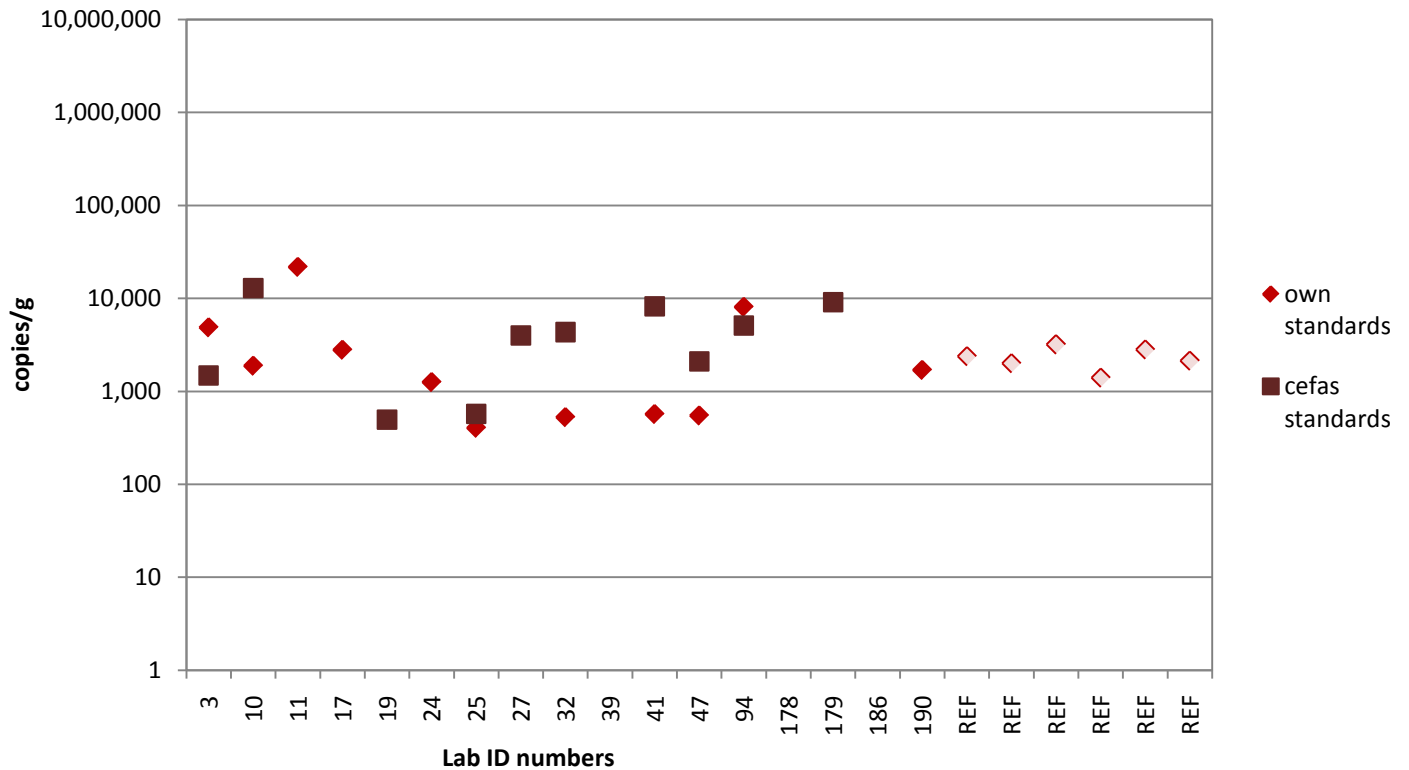
A - Quantity determined using routine method, B - Quantity determined using standard material provided, ND – not detected, x – not tested

**Appendix V:** Participants' and reference quantities for each target.

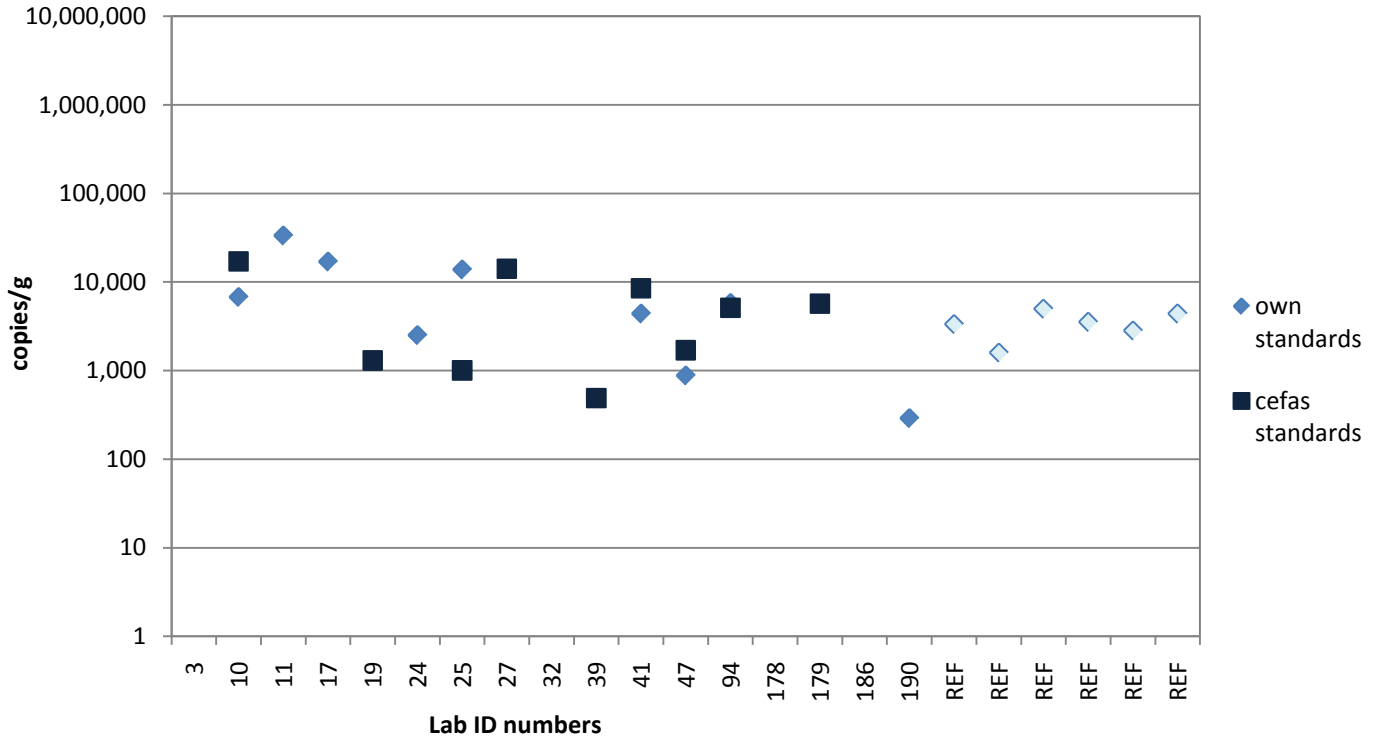
Shellfish sample 1 - GI



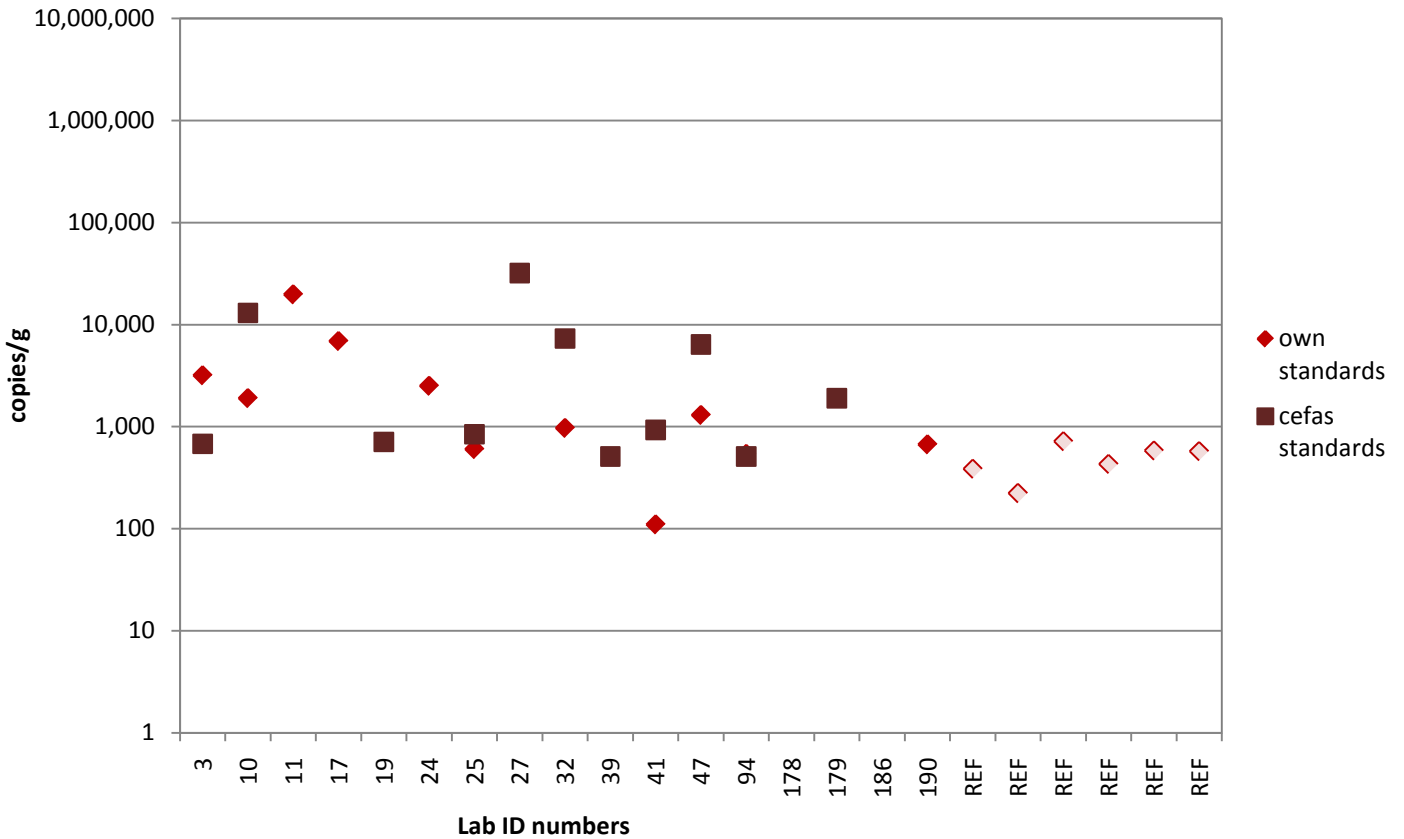
Shellfish sample 1 – GII



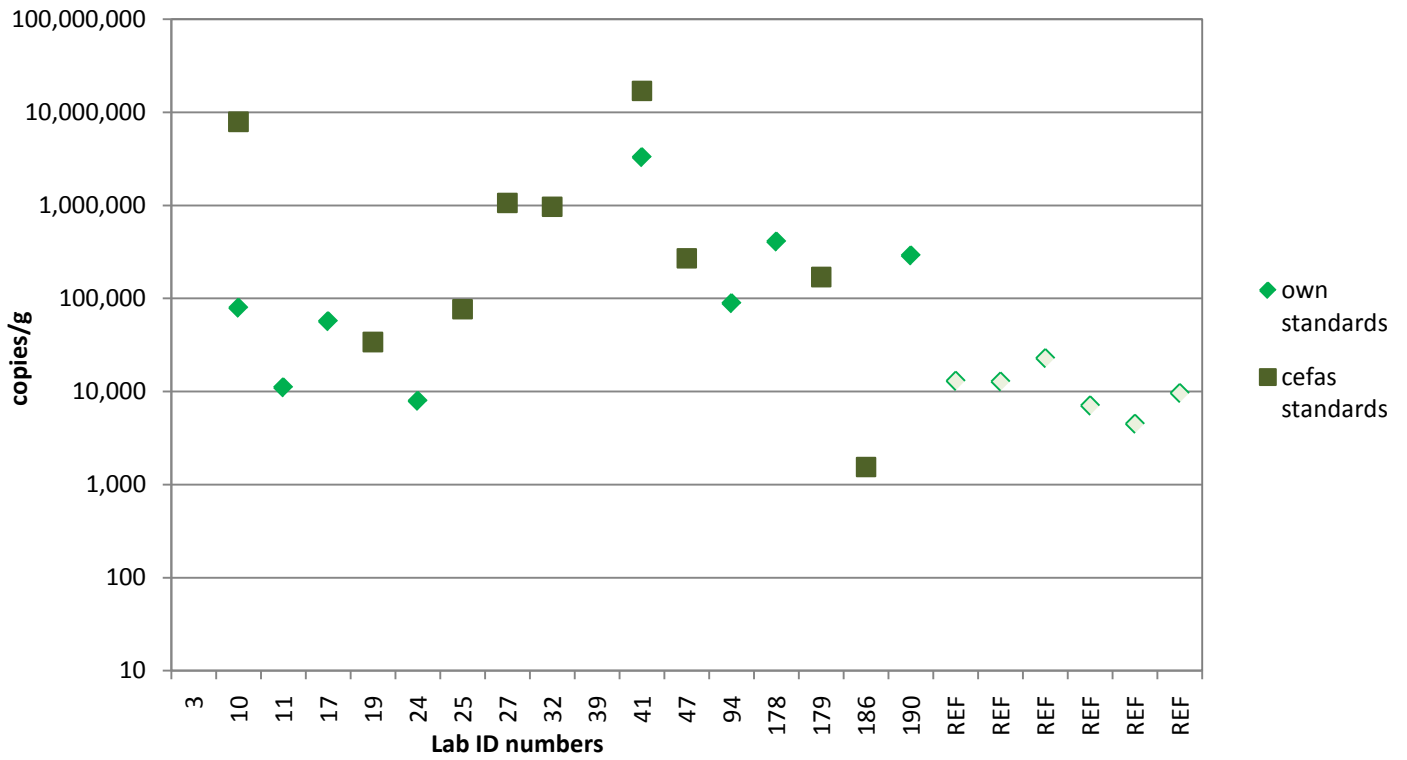
Shellfish Sample 2 – GI



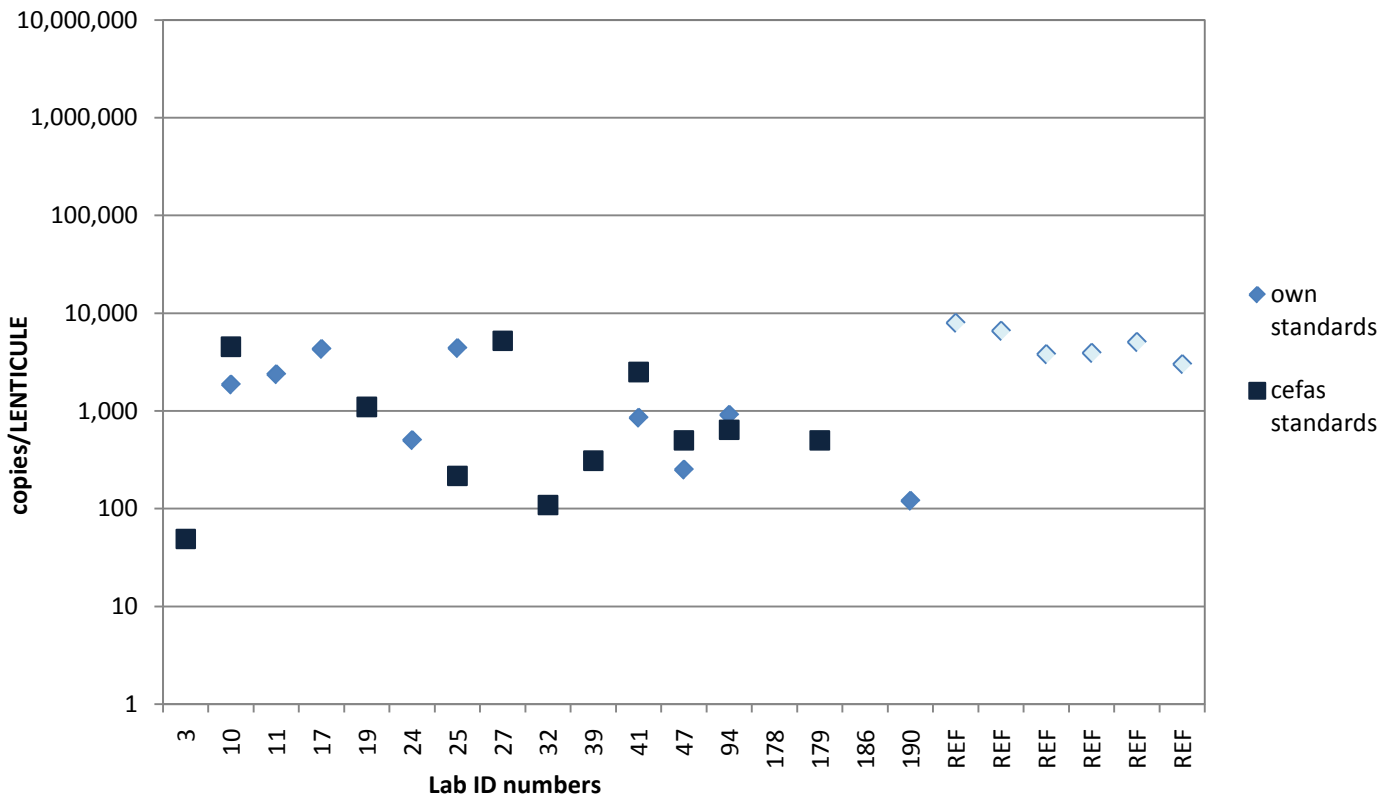
Shellfish Sample 2 – GII



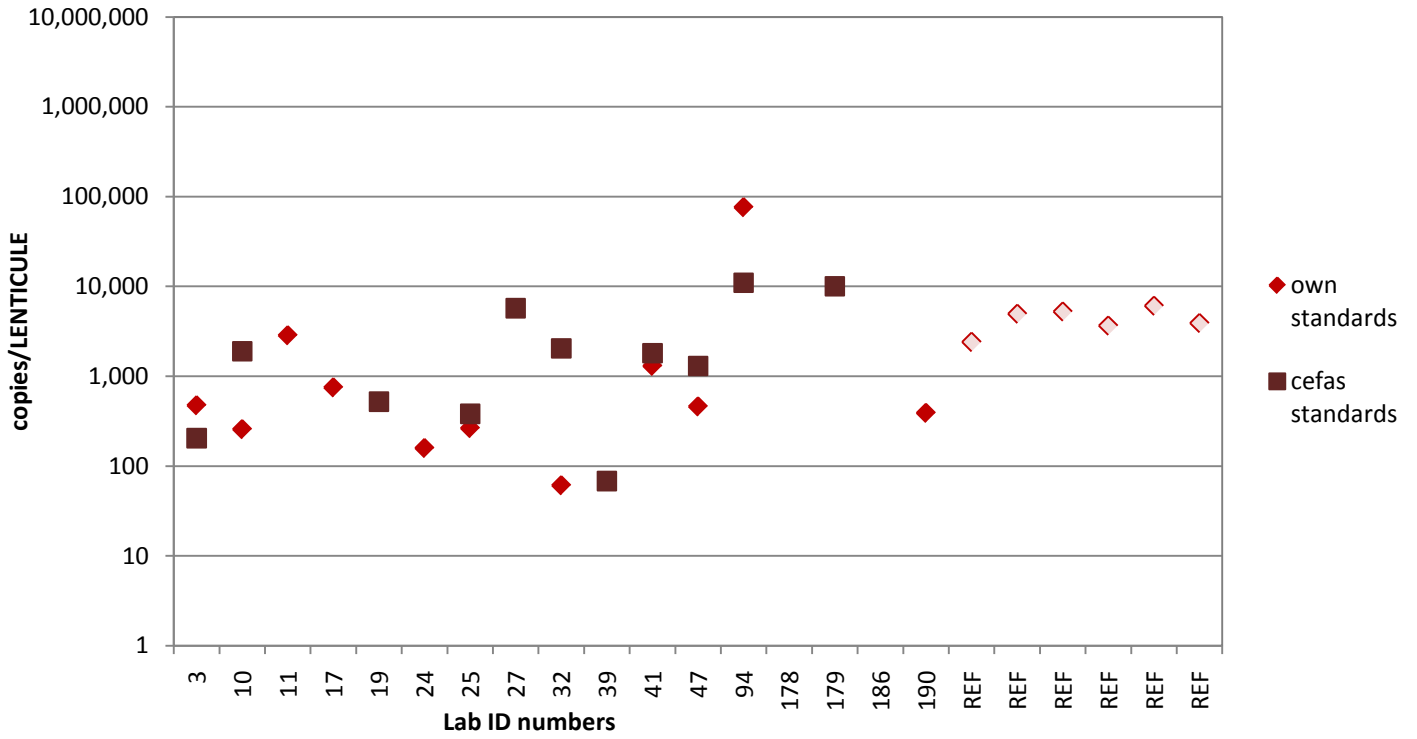
Shellfish Sample 2 - HAV



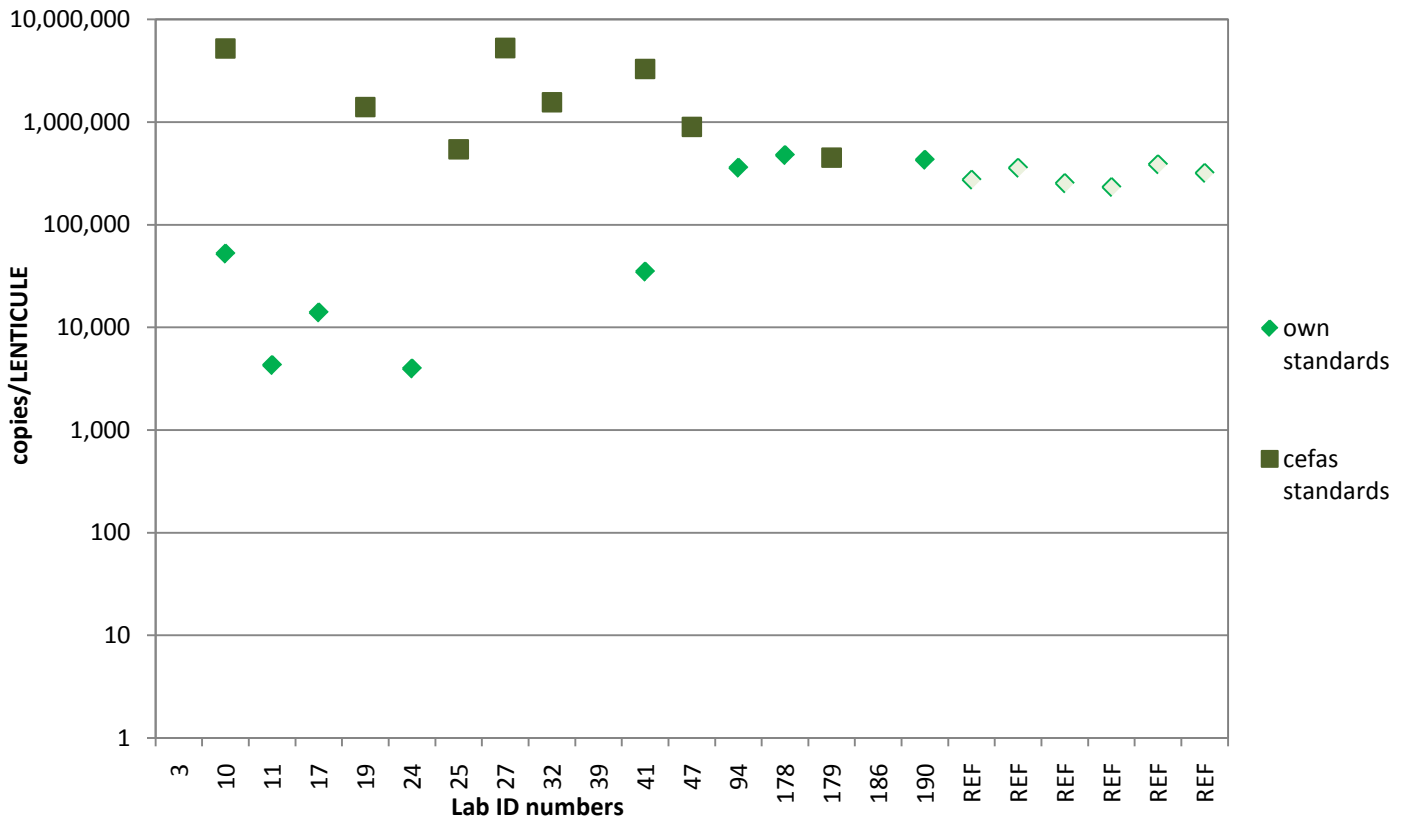
LENTICULE 2 - GI



LENTICULE 2 - GII



LENTICULE 2 - HAV



**Appendix VI: Results and methods used for shellfish samples.**

For key to method codes see next page.

LAB ID	ACC SF <sup>a</sup>	SF1			SF2			Virus extraction	RNA extraction	RT-PCR method <sup>b</sup>	RT-PCR reagents <sup>b</sup>	Primers		
		GI	GII	HAV	GI	GII	HAV					GI	GII	HAV
10	100.00%	+	+	-	+	+	+	A <sup>c</sup>	E	K	O	AA	CC	CC
11	100.00%	+	+	-	+	+	+	A	F	K	O	BB	BB	CC
17	100.00%	+	+	-	+	+	+	A	E	K	O	CC	CC	CC
24	100.00%	+	+	-	+	+	+	A	G	L	P	DD	BB	CC
25	100.00%	+	+	-	+	+	+	A	E	K	O	CC	CC	CC
27	100.00%	+	+	-	+	+	+	A	F	K	O	AA	CC	CC
41	100.00%	+	+	-	+	+	+	B	E	K	O	CC	CC	CC
47	100.00%	+	+	-	+	+	+	A	E	K	Q	CC	CC	CC
94	100.00%	+	+	-	+	+	+	B	E	K	R	EE	EE	EE
179	100.00%	+	+	-	+	+	+	A	E	K	Q	FF	CC	CC
190	100.00%	+	+	-	+	+	+	A	E	K	R	EE	EE	EE
19	83.33%	- <sup>d</sup>	+	-	+	+	+	A	E	K	O	CC	CC	CC
32	83.33%	+	+	-	-	+	+	A	E	K	O	CC	CC	CC
98	83.33%	+	+	-	+	-	+	A	E	K	R	EE	EE	EE
3	75.00%	+	+	NT <sup>e</sup>	-	+	NT	A	E	K	O	CC	CC	-
2	66.67%	-	+	-	-	+	+	B	H	M	S	BB	BB	JJ
7	66.67%	+	-	-	+	-	+	A	E	N	T	GG	GG	KK
39	66.67%	-	-	-	+	+	+	A	F	K	U	BB	BB	LL
178	66.67%	-	+	-	-	+	+	B	H	K	V	HH	BB	JJ
95	50.00%	-	-	-	+	-	+	A	F	L	W	BB	BB	MM
147	50.00%	-	+	-	-	-	+	A	F	L	X	CC	CC	EE
48	33.33%	-	-	-	-	-	+	B	H	K	S	II	II	CC
113	25.00%	-	-	NT	-	+	NT	C	F	K	S	BB	BB	-
186	16.67%	-	-	+	-	-	+	A	H	L	Y	CC	CC	CC
43	16.67%	-	-	-	-	-	-	D	J	K	S	BB	BB	LL

a - ACC SF = accuracy for shellfish samples 1 &amp; 2

b - where labs used different methods for different targets, method used with majority of targets shown

c - cells highlighted in grey indicate method taken or adapted from CEN bench protocols

d - cells highlighted in yellow indicate false negative results, cells highlighted in red indicate false positive results

e - NT = not tested



## Key to method codes

Virus extraction methods	
A	Proteinase K
B	Glycine buffer, PEG precipitation
C	Ultracentrifugation/choloroform extraction
D	MagNa Lyser Green Beads (Roche)
RNA extraction methods	
E	NucliSens Magnetic extraction reagents (BioMerieux)
F	QIAamp/Rneasy kits (Qiagen)
G	High Pure Viral Nucleic Acid (Roche)
H	Trizol/TriReagent methods
J	Total RNA Isolation NucleoSpin RNA II Kit (Macherey-Nagel)
RT-PCR methods	
K	real-time, one-step
L	real-time, two-step
M	conventional, one-step
N	conventional, two-step
RT-PCR reagents	
O	RNA Ultrasense (Invitrogen)
P	Superscript III & Platinum® qPCR SuperMix-UDG (Invitrogen)
Q	Quantitect (Qiagen)
R	ceeram Tools
S	OneStep RT-PCR kit (Qiagen)
T	MuLV & Ampli Taq Gold (Applied Biosystems)
U	SuperScript™ III Platinum® One-Step qRT-PCR System (Invitrogen)
V	Brilliant II QRT-PCR one-step kit (Agilent)
W	High Capacity cDNA RT Kit & ABI Taqman Universal Mastermix (Applied Biosystems)
X	Transcriptor high fidelity cDNA synthesis (Roche) & TaqMan Universal MasterMix (Applied Biosystems)
Y	Thermoscript RT-PCR (Invitrogen)
Primers/probes	
AA	Primers; CEN. Probe; Hohne and Schreier, 2006
BB	Kageyama et al., 2003
CC	CEN bench protocols
DD	Wolf et al., 2010
EE	ceeram Tools
FF	Primers; Ifremer (detailed in Lowther et al., 2010). Probe; Jothikumar et al., 2005
GG	Kojima et al., 2002
HH	Primers; Kageyama et al., 2003. Probe; Vega et al., 2011
II	Hohne and Schreier, 2006
JJ	Guevremont et al., 2006
KK	Bosch et al., 2000
LL	Cromeans et al., 1997
MM	Goswami et al., 1993

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### 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.co.uk](http://www.cefas.co.uk)

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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

