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Swedish Food Agency

European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

FINAL REPORT

PROFICIENCY TESTING SCHEME EFV06, 2021

Quantification of norovirus and hepatitis A virus in bivalve molluscan shellfish

Final Report – Version 1 (2021/12/09)

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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 EFV05, organised by the EURL for Foodborne Viruses.

Distribution was made 26th of April 2021 to 23 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, is therefore available at EURL homepage. External control (EC) RNA, double-stranded (ds) DNA 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 06

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 Sydney (capsid sequence)

**HAV was excluded from the PT. See results and discussion.*

Sample A, B and C were spiked in various levels. Concentration values are shown in Table 2.

Table 2: Spiking of PT EFV 06 samples

Sample	Norovirus GI	Norovirus GII	HAV**
21EFV06 A	$\approx 10^5$*	$\approx 10^3$*	–
21EFV06 B	$\approx 10^4$*	$\approx 10^5$*	$\approx 5\times 10^4$*
21EFV06 C	$\approx 10^3$*	$\approx 10^4$*	$\approx 5\times 10^3$*

**Detectable virus genome copies inoculated to each sample*
*** HAV was excluded from the PT. See results and discussion.*

PREPARATION OF SAMPLES

Approximately 600 European oysters (Ostrea edulis) were purchased from a producer 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 two days before dispatch date.

DISTRIBUTION OF THE PROFIECY TEST ITEMS

Samples were dispatched on dry ice by courier in accordance with IATA packing instructions 650 for UN3373, on April 26th. All 23 laboratories received three frozen samples, EC RNA, process control virus (mengovirus) and double stranded DNA standards.

Instruction sheet and results form were sent by email to the contact person(s) at each laboratory. The deadline for submitting the results was May 11th.

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.

REFERENCE RESULTS- HOMOGENEITY AND STABILITY OF VIRUS LEVELS IN OYSTER SAMPLES

In order to mimic realistic shipping conditions, storage conditions at the participating laboratories, stability of virus levels as well testing the homogeneity, twelve random samples each of 21EFV06A, 21EFV06B and 21EFV06C were tested. Two samples of each were tested immediately after the inoculation (April 23th), and the rest of samples were stored in -20 °C for 72 hours. Two samples of each were tested on the dispatch date (d0, April 26th 2021) and the rest of samples were transferred to dry ice container on the dispatch date for 24 hours. Two samples of each were tested directly the day after (day 1), and the rest of samples were stored in -20 °C and tested at day 2, 3 and 4. Samples were analysed according to EURL SOP based on ISO 15216-1 for the quantification of target viruses respectively. The results (d0- d4) are shown in Table 3 and 4, with box and whisker plots included in Graph 1. The results of day 4 were used in performance assessment and scoring presented later in this report. Inhibition and extraction efficiency were calculated for all the reference samples. PT samples are considered to be homogenous enough for noroviruses and for trial 05 purposes. HAV results (sample B) were excluded from this PT since the samples were not homogeneous enough. The problem is discussed later in this report in the results and discussion section.

Table 3: Qualitative results for reference samples for PT EFV 06

Sample	Norovirus GI	Norovirus GII	HAV*
21EFV06 A	Detected	detected	Excluded
21EFV06 B	Detected	detected	Excluded
21EFV06 C	Detected	detected	Excluded

* *HAV results (sample C) were excluded from the PT. See results and discussion.*

Table 4: Quantitative results for ten reference samples for PT EFV 06

Ranges based on a 95 % confidence limit determined as two geometric standard deviations above and below the geometric mean (d0- d4).

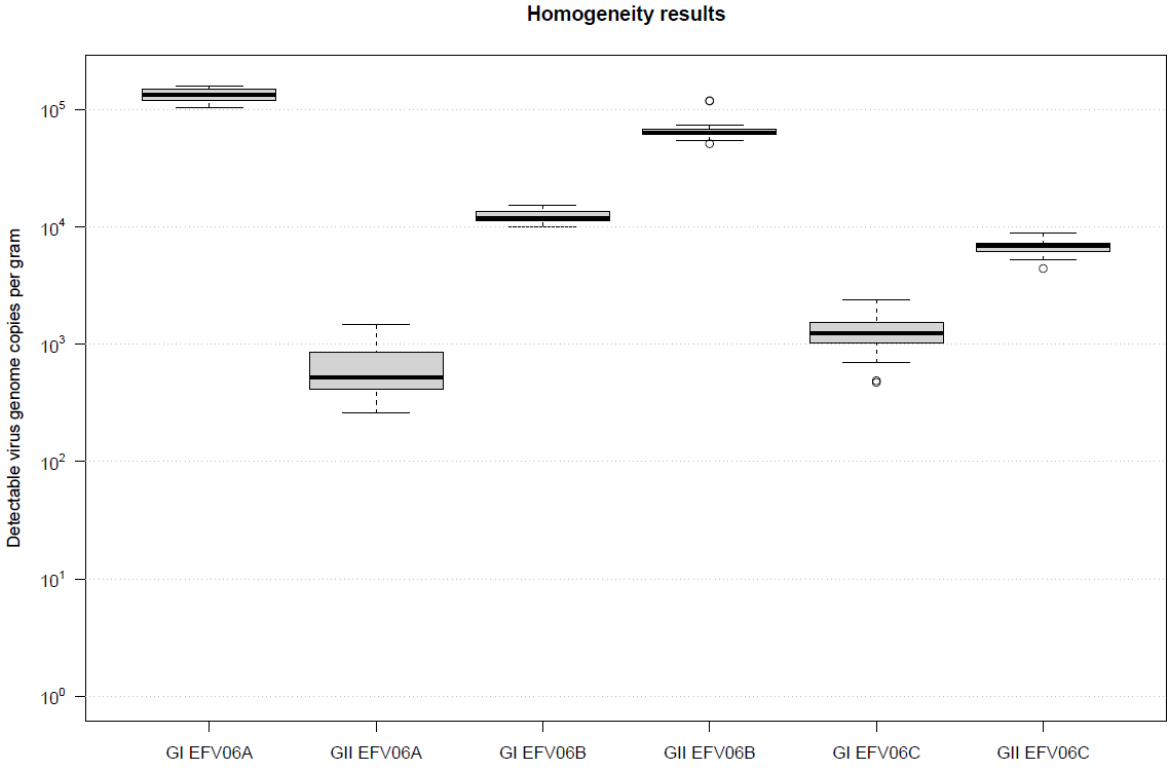
Sample	Norovirus GI	Norovirus GII	HAV**
21EFV06 A	$1.03 \times 10^5 – 1.72 \times 10^5$*	$3.13 \times 10^2 – 1.20 \times 10^3$*	Excluded
21EFV06 B	$9.89 \times 10^3 – 1.52 \times 10^4$*	$4.44 \times 10^4 – 1.03 \times 10^5$*	Excluded
21EFV06 C	$6.95 \times 10^2 – 2.20 \times 10^3$*	$5.06 \times 10^3 – 8.80 \times 10^3$*	Excluded

**detectable virus genome copies per gram sample*

*** HAV was excluded from the PT. See results and discussion.*

Graph 1: Box and whisker plots for homogeneity test of samples 21EFV06 A, B and C

The box includes 50 % of the results from 10 samples of each A and B and for C. 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.[1]



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 not enough 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 participants' 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 with values in coming PT schemes. The values of SD used as σ_{pt} were 0.15, 0.1, 0.1, for Norovirus GI and 0.1, 0.25 and 0.15 for Norovirus GII in sample A, B and C, 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.

[1] R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

The outcome is given in Table 5 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 5: Homogeneity test

Virus type	σ_{pt}	Homogenous? $s_s < 0.3*\sigma_{pt}$	Homogenous? $s_s < \sqrt{c}$
GI EFV06A	0.10	no	no
	0.15	no	yes
	0.20	yes	yes
	0.405	yes	yes
GI EFV06B	0.10	no	yes
	0.20	yes	yes
	0.465	yes	yes
GI EFV06C	0.10	yes	yes
	0.363	yes	yes
GII EFV06A	0.10	yes	yes
	0.488	yes	yes
GII EFV06B	0.20	no	no
	0.25	no	yes
	0.30	yes	yes
	0.410	yes	yes
GII EFV06C	0.10	no	yes
	0.15	no	yes
	0.20	yes	yes
	0.458	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, green fields indicate homogeneity of the samples according to both criteria and red indicates no homogeneity at given **σ_{pt}**.

RESULTS AND DISCUSSION

Samples were sent to 23 laboratories and 22 laboratories returned their results (including 19 NRLs and one in the process of becoming NRL). Information provided by laboratories showed that samples temperature upon arrival was below -20° C. The majority received the samples the day after dispatch (on April 27th), four laboratories on April 28th and one laboratory on April 29th. The majority of laboratories analysed the samples within the first week after the dispatch date.

In total, no false negative results were reported by the laboratories for sample B. In sample A, which was inoculated with the lowest copies of norovirus GII comparing to samples B and C, 3 false negative results were reported. One laboratory reported detect GII with EURL standards. In sample C, two false negative results for norovirus GI and one false negative result for norovirus GII were reported. Overview of results is demonstrated in Table 6.

Despite the fact that sample B and C which were inoculated with all the target viruses proved to be not homogenous for HAV, the majority of laboratories could detect and quantify it respectively. The results are presented in annex E. Further analysis by EURL demonstrated that the particular HAV stock used for inoculating both lettuce and oyster PT samples in 2020 and 2021, degrades in oyster samples.

The results of references samples analysed at day 4 (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 6: Overview of participants´ results for samples 21EFV06 A, B and C

Target viruses	N	Sample 21EFV06 A				Sample 21EFV06 B				Sample 21EFV06 C			
		T	FP	FN	NV	T	FP	FN	NV	T	FP	FN	NV
Norovirus GI	22	22	-	0	-	22	-	0	-	20	-	2	-
Norovirus GII	22	19(20)*	-	3(2)*	-	22	-	0	-	21	-	1	-
Hepatitis A virus	Excluded												

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, one lab reported FN results with own standards and T results with EURL standards.

PERFORMANCE ASSESMENT

PRESENCE- ABSENCE

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 four for HAV and six for norovirus GI and GII (Table 8).

QUANTITATIVE RESULTS

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 7.

Table 7: Calculated data used for scoring assessment

Quantity	21EFV06 A GI	21EFV06 B GI	21EFV06 C GI	21EFV06 A GII	21EFV06 B GII	21EFV06 C GII
Average	5.035	4.014	3.006	2.832	4.954	3.917
SD	0.405	0.465	0.363	0.488	0.410	0.458

-Values in log10 copies/g

- The results of references samples analysed at day 4 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. In Graphs 2, 3, 4, 5, 6 and 7 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 presence/absence score for each laboratory (for each target virus, excluding HAV), taking into account the results of all three samples is therefore six for GI and GII.
The results of references samples analysed at day 4 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 8: Calculated data used for scoring assessment

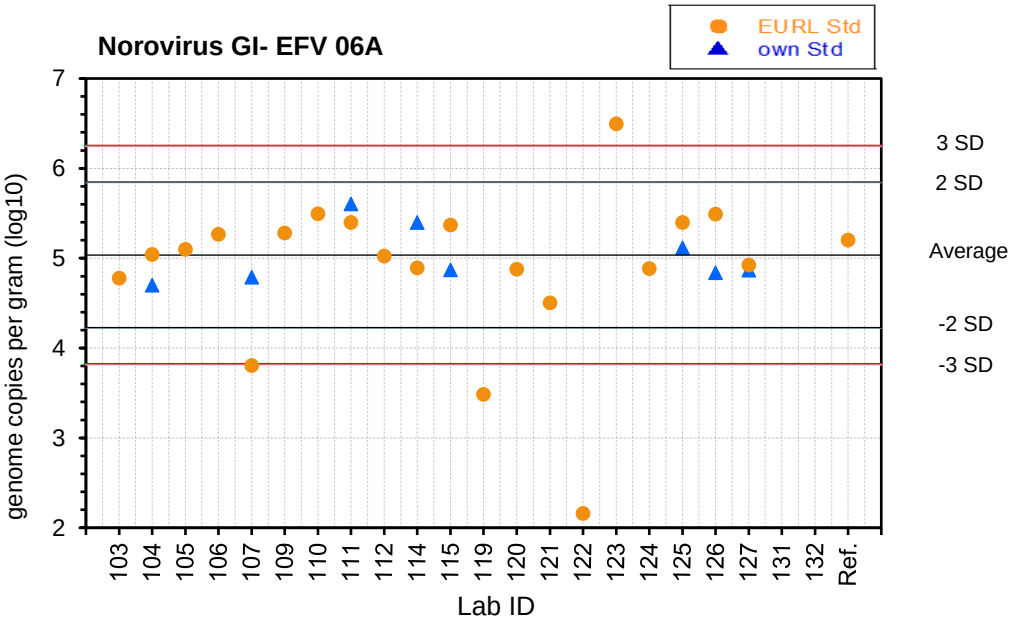
	Presence/absence		Quantitative	
Lab ID	GI	GII	GI	GII
103	6 out of 6	6 out of 6	6 out of 6	6 out of 6
104*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
105*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
106*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
107*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
109*	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	6 out of 6
111*	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
114*	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	6 out of 6
119*	4^{fn} out of 6	6 out of 6	4^{fn} out of 6	6 out of 6
120*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
121*	6 out of 6	4^{fn} out of 6	6 out of 6	4^{fn} out of 6
122*	4^{fn} out of 6	6 out of 6	4^{fn} out of 6	6 out of 6
123*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
124*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
125	6 out of 6	4^{fn} out of 6 (6 out of 6)[1]	6 out of 6	4^{fn} out of 6 (6 out of 6)[1]
126*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
127*	6 out of 6	6 out of 6	6 out of 6	6 out of 6
131*	6 out of 6	6 out of 6	-	-
132*	6 out of 6	4^{fn} out of 6	4^{nq} out of 6	$2^{fn, nq}$ out of 6

** Designated EU/EFTA member state NRL*

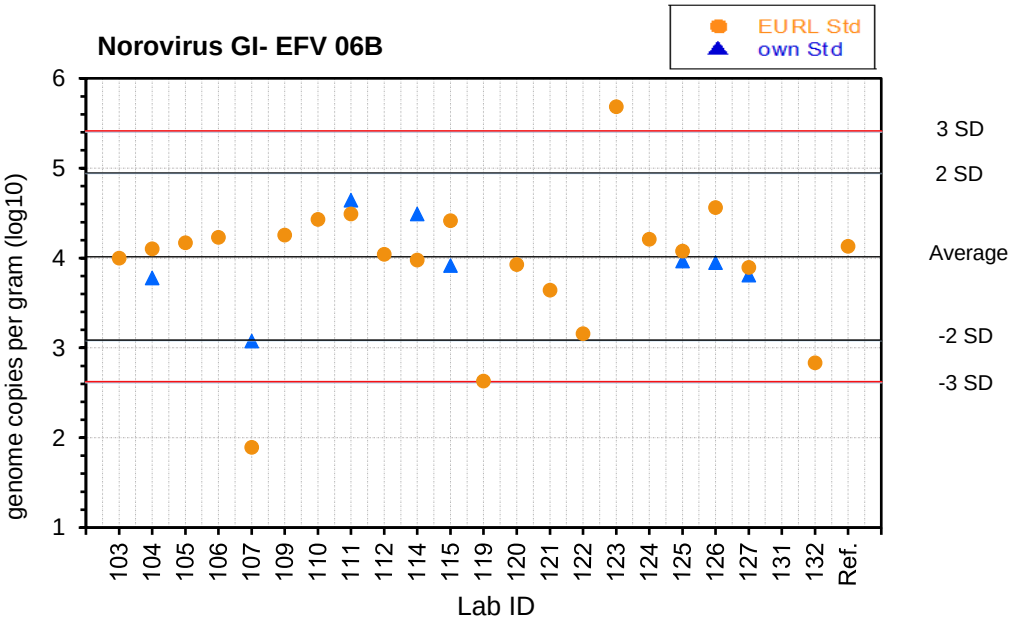
^{fn}: false negative, ^{nq}: not quantifiable due to not valid results and therefore excluded from scoring

[1]: Lab 125 reported true positive results with EURL standards, Lab 131: did not quantitative analysis.

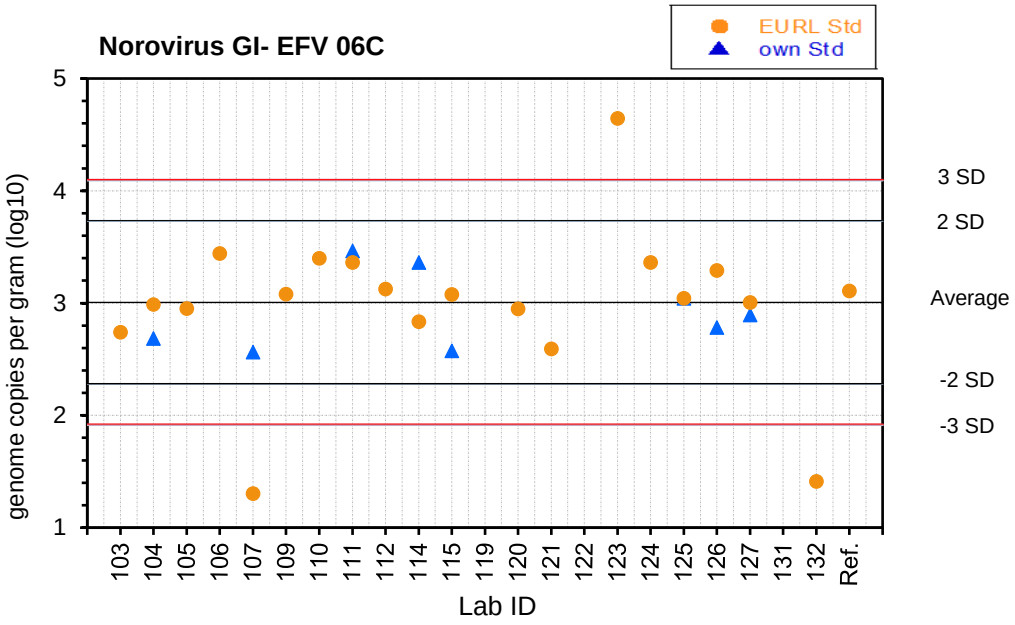
Graph 2: Distribution of results for norovirus GI in 21EFV06A



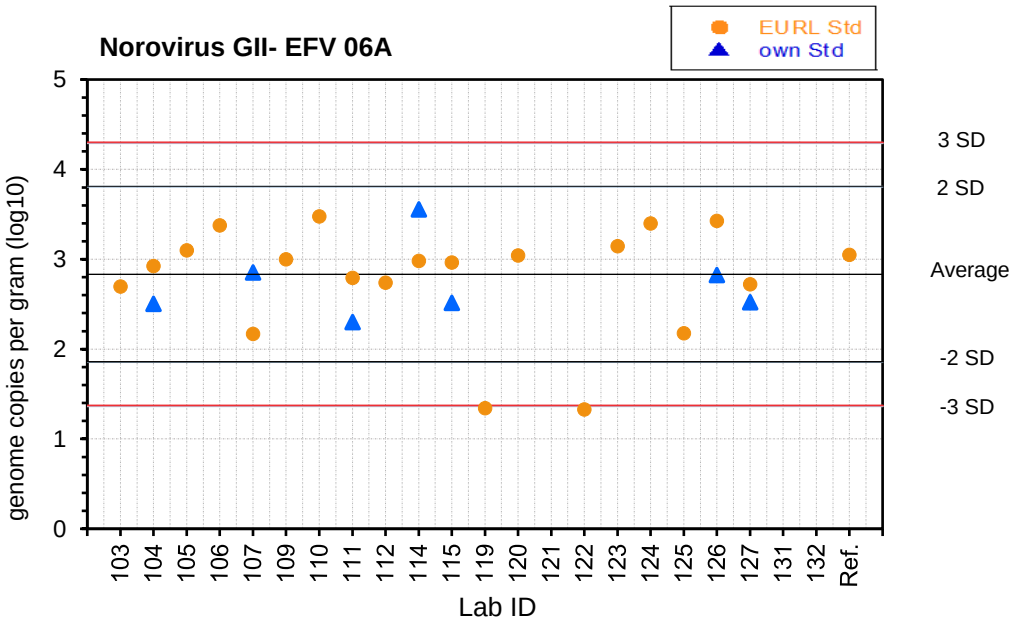
Graph 3: Distribution of results for norovirus GI in 21EFV06B



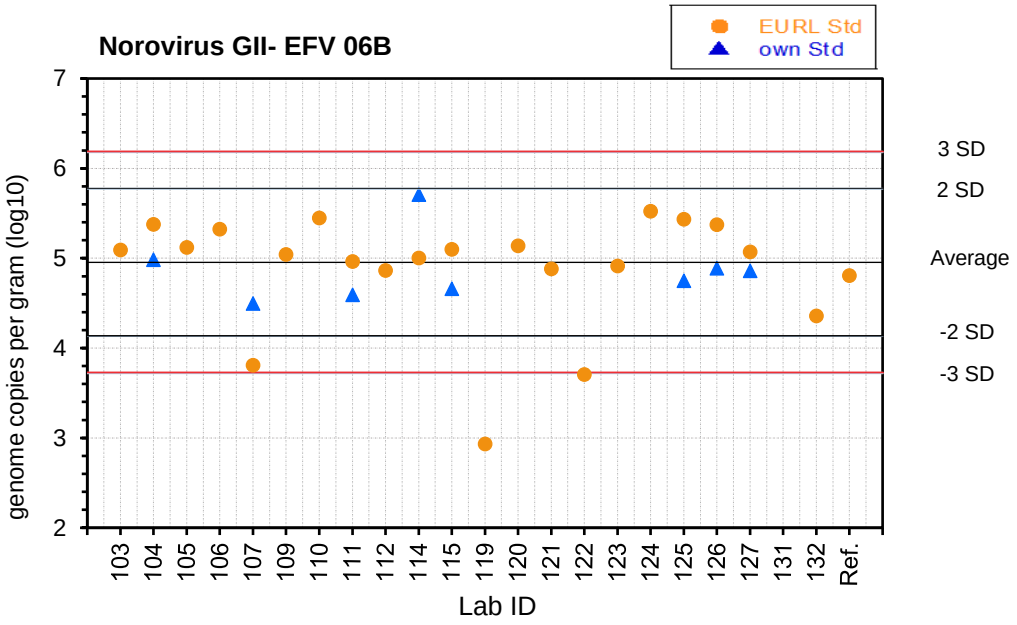
Graph 4: Distribution of results for GI in 21EFV06C



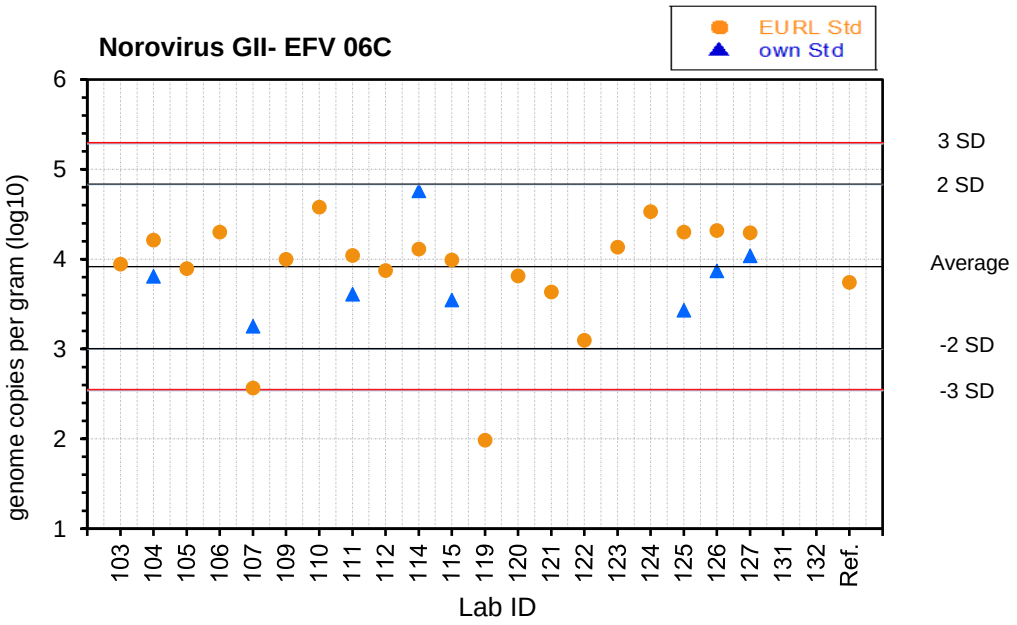
Graph 5: Distribution of results for GII in 21EFV06A



Graph 6: Distribution of results for GII in 21EFV06B



Graph 7: Distribution of results for GII in 21EFV06C



INHIBITION AND EFFICIENCY RESULTS

The results were also evaluated based on inhibition and extraction efficiency outcomes. Only one laboratory (ID: 132) reported unacceptable extraction efficiency and inhibition in samples A and therefore their quantification results were excluded from the scoring. Laboratory reported their extraction results as valid without reporting the actual values.

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.

According to ISO 15216-1 and 2, negative results are not valid in absence of inhibition (>2 or >75%) or/and extraction efficiency (<1%) 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 as detected for norovirus GI and GII in samples A, B and C are valid regardless the inhibition and extraction efficiency values, since the respective samples were inoculated for the respective target viruses. Results are presented in Annex C.

METHODS USED BY THE PARTICIPANTS

Eleven laboratories were accredited according to ISO/IEC 17025 for quantitative detection of norovirus GI, norovirus GII and eight for HAV. All the laboratories followed ISO 15216-1 with exception of one laboratory performed a modified version of ISO 15216-1. One laboratory does not perform quantification for any of target viruses and another laboratory does not perform quantitative detection of HAV. Detailed information on the methodologies used is shown in Appendix D.

CONCLUSION

The aim of PT EFV05 organized in winter of 2021 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-two laboratories submitted their results for this PT and 77 % of the participating laboratories obtained full satisfactory results.

Annex A

Participants' results

with EURL standards | with own standards | false results

Lab. ID No.	21EFV06 A				21EFV06 B				21EFV06 C			
	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)
103	28.97	5.96E+04	35.01	4.99E+02	31.41	9.98E+03	27.50	1.24E+05	35.39	5.47E+02	31.07	8.84E+03
104*	29.15	1.10E+05	37.07	8.39E+02	32.40	1.26E+04	28.38	2.38E+05	36.19	9.66E+02	32.52	1.64E+04
104*	29.15	4.99E+04	37.07	3.21E+02	32.40	5.97E+03	28.38	9.60E+04	36.19	4.83E+02	32.52	6.46E+03
105*	28.60	1.25E+05	33.70	1.26E+03	31.70	1.48E+04	27.00	1.32E+05	35.70	8.93E+02	31.00	7.88E+03
106*	29.29	1.85E+05	35.74	2.38E+03	32.99	1.71E+04	28.82	2.10E+05	35.77	2.76E+03	32.46	2.01E+04
107*	28.82	6.37E+03	34.20	1.48E+02	35.37	7.80E+01	28.59	6.44E+03	37.46	2.00E+01	32.85	3.70E+02
107*	28.82	6.13E+04	34.20	7.17E+02	35.37	1.19E+03	28.59	3.13E+04	37.46	3.64E+02	32.85	1.79E+03
109*	29.04	1.90E+05	35.13	1.00E+03	32.42	1.80E+04	27.70	1.10E+05	36.53	1.20E+03	30.59	1.00E+04
110*	26.05	3.10E+05	32.34	3.00E+03	29.24	2.70E+04	26.28	2.80E+05	32.53	2.50E+03	29.04	3.80E+04
111*	29.18	2.50E+05	36.65	6.20E+02	32.29	3.10E+04	29.03	9.20E+04	36.00	2.30E+03	32.15	1.10E+04
111*	29.18	4.00E+05	36.65	2.00E+02	32.29	4.40E+04	29.03	3.90E+04	36.00	2.90E+03	32.15	4.10E+03
112*	28.64	1.06E+05	34.83	5.51E+02	31.80	1.10E+04	27.91	7.31E+04	34.76	1.33E+03	31.14	7.48E+03
114*	29.36	7.80E+04	35.09	9.60E+02	32.51	9.50E+03	27.90	1.00E+05	36.59	6.80E+02	31.06	1.30E+04
114*	29.36	2.50E+05	35.09	3.60E+03	32.51	3.10E+04	27.90	5.10E+05	36.59	2.30E+03	31.06	5.80E+04
115	29.35	2.33E+05	38.24	9.24E+02	32.56	2.60E+04	30.66	1.25E+05	37.23	1.19E+03	34.46	9.84E+03
115	29.35	7.40E+04	38.24	3.28E+02	32.56	8.25E+03	30.66	4.55E+04	37.23	3.76E+02	34.46	3.53E+03
119*	33.85	3.05E+03	37.62	2.20E+01	36.58	4.29E+02	33.22	8.55E+02	ND	<10	36.47	9.72E+01
120*	30.53	7.55E+04	34.76	1.10E+03	34.10	8.45E+03	27.91	1.37E+05	38.20	8.88E+02	36.44	6.54E+03
121*	32.18	3.17E+04	ND	ND	34.99	4.41E+03	30.10	7.61E+04	38.68	3.89E+02	34.26	4.31E+03
122*	33.46	1.44E+02	37.33	2.13E+01	40.61	1.44E+03	34.42	5.08E+03	ND	ND	33.40	1.25E+03

** Designated EU/EFTA member state NRL, [e] excluded as a result of unacceptable extraction efficiency results. ND: reported as not detected,*

[1]Reported as not detected; the Cq value indicated is the maximum cycles recommended in ISO 15216.

Lab. ID No.	21EFV06 A				21EFV06 B				21EFV06 C			
	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)
123*	26.4	3.13 E+06	34.70	1.40 E+03	29.50	4.84 E+05	28.30	8.15 E+04	33.10	4.40 E+04	31.10	1.37 E+04
124*	32.12	7.66E+04	37.33	2.51E+03	34.49	1.62E+04	30.36	3.31E+05	37.47	2.30E+03	33.54	3.40E+04
125	27.24	2.50E+05	37.90	1.50E+02	31.47	1.20E+04	26.33	2.70E+05	34.80	1.10E+03	30.33	2.00E+04
125	27.24	1.30E+05	ND	ND	31.47	9.30E+03	27.37	5.60E+04	34.80	1.10E+03	31.99	2.70E+03
126*	28.96	3.09E+05	37.28	2.68E+03	32.46	3.66E+04	30.37	2.35E+05	37.09	1.95E+03	34.02	2.10E+04
126*	29.91	6.87E+04	36.92	6.68E+02	33.12	8.89E+03	29.79	7.71E+04	37.13	6.07E+02	33.29	7.46E+03
127*	28.62	8.40E+04	35.63	5.28E+02	32.16	7.89E+03	27.54	1.17E+05	35.14	1.01E+03	30.18	1.97E+04
127*	28.62	7.34E+04	35.63	3.32E+02	32.16	6.46E+03	27.54	7.21E+04	35.14	7.83E+02	30.18	1.09E+04
131*	32.33	NR	37.80	NR	36.20	NR	32.70	NR	38.30	NR	36.10	NR
132*	35.05	5.99E+03[e]	ND	ND	37.74	6.82E+02	32.20	2.28E+04	41.76	2.57E+01	ND	ND
Ref.**	27.93	1.59E+05	33.64	1.12E+03	31.57	1.35E+04	27.69	6.38E+04	35.04	1.28E+03	31.29	5.55E+03

** Designated EU/EFTA member state NRL, ** Reference results from day 4*

[e]: excluded as a result of unacceptable extraction efficiency results ND: reported as not detected

NR: Lab 131 did not quantitative analysis

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	**GI** 21EFV06 A		**GI** 21EFV06 B		**GI** 21EFV06 C		**GII** 21EFV06 A		**GII** 21EFV06 B		**GII** 21EFV06 C	
	EURL STD	*Own STD*	*EURL STD*	*Own STD*	*EURL STD*	*Own STD*	*EURL STD*	*Own STD*	*EURL STD*	*Own STD*	*EURL STD*	*Own STD*
103	-0.639		-0.032		-0.738		-0.275		0.337		0.064	
104*	0.017	-0.830	0.186	-0.511	-0.058	-0.886	0.187	-0.667	1.030	0.069	0.650	-0.234
105*	0.154		0.336		-0.152		0.549		0.406		-0.046	
106*	0.572		0.469		1.199		1.114		0.899		0.840	
107*	-3.037	-0.610	-4.561	-2.018	-4.693	-1.224	-1.356	0.048	-2.790	-1.116	-2.947	-1.450
109*	0.603		0.519		0.202		0.343		0.213		0.180	
110*	1.128		0.898		1.079		1.321		1.202		1.447	
111*	0.897	1.401	1.027	1.354	0.979	1.257	-0.082	-1.088	0.024	-0.884	0.271	-0.665
112*	-0.027		0.063		0.326		-0.187		-0.219		-0.095	
114*	-0.352	0.897	-0.078	1.027	-0.477	0.979	0.307	1.483	0.112	1.836	0.429	1.848
115	0.821	-0.408	0.864	-0.209	0.193	-1.186	0.273	-0.648	0.353	-0.720	0.165	-0.807
119*	-3.826		-2.970		FN		-3.052		-4.926		-4.216	
120*	-0.386		-0.187		-0.158		0.429		0.446		-0.222	
121*	-1.317		-0.794		-1.144		FN		-0.177		-0.617	
122*	-7.097		-1.837		FN		-3.080		-3.041		-1.793	
123*	3.606		3.592		4.508		0.643		-0.104		0.479	
124*	-0.371		0.421		0.979		1.162		1.379		1.341	
125	0.897	0.196	0.141	-0.097	0.098	0.098	-1.344	FN	1.163	-0.501	0.838	-1.062
126*	1.123	-0.487	1.182	-0.140	0.782	-0.613	1.221	-0.015	1.018	-0.163	0.883	-0.097
127*	-0.272	-0.417	-0.251	-0.438	-0.004	-0.309	-0.225	-0.637	0.279	-0.234	0.824	0.262
131*		NR		NR		NR		NR		NR		NR
132*	NQ i		-2.537		-4.393		FN		-1.452		FN	
Ref.	0.412		0.252		0.279		0.444		-0.363		-0.378	

** Designated EU/EFTA member state NRL, FN: false negative, NQ: non-quantifiable (reported result is excluded from scoring as the results of unacceptable extraction efficiency, STD: standard.*

Annex C

Inhibition and extraction efficiency results for sample 20EFV05 A

Lab. ID	Inhibition GI[t]	Inhibition GII[t]	Efficiency	Valid/ Not valid Presence/absence GI[t]	Valid/ Not valid Presence/absence GII[t]	Valid/Not valid Quantitative GI[t]	Valid/Not valid Quantitative GII[t]
103	A	A	A	V	V	V	V
104*	A	A	A	V	V	V	V
105*	A	A	A	V	V	V	V
106*	A	A	A	V	V	V	V
107*	A	A	A	V	V	V	V
109*	A	A	A	V	V	V	V
110*	A	A	A	V	V	V	V
111*	A	A	A	V	V	V	V
112*	A	A	A	V	V	V	V
114*	A	A	A	V	V	V	V
115	A	A	A	V	V	V	V
119*	A	A	A	V	V	V	V
120*	A	A	A	V	V	V	V
121*	A	A FN	A	V	FN	V	FN
122*	A	A	A	V	V	V	V
123*	A	A	A	V	V	V	V
124*	A	A	A	V	V	V	V
125	A	A FN	A	V	A FN	V	A FN
126*	A	A	A	V	V	V	V
127*	A	A	A	V	V	V	V
131*	A	A	NR	V	V	-	-
132*	A	A FN	U	V	FN	NV	FN

** Designated EU/EFTA member state NRL*

A: Acceptable, FN: false negative, NR: not reported, [t]: target virus, NV: not valid, V: valid results

Lab 125: true positive results with EURL standards, Lab 131: did not quantitative analysis.

Inhibition and extraction efficiency results for sample 20EFV05 B

	Inhibition		Efficiency	Valid/ Not valid Presence/absence		Valid/Not valid Quantitative	
Lab. ID	GI[t]	GII[t]		GI[t]	GII[t]	GI[t]	GII[t]
103	A	A	A	V	V	V	V
104*	A	A	A	V	V	V	V
105*	A	A	A	V	V	V	V
106*	A	A	A	V	V	V	V
107*	A	A	A	V	V	V	V
109*	A	A	A	V	V	V	V
110*	A	A	A	V	V	V	V
111*	A	A	A	V	V	V	V
112*	A	A	A	V	V	V	V
114*	A	A	A	V	V	V	V
115	A	A	A	V	V	V	V
119*	A	A	A	V	V	V	V
120*	A	A	A	V	V	V	V
121*	A	A	A	V	V	V	V
122*	A[d]	A[d]	A	V	V	V	V
123*	A	A	A	V	V	V	V
124*	A	A	A	V	V	V	V
125	A	A	A	V	V	V	V
126*	A	A	A	A	V	V	V
127*	A	A	A	A	V	V	V
131*	A	A	NR	V	V	-	-
132*	A	A FN	NR	A	V	FN	NV

** Designated EU/EFTA member state NRL*

A: Acceptable, [d]: diluted samples, FN: false negative, NR: not reported, [t]: target virus, V: valid results

Lab 131: did not quantitative analysis

Inhibition and extraction efficiency results for sample 20EFV05 C

	Inhibition		Efficiency	Valid/ Not valid Presence/absence		Valid/Not valid Quantitative	
Lab. ID	GI^t	GII^t		GI^t	GII^t	GI^t	GII^t
103	A	A	A	V	V	V	V
104*	A	A	A	V	V	V	V
105*	A	A	A	V	V	V	V
106*	A	A	A	V	V	V	V
107*	A	A	A	V	V	V	V
109*	A	A	A	V	V	V	V
110*	A	A	A	V	V	V	V
111*	A	A	A	V	V	V	V
112*	A	A	A	V	V	V	V
114*	A	A	A	V	V	V	V
115	A	A	A	V	V	V	V
119*	A FN	A	A	V	V	V	V
120*	A	A	A	V	V	V	V
121*	A	A	A	V	V	V	V
122*	A FN	A	A	V	V	V	V
123*	A	A	A	V	V	V	V
124*	A	A	A	V	V	V	V
125	A	A	A	V	V	V	V
126*	A	A	A	V	V	V	V
127*	A	A	A	V	V	V	V
131*	A	A	NR	V	V	-	-
132*	A	A FN	NR	V	FN	NV	FN

** Designated EU/EFTA member state NRL*

A: Acceptable, FN: false negative, NR: not reported, ^{t}: target virus, V: valid results

Lab 131: did not quantitative analysis

Annex D

General information on methods

Lab. ID	1	2	3	4	5	6	7
103	A	D	H	J	R	UV	X
104*	A	D	H	J	R	UV	W
105*	A	D	H	J	R TM9	UV	Wi
106*	A	D	H	J	R		Y or Yr?
107*	A	E	H	P	S	UV	Za
109*	A	D	H	J	R		Yy
110*	A	F	H	M	R TM9		W
111*	A	D	H	N	R		Y
112*	A	D	H	J	R		Yr
114*	A	D	Hh	J	R	UV	Z
115	A	D	H	J	R TM9	UV	Zb
119*	A	D	H	J	R	U	ZzQq
120*	A	D	H	J	R TM9,		X
121*	A	D	H	J	R	UV	Zq
122*	A	D	H	O	R		X
123*	A	D	H	J	R		X
124*	A	D	H	J	R TM9		Wr
125	A	D	H	N	R	U	W
126*	A, C	D	H	J	R TM9	UV	Y or Yr
127	B	D	H	J	R	U	X, Xa
131*	A, C	D	H	M	R		Yr
132*	A	D	H	J	R		Zqq

** 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 methods/reagents	
D	NucliSens® (BioMérieux)
E	NucliSens® (BioMérieux), TANBead Maelstrom™ 8 Autostage
F	NucliSens® (BioMérieux), alternative robot system QuikPick Tool
3. PCR method RT-PCR	
H	One step
Hh	Two step
4. RT-PCR reagents	
J	RNA UltraSense™ One-Step Quantitative RT-PCR System
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	GoTaq® Probe 1-Step RT-qPCR System
5. Primers and probes	
R	ISO 15216 *(The probe, NVGG1p or TM9, for norovirus GI was not asked to be specified)*
S	Modified ISO 15216

6. Accreditation	
U	Norovirus
V	HAV
7. PCR system	
W	CFX96™ Real-Time PCR Detection System (Biorad)
X	AriaMx Real-time PCR System
Y	Applied Biosystems™ 7500 Fast Real-Time PCR System
Z	Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System
Xa	Mx3000P qPCR Systems
Wi	LightCycler® 96 System (Roche)
Wr	LightCycler® 480 Instrument (Roche)
Yy	Applied Biosystems™ 7900HT Fast Real-Time PCR System
Yr	Applied Biosystems™ 7500 Real-Time PCR System
Za	Rotor-Gene Q (Qiagen)
Zb	Stratagene MX3005P® QPCR System
Zq	Applied Biosystems™ QuantStudio™ 5
Zqq	Applied Biosystems™ QuantStudio™ 3
Zzqq	Applied Biosystems™ QuantStudio™ 6

Annex E

Excluded HAV results in sample B and C reported by participants.

Since EURL did not detect HAV in any samples, it was not possible to include any HAV results (detected or not detected) in the scoring. Sample B and C were inoculated with HAV meaning that if included in the scoring, all the laboratories that reported correct detected/not detected results would have full score 6/6.

Lab. ID No.	21EFV06 B			21EFV06 C		
	Detection	HAV (Cq)	HAV(c/g)	Detection	HAV (Cq)	HAV(c/g)
103	D	33.57	2.19E+03	D	36.88	1.11E+02
104*	D	33.34	4.36E+03	ND	ND	ND
104*	D	33.34	1.10E+03	ND	ND	ND
105*	NP	NP	NP	NP	NP	NP
106*	D	33.60	1.18E+04	D	37.5	9.72E+02
107*	D	35.18	1.77E+02	ND	ND	ND
107*	D	35.18	3.00E+01	ND	ND	ND
109*	D	34.14	2.00E+04	D	36.78	2.70E+03
110*	D	32.08	3.30E+03	ND	ND	ND
111*	D	34.21	2.10E+03	D	36.15	4.90E+02
111*	D	34.21	1.80E+03	D	36.15	4.10E+02
112*	D	35.80	1.64E+03	D	37.58	4.52E+02
114*	D	32.64	2.80E+03	D	40.55[a]	1.30E+02
114*	D	32.64	1.40E+04	D	40.55[a]	6.10E+02
115	D	32.93	8.02E+03	D	36.59	6.33E+02
115	D	32.93	2.59E+03	D	36.59	<392
119*	NP	NP	NP	NP	NP	NP
120*	NP	NP	NP	NP	NP	NP
121*	D	36.69	9.41E+02	D	38.89	2.01E+02
122*	NP	NP	NP	NP	NP	NP
123*	NP	NP	NP	NP	NP	NP
124*	D	34.6	8.74E+03	D	37.26	1.42E+03
125	D	34.05	2.90E+03	D	37.51	2.80E+02
125	D	34.05	8.80E+02	D	37.51	9.30E+01
126*	NP	NR[1]	NR[1]	NP	NR[1]	NR[1]
126*	NP	NR[1]	NR[1]	NP	NR[1]	NR[1]
127*	D	34.58	1.73E+03	D	37.15	2.49E+02
127*	D	34.58	1.24E+03	D	37.15	2.00E+02
131*	NP	NR[1]	NR[1]	NP	NR[1]	NR[1]
132*	NP	NP	NP	NP	NP	NP

** Designated EU/EFTA member state NRL, a: average cq of reported cq, i: unacceptable inhibition, D: detected, ND: not detected, NP: not performed, NR[1]: only qualitative results were reported since Lab 126 do not perform quantitative analysis for HAV and Lab 131 did not perform quantitative analysis at all.*