Livsmedelsverket
Swedish Food Agency

European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

Final REPORT

PROFICIENCY TESTING SCHEME EFV03, 2019

Quantification of norovirus and hepatitis A virus in bivalve molluscan shellfish

Final Report – Version 2 (2020/04/28)

Swedish Food Agency Reference: 2019/02376

Document approved by:	Magnus Simonsson	*Review date:*	2020-05-13
Document checked by:	Tommy Šlapokas	*Classification:*	Official
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Version 2 differs from version 1 in table 7, column 7, rows 22 and 23			

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INTRODUCTION

The Swedish Food Agency has been appointed European Union Reference Laboratory (EURL) for Foodborne Viruses according to Regulation (EU) 2017/625, since 2018. Under Article 94, the EURL is responsible for organizing Proficiency Tests (PTs) for the National Reference Laboratories (NRLs) for Foodborne Viruses. Participation in EURL PTs is mandatory for relevant NRLs in each Member State appointed in line with Regulation (EU) 2017/625.

This report describes the performance of NRLs for detection and enumeration of viral contamination of bivalve molluscan shellfish in PT scheme EFV03, organised by the EURL for Foodborne Viruses.

Distribution was made 5th of November 2019 to 24 laboratories that signed up to take part in the PT and was designed for the quantitative detection of hepatitis A virus (HAV) and norovirus genogroup I (GI) and genogroup II (GII) in three samples of frozen oyster hepatopancreas.

The participating laboratories were requested to examine the samples using their routine method, however the EURL recommended to analyse the samples according to ISO 15216-1. A Standard Operating Procedure (SOP) for quantitative detection of norovirus and hepatitis A virus in bivalve molluscan shellfish, based on ISO 15216-1, was therefore provided. External control (EC) RNA and process control virus were distributed together with PT sample to all the participants.

In order to ensure confidentiality, all participants are assigned a unique laboratory identification number. Only staff within the PT team and the laboratory itself have access to this ID. However, results from NRLs appointed in line with Regulation (EU) 2017/625 will be disclosed to DG SANTE for performance assessment.

SAMPLES

Materials dispatched consisted of artificially contaminated frozen oyster digestive glands inoculated with characterised norovirus GI and GII from human faecal material and HAV from cell culture supernatant. Detailed information of the viruses used for preparation of the samples is demonstrated in Table 1.

Table 1: Description of the viruses used for the PT EFV 03

Viruses	Origin	Strain ID/genotype
Hepatitis A virus	Cell culture supernatant	ATCC® VR-1402™ (HM 175/18f)
Norovirus genogroup I	Faecal material	GI.3 (capsid sequence)
Norovirus genogroup II	Faecal material	GII.4 (capsid sequence)

Sample A, B and C were spiked with approximately 10^5, 10^5 and 10^4 virus genome copies per 2 gram oyster digestive glands respectively. Concentration values are shown in Table 2.

Table 2: Spiking of PT EFV 02 samples

Sample	Norovirus GI	Norovirus GII	HAV
19EFV03 A	10^5 c/s*	–	–
19EFV03 B	–	10^5 c/s*	–
19EFV03 C	–	–	10^4 c/s*

* *Detectable virus genome copies spiked to each sample*

PREPARATION OF SAMPLES

Approximately 500 European oysters (Ostrea edulis) were purchased from a retail in Sweden. A homogenous mixture was prepared by shucking the oysters, separating the digestive glands, removing adipose tissues and finally blending and pooling the material together. The mixture was then divided in 2 gram aliquots and each aliquot was spiked with the target viruses and stored in -20° C for approximately 10 days before dispatch date.

DISTRIBUTION OF THE PROFIENCY TEST ITEMS

Samples were dispatched on dry ice by courier in accordance with IATA packing instructions 650 for UN3373, on November 5th. All 24 laboratories received three frozen samples, EC RNA, process control virus (mengovirus) and double stranded DNA standards. The standards were designed by the EURL and differ from standards previously distributed by Centre for Environment, Fisheries and Aquaculture Science (Cefas). The EURL standards have larger target sequence inserts, which provide a better flexibility for future primer designs. It is also expected that EC RNA produced from these plasmids are less prone to degradation.

Instruction sheet and results form were sent by email to the contact person(s) at each laboratory. The deadline for submitting the results was November 28th.

QUALITY CONTROL

Frozen oysters digestive glands used to produce the test items were tested negative for HAV, norovirus GI and norovirus GII. Spiked samples were examined for homogeneity and stability. Inhibition and extraction efficiency were acceptable for all the samples used for homogeneity and stability test.

STABILITY OF VIRUS LEVELS IN OYSTER SAMPLES

In order to investigate the stability of spiked viruses in samples stored in freezer, a study was conducted prior to dispatch. Thirty-six minced hepatopancreas samples (2 gram) were produced from approximately 220 oysters and were spiked with all target viruses (12 samples of each type). Six samples (two of each sample type) were analysed immediately after spiking (day -1) and the rest were kept on dry ice overnight. Six samples were tested directly after the overnight storage (day 0) and the rest of samples were stored in -20 °C and analysed at day 1, 6, 13 and 21.

The results showed that the level of detectable virus genome copies after 24 hours storage on dry ice (d0) was similar to the one before the storage. Moreover, virus levels had no tendency to decrease when stored at -20 °C.

REFERENCE RESULTS AND HOMOGENEITY OF VIRUS LEVELS IN OYSTER SAMPLES

In order to mimic realistic shipping conditions as well as storage conditions at the participating laboratories, ten samples each of 19EFV03A, 19EFV03B and 19EFV03C were stored on dry ice on the dispatch date (November 5th 2019) for 24 hours. Two samples of each were tested directly the day after (day 0), and the rest of samples were stored in -20 °C and tested at day 2, 5, 12 and 13. Samples were analysed according to EURL SOP based on ISO 15216-1 for the quantification of target viruses respectively. The results are shown in Table 3, with box and whisker plots included in Graph 1. The results of day 5 were used in performance assessment and scoring presented later in this report.

Table 3: Quantitative results for ten reference samples for PT EFV 03

Ranges based on a 95 % confidence limit determined as two geometric standard deviations above and below the geometric mean.

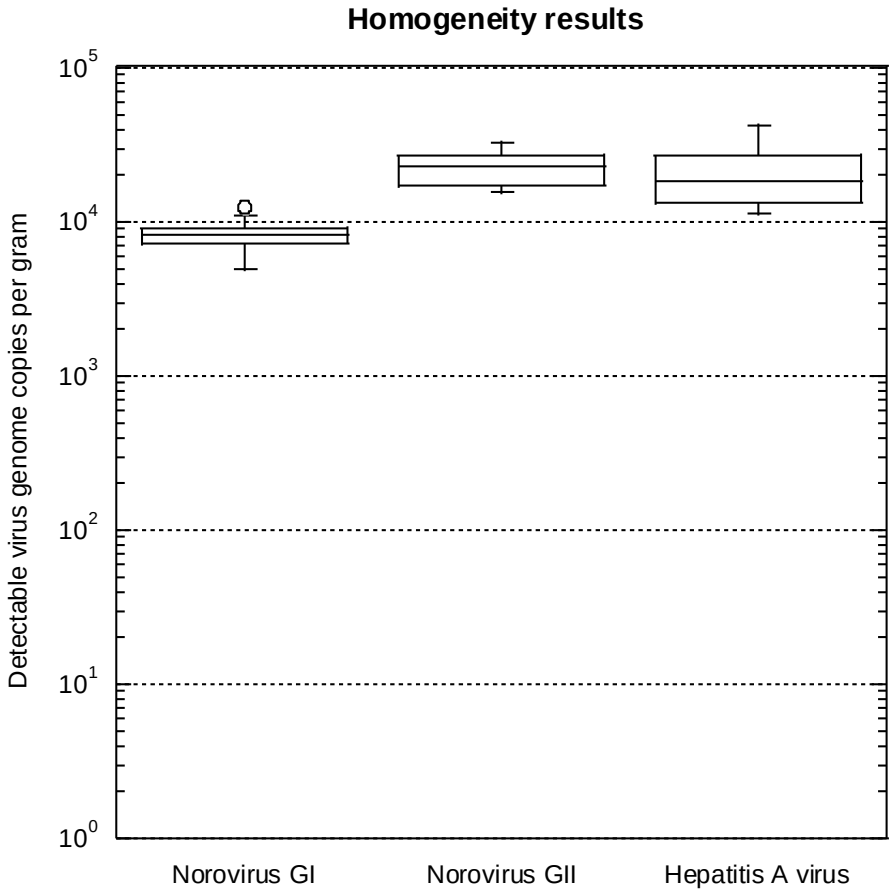
Sample	Norovirus GI	Norovirus GII	HAV**
19EFV03 A	4.9 x 10^3 – 1.4 x 10^4 c/g*	not detected	not detected
19EFV03 B	not detected	1.3 x 10^4 – 3.8 x 10^4 c/g*	not detected
19EFV03 C	not detected	not detected	2.1 x 10^3 – 8.9 x 10^4 c/g*

* *detectable virus genome copies per gram sample*

***results for HAV day 12 are not included due to problems during the extraction procedure.*

Graph 1: Box and whisker plots for homogeneity test of samples 19EFV03 A, B and C

The box includes 50 % of the results from 10 samples for samples A and B and 8 samples for C (samples 6 and 7 were excluded due to problems that occurred during the extraction). 25 % of the results set above the median, 25 % of the results set below the median and the remaining 50 % are illustrated by lines outside the box. A circle in the plot indicates a value that deviates from the other values but is not defined as an outlier.



The assessment of homogeneity (presented in Annex C) is in principle based on ISO 13528:2015 (Statistical methods for use in proficiency testing of interlaboratory comparison), by use of analysis of variance (ANOVA) and further steps. The homogeneity test was not performed under repeatability conditions, since it was not possible to analyse all the samples made for the homogeneity test at one occasion and at the same time.

As there are no previous values of standard deviation for proficiency assessment (σ_{pt}) available for virus types used in the current PT, the principles of point d in clause B.2.4 of Annex B in the standard are applied. This means that the check of homogeneity against criteria is performed by use of the consensus standard deviation (SD) from the participant results. The SD for each virus type is obtained as the robust standard deviation by application of Algorithm A (Huber's method) according to Annex C, clause C.3.1 in the standard. The SD values obtained are used as tentative values of σ_{pt}, to be compared to values in coming PT schemes. The values of SD used as σ_{pt} were 0.33, 0.35 and 0.83, for Norovirus GI, Norovirus GII and HAV, respectively. These values were used to determine two criteria

to check if the between sample standard deviation from ANOVA (s_s) represent homogenous samples. This was done according to ISO 13528, Annex C, clauses B.2.2 and B.2.3. At least one of the two criteria should be fulfilled to consider the samples to be homogeneous. The outcome is given in Table 4 showing that all samples were homogenous using the above indicated σ_{pt} values, at least according to criterion 2. Other values of σ_{pt} are also shown in the table as a comparison to indicate where the limits for satisfaction of the criteria are.

The two homogeneity criteria used where

1. σ_{pt} (the standard deviation for proficiency testing) is compared with s_s (the between sample standard deviation from the ANOVA). The samples are regarded as homogeneous when $s_s < 0.3*\sigma_{pt}$ according to clause B.2.2 of ISO 13528, Annex B.
2. s_s is compared with $\sqrt{c}$; the samples are regarded as homogeneous when $s_s < \sqrt{c}$ according to clause B.2.3 of ISO 13528, Annex B; this criterion is the least conservative one.

Table 4: Homogeneity test

Virus type	σ_{pt}	Homogenous? $s_s < 0.3*\sigma_{pt}$	Homogenous? $s_s < \sqrt{c}$
GI	0,20	no	No
	0,25	no	Yes
	0,30	no	Yes
	0,33	**no**	**Yes**
	0,35	yes	Yes
GII	0,20	no	No
	0,25	no	Yes
	0,30	no	Yes
	0,35	**no**	**Yes**
	0,37	yes	Yes
HAV	0,45	no	No
	0,50	no	Yes
	0,55	no	Yes
	0,60	no	Yes
	0,65	no	Yes
	0,705	no	Yes
	0,75	yes	Yes
	0,80	yes	Yes
	0,83	**yes**	**Yes**

σ_{pt}: standard deviation for proficiency testing, **s_s**: the between sample standard deviation from the ANOVA that is compared with **$3*\sigma_{pt}$** as well as with **$\sqrt{c}$** according to ISO 13528, Annex B; figures in bold are the consensus values of **σ_{pt}** from participant results; yellow indicate homogeneity according to one criterion and green fields indicate homogeneity of the samples according to both criteria.

RESULTS AND DISCUSSION

Twenty-four laboratories, including 19 NRLs participated in the current PT and all except one returned their results.

Despite the fact that only one false positive result was reported, some of the true negative results were actually not valid due to unacceptable inhibition and/or extraction efficiency. Since re-testing was not possible, such non-valid results were accounted as correct in the scoring of participants. Overview of results are demonstrated in Table 5.

One laboratory (nr.108) only reported qualitative results for all the target viruses. Two laboratories (nr.122 and nr.126) did not report quantitative detection results for HAV (those laboratories do not perform HAV quantification). Another laboratory (nr.115) reported that norovirus GI in sample A was not quantifiable due to low extraction efficiency. One laboratory (nr.110) reported low efficiency in all samples and therefore their quantification results were excluded from the scoring.

The results show that HAV results generated from EURL standard are around $1\log_{10}$ higher than HAV results generated by participant's own standard. The cause/causes behind this difference will be investigated by the EURL in the near future. However, it stresses the importance of standardised reference material.

The results of references samples analysed at day 5 (assumed to be the closest analysis date to the majority of participants) are presented as Ref. Detailed information about the participating laboratories results can be found in Annex A.

Table 5: Overview of participants´ results for samples 19EFV03 A, B and C

Target viruses	N	Sample 19EFV03 A				Sample 19EFV03 B				Sample 19EFV03 C			
		T	FP	FN	NV	T	FP	FN	NV	T	FP	FN	NV
Norovirus GI	23	23	-	0	0	23	0	-	2	23	0	-	4
Norovirus GII	23	22	1	-	4	23	-	0	0	23	0	-	4
Hepatitis A virus	23	23	0	-	4	23	0	-	2	23	-	0	0

N: Number of laboratories that reported results for the analysis, T: true results, FP: False positive, FN: False negative, NV: Not valid negative results, -: not possible outcome

PERFORMANCE ASSESMENT

All the results were firstly assessed as presence–absence data in concordance with intended results as followed:

- 2 points: correct result for each target virus, regardless valid or non-valid results for negative samples.
- 0 points: Incorrect results for each target virus

The maximum score for each laboratory (for each target virus), taking into account the results of all three samples is therefore six (Table 7).

In order to asses a comparison of the quantitative results and provide a tool to laboratories when following up their results, all the results were converted to scores. Average and standard deviation is obtained as the robust average and robust standard deviation by application of Algorithm A (Huber's method) according to Annex C, clause C.3.1 in ISO 13528:2015 and are presented in Table 6.

Table 6: Calculated data used for scoring assessment

Quantity	19EFV03 A	19EFV03 B	19EFV03 C
Average	4,177	4,435	4,094
SD	0,411	0,358	0,717

-Values in log10 copies/g

- The results of references samples analysed at day 5 are included

Since all the laboratories received EURL quantification standards together with PT materials, some participants provided two sets of results determined by both EURL and their own standards. In such cases, only the results using their own standards were considered for performance scoring, since it is part of the laboratories own routine. An exception was made in case of lab 128, which only indicated Cq values when reporting own standards results. However, all the required results were reported with the use of EURL standards. In this case, results from EURL standards were used for the quantitative results assessment and scoring. In Graphs 2, 3 and 4 all participants' results are presented.

The results for intended positive results were assessed and scored as followed:

- 2 points: Satisfactory - Difference between result and participants' average (absolute value)<2 SD
  True negative results
- 1 point: Questionable – 2 SD <Difference between result and participants' average (absolute value) ≤3 SD
  Non-valid true positive results reported as unquantifiable
- 0 points: Unsatisfactory - Difference between result and participants' average (absolute value) >3 SD
  False positive results
  False negative results

The maximum score for each laboratory (for each target virus), taking into account the results of all three samples is therefore six (Table 7).

The results of references samples analysed at day 5 were included in the score calculations and are presented as Ref. in Annex B as well as the score Graphs 2, 3 and 4.

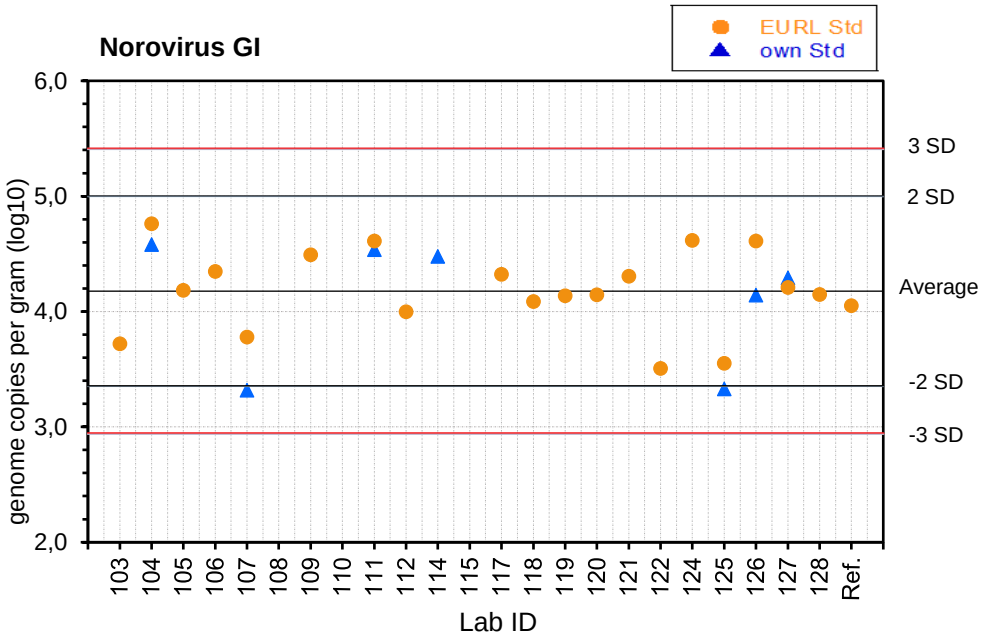
Table 7: Calculated data used for scoring assessment

	Presence/absence			Quantitative		
Lab ID	GI	GII	HAV	GI	GII	HAV
103	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
104*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
105*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
106*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
107*	6 out of 6	6 out of 6	6 out of 6	5 out of 6	6 out of 6	6 out of 6
108*	6 out of 6	6 out of 6	6 out of 6	NE[1]	NE[1]	NE[1]
109*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
110*	6 out of 6	6 out of 6	6 out of 6	NQ	NQ	NQ
111*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
112*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
114*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
115*	6 out of 6	6 out of 6	6 out of 6	4 out of 4[nq]	6 out of 6	6 out of 6
117*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
118*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
119	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
120	6 out of 6	4 out of 6[fp]	6 out of 6	6 out of 6	4 out of 6	6 out of 6
121*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
122*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	NE[2]
124*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
125	6 out of 6	6 out of 6	6 out of 6	5 out of 6	5 out of 6	6 out of 6
126*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	NE[2]
127*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
128*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6

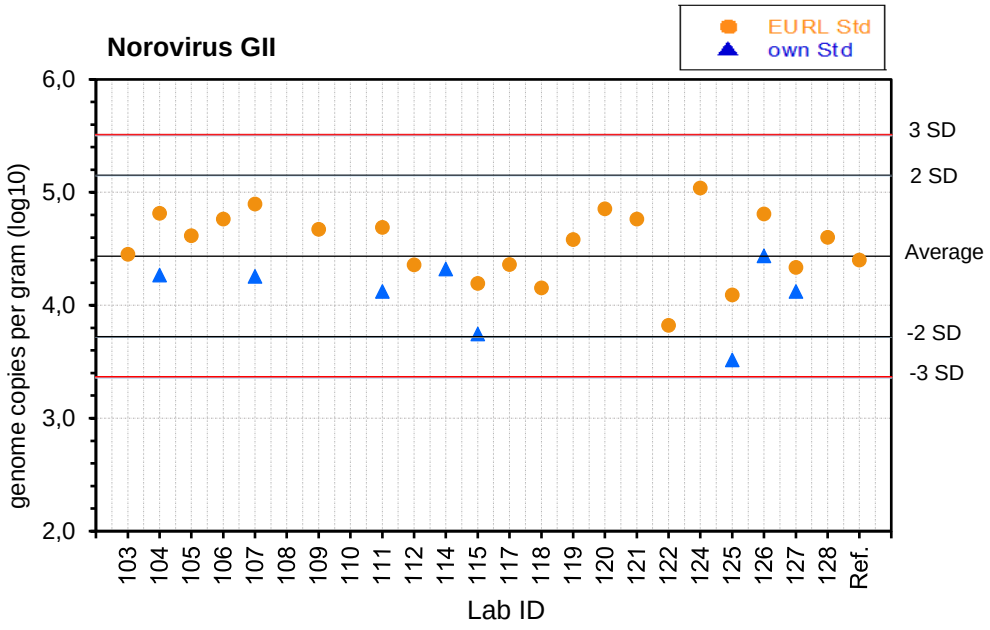
** Designated EU/EFTA member state NRL*

[fp]: false positive, [nq]: reported as not quantifiable in one sample and therefore excluded from scoring, NQ: not quantifiable, NE: not examined ([1]: did not perform any quantification, [2]: do not perform quantification for HAV)

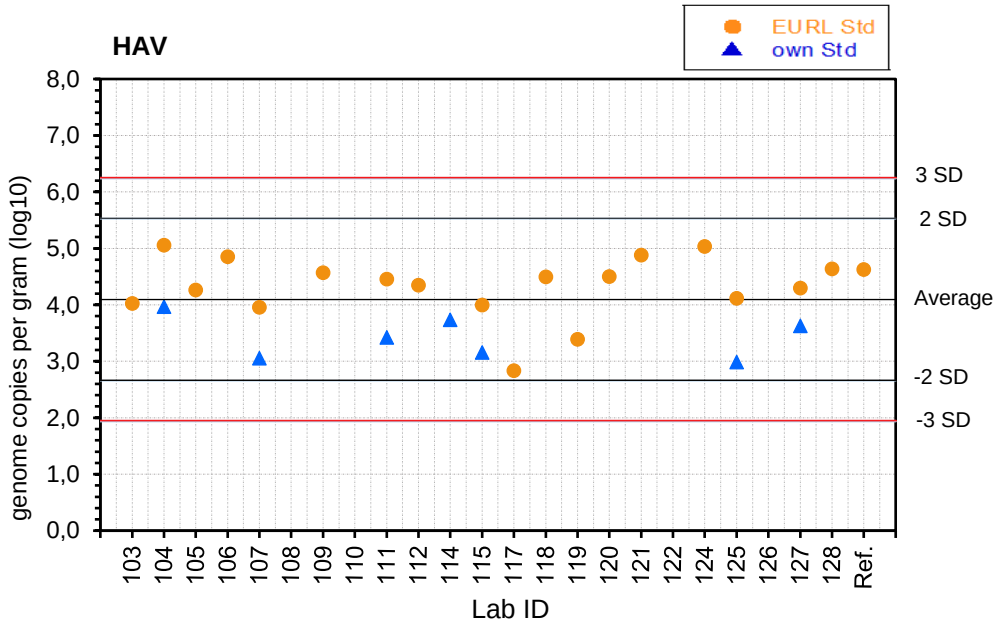
Graph 2: Distribution of results for norovirus GI in 19EFV03A



Graph 3: Distribution of results for norovirus GII in 19EFV03B



Graph 4: Distribution of results for HAV in 19EFV03C



INHIBITION and EFFICIENCY RESULTS

The results were also evaluated based on inhibition and extraction efficiency outcomes. Since it was not possible to provide the laboratories with a retest option, this evaluation is not a part of performance assessment and scoring (except for true positive results, which were not quantifiable due to unacceptable inhibition and/or extraction efficiency). However, it can provide a guidance for valid reporting in official control according to ISO 15216-1.

All the laboratories with the exception of one (only for norovirus GI in sample A) reported acceptable inhibition results (≤75 %). However, one laboratory failed to report the inhibition results for sample C. The majority of laboratories, 18 out of 23, reported acceptable extraction efficiency values (≥1 %). According to ISO 15216-1, negative results are not valid in absence of inhibition or/and extraction efficiency values as well as in case of unacceptable inhibition or/and extraction efficiency results and shall be reported as invalid. Positive results on the other hand could be considered valid despite unacceptable inhibition and extraction efficiency results and shall be reported as "virus genome detected in (the amount of sample tested) g followed by "not quantifiable". All the results reported for norovirus GI in sample A, norovirus GII in sample B and hepatitis A virus in sample C are valid regardless the inhibition and extraction efficiency values, since the respective samples were positive for the respective target viruses. Only one laboratory reported their invalid positive results correctly according to ISO 15216-1 and as not quantifiable (lab 115). If a valid result is not obtained, results shall be expressed as invalid. However, the participants were not asked to report the results according to ISO 15216-1. Such results were excluded from the calculation of participants' mean results. Results are presented in Annex C.

METHODS USED BY THE PARTICIPANTS

Ten laboratories were accredited according to ISO/IEC 17025-2 for quantitative detection of norovirus GI, norovirus GII and eight for HAV and the majority followed ISO 15216-1. One laboratory used an in house method adapted from ISO 15216 and one laboratory applied a modified version of ISO 15216-1. Detailed information on the methodologies used is shown in Annex D.

CONCLUSION

The aim of PT EFV03 organized by EURL for Foodborne Viruses was to assess the NRLs capabilities for quantitative detection of HAV, norovirus GI and norovirus GII in frozen minced oyster hepatopancreas samples.

Twenty-three laboratories participated in the PT and the majority of the participating laboratories obtained satisfactory results. Moreover, the majority of laboratories correctly reported the inhibition and extraction efficiency values. The proportion of valid results reported has been improved compared to previous PT distributions.

ANNEX A

Participants' results

with EURL standards, with own standards, false results

Lab. ID No.	19EFV03 A				19EFV03 B				19EFV03 C			
	GI (Cq)	GI (c/g)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	GII (c/g)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	HAV (c/g)
103	31,48	5,23E+03				29,8	2,83E+04				33,62	1,05E+04
104*	31,41/31,2	5,75E+04				31,42/31,88	6,51E+04				30,35	1,13E+05
104*	31,41/31,2	3,80E+04				31,42/31,88	1,85E+04				30,35	9,30E+03
105*	31,51	1,52E+04				28,9	4,14E+04				34,3	1,82E+04
106*	31,96	2,22E+04				32,25	5,80E+04				33,56	7,06E+04
107*	29,44	5,99E+03				26,82	7,88E+04				31,95	8,98E+03
107*		9,98E+03[d]					1,32E+04					2,15E+03
107*	29,44	2,07E+03				26,82	1,81E+04				31,95	1,13E+03
107*		9,98E+03[d]				26,82	1,32E+04					2,15E+03
108*	27,46					26,82					29,93	
109*	30,48	3,10E+04				29,29	4,70E+04				32,57	3,70E+04
110*	32,63	7,20E+03				30,95	1,70E+04				36,15	1,80E+03
110*	32,63	2,00E+04				30,95	2,30E+04				36,15	4,50E+02
111*	31,67/31,61	4,06E+04				31,63/31,73	4,88E+04				33,5418	2,85E+04
111*	31,67/31,61	3,43E+04				31,63/31,73	1,33E+04				33,5418	2,63E+03
112*	32,91/33,02	9,96E+03				31,92/32,26	2,27E+04				34,99	2,22E+04
114*	31,34/31,2	3,00E+04	45	45	45	30,15/30,03	2,10E+04	45	45	45	34,26	5,40E+03
115*	32,75	NQ				32,60	1,56E+04				34,83	9,87E+03
115*	32,75	NQ				32,60	5,58E+03				34,83	1,42E+03

** Designated EU/EFTA member state NRL, d: dd PCR*

Lab. ID No.	19EFV03 A				19EFV03 B				19EFV03 C			
	GI (Cq)	GI (c/g)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	GII (c/g)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	HAV (c/g)
117*	28,4	2,10E+04				28,95	2,30E+04				32,97	6,80E+02
118*	33,5	1,22E+04				34,39	1,43E+04				33,86	3,10E+04
119	31,92	1,37E+04				29,79	3,83E+04				35,94	2,45E+03
120	32,29	1,39E+04	41,53			29,78	7,14E+04				32,99	3,14E+04
121*	34,21	2,02E+04				30,65/30,52	5,80E+04				33,58	7,57E+04
122*	40,26	3,21E+03				36,07	6,63E+03				29,25	
124*	31,61	4,14E+04				31,16	1,09E+05				32,83	1,08E+05
125	31,9	3,54E+03				31,25	1,24E+04				33,33	1,31E+04
125	31,9	2,12E+03				31,25	3,28E+03				33,33	9,72E+02
126*	32,32	4,07E+04				30,86	6,42E+04				32,86	
126*	32,32	1,39E+04				30,86	2,74E+04				32,86	
127*	30,02	1,61E+04				29,76	2,16E+04				31,97	1,97E+04
127*	30,02	1,96E+04				29,76	1,33E+04				31,97	4,23E+03
128*	32,18	1,40E+04				32,72	4,00E+04				33,82	4,30E+04
128*	32,22					41,39					37,02	
Ref.**	30,99	1,12E+04				29,35	2,51E+04				33,39	4,18E+04

** Designated EU/EFTA member state NRL, ** Reference results from day 5*

ANNEX B

Differences between participants' results and the participants' mean presented in terms of SD

All the laboratories received EURL quantification standards together with PT materials, therefore some participants provided two sets of results determined by both EURL and their own standards. In such cases, only the results using their own standards were considered for performance scoring. However, all the results are presented in the table.

2 SD< ≤3 SD, -3 SD≤ <-2 SD, >3 SD, <-3 SD

Lab ID	19EFV03 A- GI		19EFV03 B- GII		19EFV03 C- HAV	
	EURL standard	Own standard	EURL standard	Own standard	EURL standard	Own standard
103	-1,101		0,072		0,013	
104*	1,429	0,992	1,084	-0,444	1,450	-0,062
105*	0,025		0,535		0,345	
106*	0,424		0,944		1,166	
107*	-0,958	-2,080	1,316	-0,470	-0,083	-1,340
108*	NE	NE	NE	NE	NE	NE
109*	0,777		0,689		0,774	
110*	NQnv	NQnv	NQnv	NQnv	NQnv	NQnv
111*	1,062	0,884	0,734	-0,845	0,616	-0,828
112*	-0,422		-0,195		0,464	
114*		0,742		-0,290		-0,391
115*	NQ	NQ	-0,651	-1,900	-0,026	-1,199
117*	0,366		-0,179		-1,646	
118*	-0,207		-0,757		0,667	
119	-0,085		0,440		-0,869	
120	-0,070		1,197		0,675	
121*	0,325		0,944		1,208	
122*	-1,617		-1,690		NQR	NQR
124*	1,082		1,710		1,421	
125	-1,513	-2,054	-0,930	-2,545	0,145	-1,430
126*	1,064	-0,070	1,067	0,033	NQR	NQR
127*	0,085	0,293	-0,845	-0,256	-0,539	0,393
128*	-0,062		0,493		0,865	
Ref.	-0,298		-0,073		0,848	

** Designated EU/EFTA member state NRL, NQ = results reported as non-quantifiable, therefore excluded from scoring, NE: not examined for quantification, NQR = no quantification results reported, NQnv= the reported results were excluded by EURL since they were not valid and should have been reported as non-quantifiable.*

ANNEX C

Inhibition and extraction efficiency results for sample 19EFV03A

	Inhibition			Efficiency	Valid/ Not valid Presence/absence			Valid/Not valid Quantitative		
Lab. ID	GI[t]	GII	HAV		GI[t]	GII	HAV	GI[t]	GII	HAV
103	A	A	A	A	V	V	V	V	V	V
104*	A	A	A	A	V	V	V	V	V	V
105*	A	A	A	A	V	V	V	V	V	V
106*	A	A	A	A	V	V	V	V	V	V
107*	A	A	A	A	V	V	V	V	V	V
108*	A	A	A	U	V	NV	NV	NV	NV	NV
109*	A	A	A	A	V	V	V	V	V	V
110*	A	A	A	U	V	NV	NV	NV	NV	NV
111*	A	A	A	A	V	V	V	V	V	V
112*	A	A	A	A	V	V	V	V	V	V
114*	A	A	A	A	V	V	V	V	V	V
115*	A	U	A	U	V	NV	NV	NV	NV	V
117*	A	A	A	A	V	V	V	V	V	V
118*	A	A	A	A	V	V	V	V	V	V
119	A	A	A	A	V	V	V	V	V	V
120	A	A[f]	A	A	V	V	V	V	V	V
121*	A	A	A	A	V	V	V	V	V	V
122*	A	A	A	A	V	V	V	V	V	V
124*	A	A	A	A	V	V	V	V	V	V
125	A	A	A	A	V	V	V	V	V	V
126*	A	A	A	A	V	V	V	V	V	V
127*	A	A	A	A	V	V	V	V	V	V
128*	A	A	A	A	V	V	V	V	V	V

** Designated EU/EFTA member state NRL*

A: Acceptable, f: false results, NR: not reported, NV: not valid, t: target virus, U: Unacceptable V: valid results

Inhibition and extraction efficiency results for sample 19EFV03B

	Inhibition			Efficiency	Valid/ Not valid Presence/absence			Valid/Not valid Quantitative		
Lab. ID	GI	GII[t]	HAV		GI	GII[t]	HAV	GI	GII[t]	HAV
103	A	A	A	A	V	V	V	V	V	V
104*	A	A	A	A	V	V	V	V	V	V
105*	A	A	A	A	V	V	V	V	V	V
106*	A	A	A	A	V	V	V	V	V	V
107*	A	A	A	A	V	V	V	V	V	V
108*	A	A	A	A	V	V	V	V	V	V
109*	A	A	A	A	V	V	V	V	V	V
110*	A	A	A	U	NV	V	NV	NV	NV	NV
111*	A	A	A	A	V	V	V	V	V	V
112*	A	A	A	A	V	V	V	V	V	V
114*	A	A	A	A	V	V	V	V	V	V
115*	A	A	A	A	V	V	V	V	V	V
117*	A	A	A	A	V	V	V	V	V	V
118*	A	A	A	A	V	V	V	V	V	V
119	A	A	A	A	V	V	V	V	V	V
120	A	A	A	A	V	V	V	V	V	V
121*	A	A	A	A	V	V	V	V	V	V
122*	A	A	A	A	V	V	V	V	V	V
124*	A	A	A	A	V	V	V	V	V	V
125	A	A	A	A	V	V	V	V	V	V
126*	A	A	A	A	V	V	V	V	V	V
127*	A	A	A	A	V	V	V	V	V	V
128*	A	A	A	A	V	V	V	V	V	V

** Designated EU/EFTA member state NRL*

A: Acceptable, f: false results, NR: not reported, NV: not valid, t: target virus, U: Unacceptable V: valid results

Inhibition and extraction efficiency results for sample 19EFV03C

	Inhibition			Efficiency	Valid/ Not valid Presence/absence			Valid/Not valid Quantitative		
Lab. ID	GI	GII	HAV^t		GI	GII	HAV^t	GI	GII	HAV^t
103	A	A	A	A	V	V	V	V	V	V
104*	A	A	A	A	V	V	V	V	V	V
105*	A	A	A	A	V	V	V	V	V	V
106*	A	A	A	A	V	V	V	V	V	V
107*	A	A	A	A	V	V	V	V	V	V
108*	A	A	A	U	NV	NV	V	NV	NV	NV
109*	A	A	A	A	V	V	V	V	V	V
110*	A	A	A	U	NV	NV	V	NV	NV	NV
111*	A	A	A	A	V	V	V	V	V	V
112*	A	A	A	A	V	V	V	V	V	V
114*	A	A	A	A	V	V	V	V	V	V
115*	A	A	A	A	V	V	V	V	V	V
117*	A	A	A	A	V	V	V	V	V	V
118*	A	A	A	A	V	V	V	V	V	V
119	A	A	A	A	V	V	V	V	V	V
120	A	A	A	A	V	V	V	V	V	V
121*	A	A	A	A	V	V	V	V	V	V
122*	NR	NR	NR	NR	NV	NV	V	NV	NV	NV
124*	A	A	A	A	V	V	V	V	V	V
125	A	A	A	A	V	V	V	V	V	V
126*	A	A	A	A	V	V	V	V	V	V
127*	A	A	A	A	V	V	V	V	V	V
128*	A	A	A	A	V	V	V	V	V	V

** Designated EU/EFTA member state NRL*

A: Acceptable, f: false results, NR: not reported, NV: not valid, t: target virus, U: Unacceptable V: valid results

ANNEX D

General information on methods

Lab. ID No.	1	2	3	4	5	6
103	A	D	H	J	R	
104*	A	D	H	J	R	U,V
105*	A	D	H	J	R	U,V
106*	A	D	H	J	R	
107*	A	D, E	H,I	K,Q	S	U,V
108*	B	D	H	L	T	
109*	A	D	H	J	R	U,V
110*	A	F	H	M	R	
111*	A	D	H	N	R	
112*	A	D	H	J	R	
114*	A	D	H	J	R	U,V
115*	A	D	H	N	R	U,V
117*	B	G	H	J	R	
118*	A	D	H	J	R	
119	A	D	H	J	R	
120	A	D	H	J	R	
121*	A	D	H	J	R	U,V
122*	A	D	H	O	R	
124*	A	D	H	J	R	
125	A	D	H	P	R	U
126*	A, C	D	H	J	R	U,V
127*	B	D	H	J	R	U
128*	A,C	D	H	J	R	

** Designated EU/EFTA member state NRL*

Key to method codes

1. Virus isolation and concentration method	
A	ISO 15216-1
B	Modified ISO 15216-1
C	ISO 15216-2
2. RNA extraction reagents	
D	NucliSens® (BioMérieux)
E	QIAamp® Airal RNA Mini Kit (Qiagen)
F	NucliSens® (BioMérieux), alternative robot system QuikPick Tool
G	NucliSens® (BioMérieux), modified
3. RNA extraction reagents	
H	One step
I	Two step
4. RT-PCR reagents	
J	RNA UltraSense™ One-Step Quantitative RT-PCR System
K	Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit
L	CeeramTools® real time RT-PCR kits (Ceeram)
M	QuantiTect® Probe RT-PCR kit (Qiagen)
N	Applied Biosystems™ TaqMan® Fast virus 1-Step Master Mix
O	SensiFAST™ Probe Hi-ROX One-Step Kit
P	Platinum™ Quantitative RT-PCR ThermoScript™ One-Step System
Q	GoTaq® Probe 1-Step RT-qPCR System

	5. Primers and probes
R	ISO 15216 *(The probe, NAGG1p or TM9, for norovirus GI was not asked to be specified)*
S	ISO 15216, with different fluorophores & quenchers
T	CeeramTools®
	6. Accreditation
U	Norovirus
V	HAV