Livsmedelsverket
Swedish Food Agency

European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

Final report

Proficiency testing scheme EFV 11, 2023

Quantification of norovirus and hepatitis A virus in bivalve molluscan shellfish

Final report- (2024.12. 04)

Swedish Food Agency Reference: 2024/01179

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Classification: Official
Location: Uppsala, Sweden

ISSN 1104-7089

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Summary

This report describes the performance evaluation of National Reference Laboratories (NRLs) in the proficiency testing (PT) EFV11 organised by the EURL for Foodborne Viruses. Distribution of samples occurred on November 27th, 2023 to 25 laboratories registered for the PT. The PT aimed to quantitatively detect hepatitis A virus (HAV) and norovirus genogroups I (GI) and II (GII) in two samples of frozen oyster hepatopancreas as well as a sample comprising twelve whole frozen oysters.

The participating laboratories were instructed to analyse the samples using their routine method, though the EURL recommended following ISO 15216-1 method. A Standard Operating Procedure (SOP) for detection of norovirus and hepatitis A virus bivalve molluscan shellfish, based on ISO 15216-1, is therefore accessible on the EURL homepage. Alongside PT samples, laboratories requesting in advance received external control (EC) RNA, double-stranded (ds) DNA and process control virus.

Confidentiality is maintained by assigning each participant a unique laboratory identification number. Only the PT team and the respective laboratory have access to this ID. However, results from NRLs appointed under Regulation (EU) 2017/625 will be disclosed to DG SANTE for performance assessment purposes.

Background

Since 2018, the Swedish Food Agency has served as the European Union Reference Laboratory (EURL) for Foodborne Viruses, designated under Regulation (EU) 2017/625. According to Article 94 of the regulation, the EURL is tasked with 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 accordance with Regulation (EU) 2017/625.

Samples

The dispatched materials included two samples of artificially contaminated frozen oyster digestive glands inoculated with characterised norovirus GI and GII sourced from human faecal material, as well as HAV from cell culture supernatant, along with 12 oysters. Table 1 provides information on the viruses used for sample preparation, while Table 2 outlines the levels of spiking for each virus.

Table 1: Description of the viruses used for the PT EFV 11

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)

Table 2: Spiking of PT EFV 11 samples

Sample	Norovirus GI	Norovirus GII	HAV
23EFV11 A	–	–	≈5×10^4*
23EFV11 B	≈5×10^3*	≈10^4*	–
23EFV11 C	–	–	–

**Detectable virus genome copies inoculated to surface of each sample*

Preparation of samples

Approximately 600 European oysters (Ostrea edulis) were purchased from a producer in Sweden. To create samples A and B, a homogenous mixture was prepared by shucking the oysters, extracting the digestive glands, removing adipose tissues, and blending and pooling the material. This mixture was divided into 2-gram aliquots, each of which was spiked with the target viruses and stored at -20°C for two days prior to the dispatch date. Sample C comprised 12 frozen oysters.

Distribution of the proficiency testing items

On November 27th, samples were dispatched on dry ice by courier following IATA packing instructions 650 for UN3373. Each of 25 laboratories received three frozen samples, EC RNA, process control virus (mengovirus) and double stranded DNA standards.

An instruction sheet and results form were emailed to the designated contact persons at each laboratory. The deadline for submitting the results was December 11th.

Quality control

The oysters used in the production of the test items tested negative for HAV, norovirus GI, and norovirus GII. Spiked samples underwent assessment for both homogeneity and stability. Inhibition and extraction efficiency met acceptable criteria across all samples used in the homogeneity and stability tests.

Reference results- Homogeneity and stability of virus levels in oyster samples

The homogeneity of dispatched samples as well as stability of virus levels were evaluated using ten random samples each of 23EFV11 A and B simulating realistic shipping and storage conditions at participating laboratories. Additionally, five samples were analysed for all the target viruses a few weeks prior to spiking and dispatching to confirm that sample 23EFV11C was not positive for the target viruses. The spiked samples were prepared two days prior to dispatch and initially stored at -20°C, then transferred to dry ice for 24 hours on the dispatch day. Three samples of each A and B were immediately tested after dry ice storage, while the remaining samples were stored in -20°C and tested after 24 hours, 48 hours, and 72 hours, respectively.

All samples were analysed according to EURL SOP based on ISO 15216-1 for quantifying the target viruses. The results of ten reference samples are detailed in Table 3 and 4, with box and whisker plots provided in Graph 1. A reference sample from day 2 was used for performance assessment and scoring, as presented in this report under Ref.

Inhibition and extraction efficiency were acceptable for all reference samples. The PT samples are considered sufficiently homogenous for noroviruses and HAV for trial 11 purposes.

Table 3: Qualitative results for reference samples for PT EFV 11

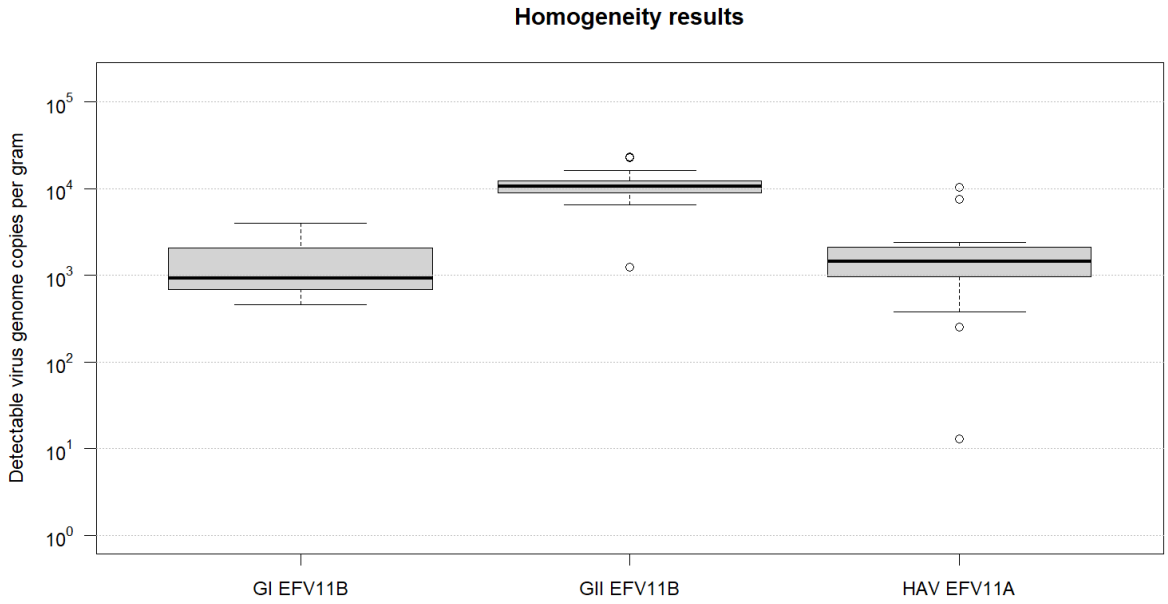
Sample	Norovirus GI	Norovirus GII	HAV
23EFV11 A	Not detected	Not detected	Detected
23EFV11 B	Detected	Detected	Not detected
23EFV11 C	Not detected	Not detected	Not detected

Table 4: Quantitative results for ten reference samples for PT EFV 11
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
23EFV11 A	Not detected	Not detected	$5.43 \times 10^1 – 2.1 \times 10^4$
23EFV11 B	$2.92 \times 10^2 – 3.32 \times 10^3$	$3.05 \times 10^3 – 3.36 \times 10^4$	Not detected
23EFV11 C	Not detected	Not detected	Not detected

**detectable virus genome copies per gram sample*

Graph 1: Box and whisker plots for homogeneity test of samples 23EFV11 C
The box includes 50 % of the results from 10 samples. 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 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

[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/.

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 to values in coming PT schemes. The values of SD used as σ_{pt} were 0.479 for norovirus GI, 0.396 for norovirus GII and 0.346 for hepatitis A virus. 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 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

***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.*

Virus type	σ_{pt}	Homogenous? $s_s < 0.3*\sigma_{pt}$	Homogenous? $s_s < \sqrt{c}$
GI EFV11B	0.3	**yes**	No
	0.5	**yes**	**Yes**
GII EFV11B	0.4	**yes**	No
	0.5	**yes**	**Yes**
HAV EFV11A	0.3	yes	no
	0.5	**yes**	**yes**

Results and discussion

Samples were distributed to 25 laboratories, including 23 NRLs and one undergoing designation. Seventeen laboratories received their samples one day after dispatch, while seven laboratories received them two days after dispatch. All laboratories returned their results, with three laboratories analysing the samples upon arrival, and the majority completing their analyses within the first two days of receipt.

The majority of laboratories reported true results; However, instances of false negative results were reported. Specifically, one false negative was reported for hepatitis A virus in sample A, two false negative results for norovirus GI in sample B, and no results for hepatitis A virus in sample C were submitted.

NRL 131 did not provide quantitative results, inhibition and extraction efficiency results for any samples and reported Cq values for some analyses. Additionally, there were between 2 to 4 non-valid negative results observed across all sample types and agents. An overview of the results is provided in Table 6.

Detailed information regarding the results of participating laboratories can be found in Annex A. Results of reference samples analysed at day 2 are presented as Ref.

Table 6: Overview of participants´ results for samples 23EFV11 A, B and C

Target viruses	N	23EFV11 A				23EFV11 B				23EFV11 C			
		T*	FP	FN	NV	T*	FP	FN	NV	T*	FP	FN	NV
Norovirus GI	25	25	0	-	2	23	-	2	-	25	0	-	2
Norovirus GII	25	25	0	-	2	25	-	0	-	25	0	-	3
Hepatitis A virus	25	24	-	1	-	24	1	-	4	24	1	-	4

**: one NRL did not report any qualitative neither quantitative results. 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 assessment

Presence- Absence

All results were evaluated as presence–absence data in concordance with intended results and according to the following criteria:

- 2 points: correct result for each target virus, irrespective of valid or non-valid negative results.
- 0 points: incorrect results for each target virus

Each laboratory could achieve a maximum score of six points for each target virus, based on the results for three viruses (GI, GII and HAV) from all three samples (see Table 8).

Quantitative results

In order to assess a comparison of quantitative results and aid laboratories in evaluating their performance, all results were converted into scores. The average and standard deviation were obtained as the robust average and robust standard deviation by applying Algorithm A (Huber's method), as per Annex C, clause C.3.1 of ISO 13528:2015. These values are presented in Table 7.

Table 7: Calculated data used for scoring assessment

Quantity	23EFV11 B GI	23EFV11 B GII	23EFV11 A HAV
Average	3.738	4.471	3.661
SD	0.478	0.391	0.346

-Values in log10 copies/g

- The results of references samples analysed at day 2 are included

Since all laboratories received EURL quantification standards along with PT materials, some participants provided two sets of results obtained by both EURL standards and their own standards. In such cases, only results obtained using the laboratories' own standards were considered for performance scoring, as it reflects their routine practices.

Quantitative results were assessed and scored according to the following criteria:

- 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 results of one reference sample analysed at day 2 were incorporated into the score calculations and are depicted as Ref.

Scoring results are illustrated in Table 9 and Graphs 2, 3 and 4.

Table 8: 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
107*	6 out of 6	6 out of 6	6 out of 6	5 out of 6	6 out of 6	5 out of 6
108*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	5 out of 6	4 out of 6
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	4 out of 6^{fn}	6 out of 6	6 out of 6	4 out of 6^{fn}
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	6 out of 6	6 out of 6	6 out of 6
119*	4 out of 6^{fn,e}	6 out of 6^{e,i}	6 out of 6^{e}	4 out of 6^{fn,e}	4 out of 6^{e,i}	5 out of 6^{e}
120*	6 out of 6	6 out of 6	0 out of 6^{i}	5 out of 6	5 out of 6	0 out of 6$^{i,\ nr}$
121*	6 out of 6	6 out of 6	6 out of 6^{i}	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	6 out of 6
123*	6 out of 6	6 out of 6	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	6 out of 6	6 out of 6
125	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 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	4 out of 6^{nr1}
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
129*	6 out of 6^{e}	6 out of 6^{e}	6 out of 6^{e}	5 out of 6^{e}	5 out of 6^{e}	6 out of 6^{e}
131*	4 out of 6^{ei,nr}	6 out of 6^{ei}	6 out of 6^{ei}	4 out of 6^{ei,nr}	4 out of 6^{ei}	4 out of 6$^{ei,\ nr}$
132*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6
133*	6 out of 6	6 out of 6	6 out of 6	6 out of 6	6 out of 6	5 out of 6
134*	4 out of 6^{fn}	4 out of 6^{fn}	6 out of 6	4 out of 6^{fn}	4 out of 6^{fn}	4 out of 6^{nr}

** Designated EU/EFTA member state NRL, ** in designation process*

^{e}: unacceptable extraction efficiency, ^{fn}: false negative, ^{fp}: false positive, ^{i}: unacceptable inhibition

NR^{1}: only qualitative results were reported since this NRL do not perform quantification analysis for HAV

Table 9: 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.

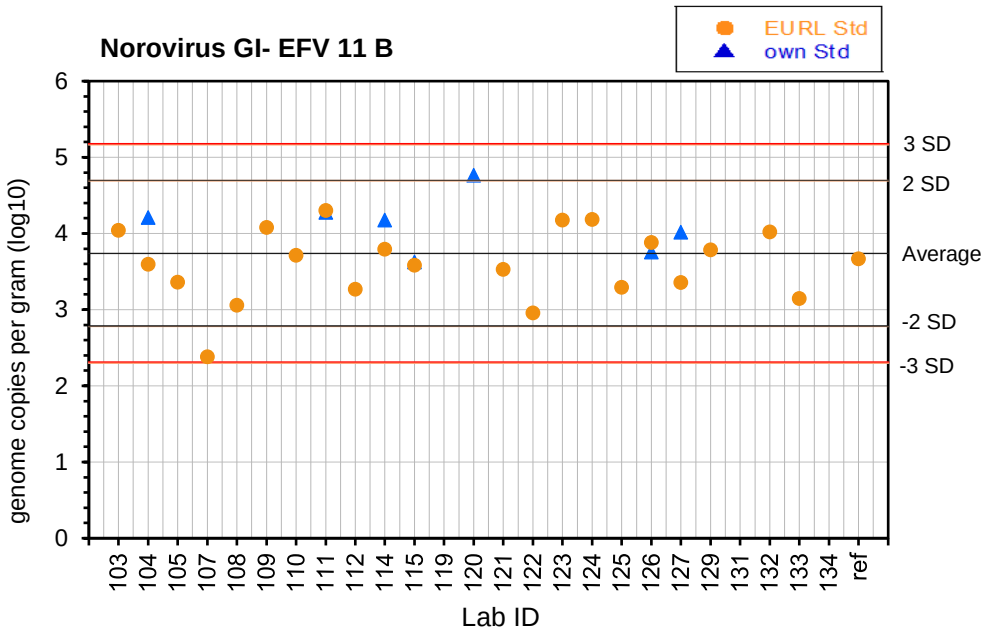
SD values are presented in table 7.

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

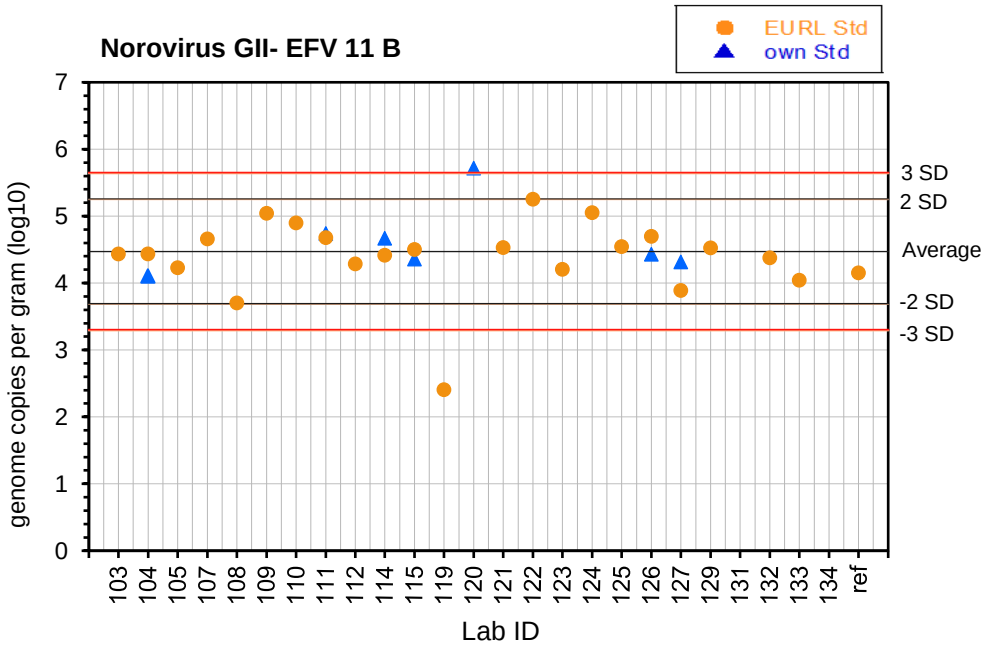
Lab ID	**GI** 23EFV11 B		**GII** 23EFV11 B		**HAV** 23EFV11 A	
	EURL STD	*Own STD*	*EURL STD*	*Own STD*	*EURL STD*	*Own STD*
103**	0,304		-0,035		0,257	
104*	-0,141	0,466	-0,036	-0,367	-0,072	-0,171
105*	-0,378		-0,239		-0,305	
107*	-1,358		0,187		-0,945	
108*	-0,682		-0,769		-1,547	
109*	0,336		0,570		0,258	
110*	-0,027		0,427		FN	
111*	0,558	0,536	0,210	0,269	0,038	-0,027
112*	-0,476		-0,182		0,479	
114*	0,049	0,433	-0,056	0,201	-0,008	0,139
115	-0,158	-0,118	0,034	-0,113	0,251	0,097
119*	FN		-2,063		-0,060	
120*		1,021		1,245		NR
121*	-0,212		0,061		-0,254	
122*	-0,787		0,785		-0,240	
123*	0,433		-0,264		0,433	
124*	0,442		0,583		0,426	
125	-0,448		0,075		-0,292	
126*	0,139	0,139	0,227	-0,038	NR[1]	NR[1]
127*	-0,387	0,274	-0,580	-0,157	-0,229	0,085
129*	0,044		0,058		-0,014	
131*	NR		NR		NR	
132*	0,278		-0,101		0,433	
133*	-0,597		-0,440		-0,661	
134*	FN		NR		NR	
Ref.	-0,075		-0,326		0,335	

** Designated EU/EFTA member state NRL, ** in designation process, FN: false negative, NR: not reported, NR[1]: only qualitative results were reported since this NRL do not perform quantification analysis for HAV*

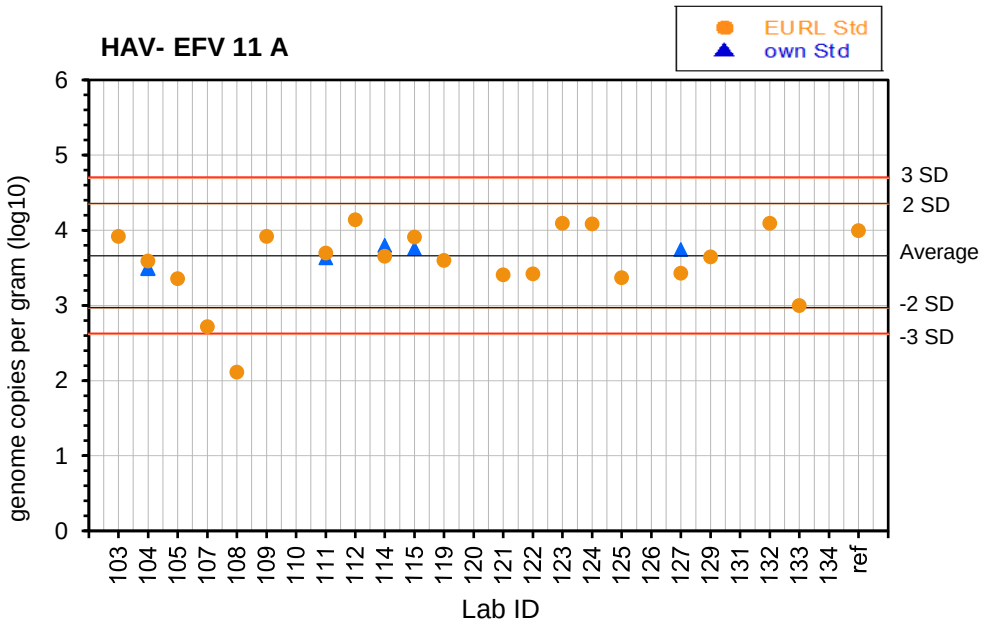
Graph 2: Distribution of results for norovirus GI in 23EFV11 B



Graph 3: Distribution of results for norovirus GII in 23EFV11 B



Graph 4: Distribution of results for HAV in 23EFV11 A



Inhibition and efficiency results

The results were additionally assessed based on inhibition and extraction efficiency outcomes. However, due to inability to offer laboratories a retest option, this evaluation was excluded from the performance assessment and scoring for qualitative results.

For quantitative results, the performance assessment and scoring followed the same procedure, with the exception of true positive results that were not quantifiable due to unacceptable inhibition and/or extraction efficiency. According to ISO 15216-1 and 2:

- Negative results are considered not valid in the absence of inhibition or/and extraction efficiency values, or in case of unacceptable inhibition (>2 Ct values or >75%) and/or extraction efficiency results (<1%), and should be reported as invalid.
- Positive results, despite unacceptable inhibition and extraction efficiency results, are considered valid and should be reported as "virus genome detected in (the amount of sample tested) g" followed by "not quantifiable".

All qualitative results reported as detected for HAV in sample A, norovirus GI, and GII in sample B are considered valid for scoring, regardless the inhibition and extraction efficiency values, since additional samples were not provided by EURL. Detailed results are presented in Annex B.

Methods used by the participants

Eleven laboratories were accredited according to ISO/IEC 17025 for the quantitative detection of norovirus GI and GII, and nine were accredited for HAV. All laboratories followed ISO 15216-1 except for one laboratory that does not perform quantitative detection of HAV. Detailed information on the methodologies used is provided in Appendix C.

Conclusion

The aim of PT EFV11, organized in November 2023 by the EURL for Foodborne Viruses, was to assess the NRLs' ability for quantitative detection of HAV, norovirus GI and norovirus GII in frozen minced oyster hepatopancreas samples and frozen oysters.

Twenty-five laboratories submitted their results for this PT. Furthermore, 80% of the participating laboratories obtained fully satisfactory results for qualitative analysis, while 60% achieved fully satisfactory results for quantitative analysis.

Annex A

Participant's results

with EURL standards with own standards false results

Lab ID	23EFV11 A				23EFV11 B					23EFV11 C		
	GI (Cq)	GII (Cq)	HAV (Cq)^{t}	HAV (c/g)^{t}	GI (Cq)^{t}	GI (c/g)^{t}	GII (Cq)^{t}	GII (c/g)^{t}	HAV (Cq)	GI (Cq)	GII (Cq	HAV (Cq)
103**	ND	ND	34.38	8.27E+03	28.26	1.10E+04	27.72	2.73E+04	ND	ND	ND	ND
104*	ND	ND	32,08	3.88E+03	32.31	3.96E+03	31.06	2.72E+04	ND	ND	ND	ND
104*	ND	ND	32.08	3.09E+03	32.31	1.62E+04	31.06	1.27E+04	ND	ND	ND	ND
105*	ND	ND	35.7	2.27E+03	33.2	2.29E+03	28.8	1.71E+04	ND	ND	ND	ND
107*	ND	ND	34.61	5.20E+02	31.91	2.40E+02	27.27	4.55E+04	ND	ND	ND	ND
108*	ND	ND	31.91	1.30E+02	31.33	1.15E+03	27.67	5.03E+03	ND	ND	ND	ND
109*	ND	ND	33.03	8.30E+03	31.74	1.2E+04	27.45	1.1E+05	ND	ND	ND	ND
110*	ND	ND	ND	ND	30.16	5.2E+03	28.06	7.9E+04	ND	ND	ND	ND
111*	ND	ND	32.57	5.0E+03	31.10	2.0E+04	29.35	4.80E+04	ND	ND	ND	ND
111*	ND	ND	32.57	4.3E+03	31.10	1.9E+04	29.35	5.5E+04	ND	ND	ND	ND
112*	ND	ND	29.33	1.38E+04	34.70	1.85E+03	31.21	1.95E+04	ND	ND	ND	ND
114*	ND	ND	32.67	4.5E+03	31.51	6.2E+03	29.72	2.6E+04	ND	ND	ND	ND
114*	ND	ND	32.67	6.3E+03	31.51	1.5E+04	29.72	4.7E+04	ND	ND	ND	ND
115	ND	ND	32.99	8.17E+03	33.19	3.85E+03	31.08	3.20E+04	ND	ND	ND	ND
115	ND	ND	32.99	5.73E+03	33.19	4.21E+03	31.08	2.28E+04	ND	ND	ND	ND
119*	ND	ND	31.50	3.99E+03	ND	ND	35.23	2.56E+02	ND	ND	ND	ND
120*	ND	ND	NR	NR	33.15	5.80E+04	28.60	5.20E+05	NR	ND	ND	NR
121*	ND	ND	34.95	2.55E+03	33.49	3.40E+03	31.27	3.41E+04	ND	ND	ND	ND
122*	ND	ND	32.75	2.64E+03	32.54	9.04E+02	28.04	1.80E+05	ND	ND	ND	ND

**Designated EU/EFTA member state NRL, ** in designation process, #Reference results from day 2, D: reported as detected, ND: reported as not detected, NR: not reported, t: target virus*

Lab ID	23EFV11 A				23EFV11 B					23EFV11 C		
	GI (Cq)	GII (Cq)	HAV (Cq)^{t}	HAV (c/g)^{t}	GI (Cq)^{t}	GI (c/g)^{t}	GII (Cq)^{t}	GII (c/g)^{t}	HAV (Cq)	GI (Cq)	GII (Cq	HAV (Cq)
123*	ND	ND	31.7	1.24E+04	30.0	1.50E+04	28.5	1.61E+04	ND	ND	ND	ND
124*	ND	ND	33.25	1.22E+04	31.95	1.53E+04	31.11	1.13E+05	ND	ND	ND	ND
125*	ND	ND	33.21	2.34E+03	33.26	1.97E+03	28.62	3.52E+04	ND	ND	ND	ND
126*	ND	ND	34.89	NR[1]	32.95	7.63E+03	29.79	4.99E+04	ND	ND	ND	ND
126*	ND	ND	34.89	NR[1]	32.95	7.63E+03	29.79	4.99E+04	ND	ND	ND	ND
127*	ND	ND	32	2.70E+03	30.60	5.69+03	28.43	2.71E+04	ND	ND	ND	ND
127*	ND	ND	32	5.57E+03	30.60	1.04E+04	28.43	2.06E+04	ND	ND	ND	ND
129*	ND	ND	33.79	4.43E+03	31.86	6.13E+03	30.96	3.38E+04	ND	ND	ND	ND
131*	ND	ND	32.48	NR	30.5	NR	36.5	NR	ND	ND	ND	ND
132*	ND	ND	30.24	1.24E+04	29.68	1.05E+04	32.12	2.40E+04	ND	ND	ND	ND
133*	ND	ND	35.05	1.00E+03	34.10	1.40E+03	31.88	1.10E+04	ND	ND	ND	ND
134*	ND	ND	31.54	NR	ND	ND	35.59	NR	ND	ND	ND	ND
Ref.[#]	ND	ND	32.92	9.91E+03	32.6	4.66E+03	27.9	1.43E+04	ND	ND	ND	ND

**Designated EU/EFTA member state NRL, ** in designation process, [#]Reference results from day 2, D: reported as detected, ND: reported as not detected, NR[1]: only qualitative results were reported since this NRL do not perform quantification analysis for HAV, [t]: target virus*

Annex B

Inhibition and extraction efficiency results

Inhibition and extraction efficiency results for sample 23EFV11 A

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV^{t}		GI	GII	HAV
103**	A	A	A	A	V	V	V
104*	A	A	A	A	V	V	V
105*	A	A	A	A	V	V	V
107*	A	A	A	A	V	V	V
108*	A	A	A	A	V	V	V
109*	A	A	A	A	V	V	V
110*	A	A	A (FN)	A	V	V	FN
111*	A	A	A	A	V	V	V
112*	A	A	A	A	V	V	V
114*	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
119*	A	A	A	U	NV	NV	V
120*	A	A	NR	A	V	V	NR
121*	A	A	U	A	V	V	V
122*	A	A	A	A	V	V	V
123*	A	A	A	A	V	V	V
124*	A	A	A	A	V	V	V
125	A	A	A	A	V	V	V
126*	A	A	A	A	V	V	V
127*	A	A	A	A	V	V	V
129*	A	A	A	A	V	V	V
131*	NR	NR	NR	U	NV	NV	V
132*	A	A	A	A	V	V	V
133*	A	A	A	A	V	V	V
134*	A	A	A	A	V	V	V

** Designated EU/EFTA member state NRL, ** in designation process*

A: Acceptable, FN: false negative, FP: false positive, NR: not reported, NV: not valid, ^{t}: target virus, U: Unacceptable, V: valid results

Inhibition and extraction efficiency results for sample 23EFV11 B

	Inhibition			Efficiency	Results		
Lab. ID	GI[t]	GII[t]	HAV		GI	GII	HAV
103**	A	A	A	A	V	V	V
104*	A	A	A	A	V	V	V
105*	A	A	A	A	V	V	V
107*	A	A	A	A	V	V	V
108*	A	A	A	A	V	V	V
109*	A	A	A	A	V	V	V
110*	A	A	A	A	V	V	V
111*	A	A	A	A	V	V	V
112*	A	A	A	A	V	V	V
114*	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
119*	A (FN)	U	A	U	FN	V	NV
120*	A	A	NR	A	V	V	NR
121*	A	A	U	A	V	V	NV
122*	A	A	A	A	A	A	A
123*	A	A	A	A	V	V	V
124*	A	A	A	A	V	V	V
125	A	A	A	A	V	V	V
126*	A	A	A	A	V	V	V
127*	A	A	A	A	V	V	V
129*	A	A	A	U	V	V	NV
131*	NR[1,2]	NR[1,2]	NR	U	V	V	NV
132*	A	A	A	A	V	V	V
133*	A	A	A	A	V	V	V
134*	A (FN)	A	A	A	FN	V	V

** Designated EU/EFTA member state NRL, ** in designation process*
A: Acceptable, FN: false negative, FP: false positive, NR: not reported, NR[1,2]: not reported 1 inhibition, 2 detected/not detected results, NV: not valid, [t]: target virus,
U: Unacceptable, V: valid results

Inhibition and extraction efficiency results for sample 23EFV11 C

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV		GI	GII	HAV
103**	A	A	A	A	V	V	V
104*	A	A	A	A	V	V	V
105*	A	A	A	A	V	V	V
107*	A	A	A	A	V	V	V
108*	A	A	A	A	V	V	V
109*	A	A	A	A	V	V	V
110*	A	A	A	A	V	V	V
111*	A	A	A	A	V	V	V
112*	A	A	A	A	V	V	V
114*	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
119*	A	A	A	U	NV	NV	NV
120*	A	A	NR	A	V	V	NR
121*	A	A	A	A	V	V	V
122*	A	A	A	A	V	V	V
123*	A	A	A	A	V	V	V
124*	A	A	A	A	V	V	V
125	A	A	A	A	V	V	V
126*	A	A	A	A	V	V	V
127*	A	A	A	A	V	V	V
129*	A	A	A	U	NV	NV	NV
131*	NR	NR	NR	U	NV	NV	NV
132*	A	A	A	A	V	V	V
133*	A	A	A	A	V	V	V
134*	A	A	A	A	V	V	V

** Designated EU/EFTA member state NRL, ** in designation process*

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

Annex C

General information on methods

Lab. ID	1	2	3	4	5	6	7
103**	A	D	H	N	R		X
104*	A	D	H	J	R	UV	W
105*	A	D	H	J	R TM9	UV	Wi
107*	A	E	H	P	R	UV	Za
108*	A	D	H	L	T		X
109*	A	D	H	O	R		Yy
110*	A	F	H	M	R TM9		W
111*	A	D	H	N	R		Yy
112*	A	E	H	J	R		Zq
114*	A	D	H	J	R	UV	Z
115	A	D	H	J	R TM9	UV	Zb
119*	A	G	H	J	R	UV	Zzqq
120*	A	D	H	J	S R TM9	UV	X
121*	A	D	H	J	R	UV	Zq
122*	A	D	H	J	R		Xx
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	Yr
127*	A	D	H	J	R	U	Xa
129*	A	D	H	L	T		W
131*	A	D	H	M	T		Zq
132*	A	D	H	J	R		Zqq
133*	A	Gg	H	Q	?		Yr
134*	A	Ff	H	J	R		Za

** Designated EU/EFTA member state NRL, ** in designation process*

Key to method codes

1. Virus isolation and concentration method	
A	ISO 15216-1
C	ISO 15216-2
2. RNA extraction methods/reagents	
D	NucliSens® (BioMérieux)
E	NucliSens® (BioMérieux), TANBead Maelstrom™
F	NucliSens® (BioMérieux), alternative robot system QuikPick Tool
G	PureLink™ Viral RNA/DNA Mini Kit
Gg	QIAamp Viral RNA Mini Kit
Ff	spin Syngen Viral Mini Kit
3. PCR method RT-PCR	
H	One step
4. RT-PCR reagents	
J	RNA UltraSense™ One-Step Quantitative RT-PCR System
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	Luna® Universal Probe One-Step RT-qPCR Kit
P	GoTaq® Probe 1-Step RT-qPCR System
Q	Norovirus Genogroups 1 and 2 genesig Advanced Kit
5. Primers and probes	
R	ISO 15216 *(The probe, NVGG1p or TM9, for norovirus GI was not asked to be specified)*
S	AriaMx

T	CeeramTools®

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

Livsmedelsverket
Swedish Food Agency

European Union Reference Laboratory
for Foodborne Viruses