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for Foodborne Viruses

EURL – FOODBORNE VIRUSES

Final report

Proficiency testing scheme EFV 09, 2022

Quantification of norovirus and hepatitis A virus in bivalve molluscan shellfish

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Summary

This report describes the performance of NRLs for quantitative detection of viral contamination in bivalve molluscan shellfish in PT scheme EFV09, organised by the EURL for Foodborne Viruses. Distribution was made on 14th of November 2022 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 detection of norovirus and hepatitis A virus 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 the participants who have requested them in advance.

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.

Background

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.

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 the sample preparation and the levels of spiking are demonstrated in Table 1 and 2 respectively.

Table 1: Description of the viruses used for the PT EFV 09

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 09 samples

Sample	Norovirus GI	Norovirus GII	HAV
22EFV09 A	–	–	–
22EFV09 B	–	–	–
22EFV09 C	≈10^4*	≈10^3*	≈10^4*

**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. 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 proficiency testing items

Samples were dispatched on dry ice by courier in accordance with IATA packing instructions 650 for UN3373, on November 14th. 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 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.

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, eighteen random samples of 22EFV09C were tested. Three samples were tested immediately after the inoculation, three samples were tested on the dispatch date (November 14th) after storing in -20 °C for 48 hours (d0), and the rest of samples were transferred to dry ice container on the dispatch date for 24 hours. Five samples were tested the day after (day 1) and the rest of samples were stored in -20 °C and five samples were tested at day 2. Two samples were tested a day after deadline (November 28th) as an extra stability check.

Samples were analysed according to EURL SOP based on ISO 15216-1 for the quantification of target viruses respectively. The results (d1- d2) are shown in Table 3 and 4, with box and whisker plots included in Graph 1. The results of one reference sample from day 2 were used in performance assessment and scoring and are presented in this report as Ref. Inhibition and extraction efficiency were calculated for all the reference samples. The PT samples are considered to be homogenous enough for noroviruses and for trial 09 purposes.

Table 3: Qualitative results for reference samples for PT EFV 09

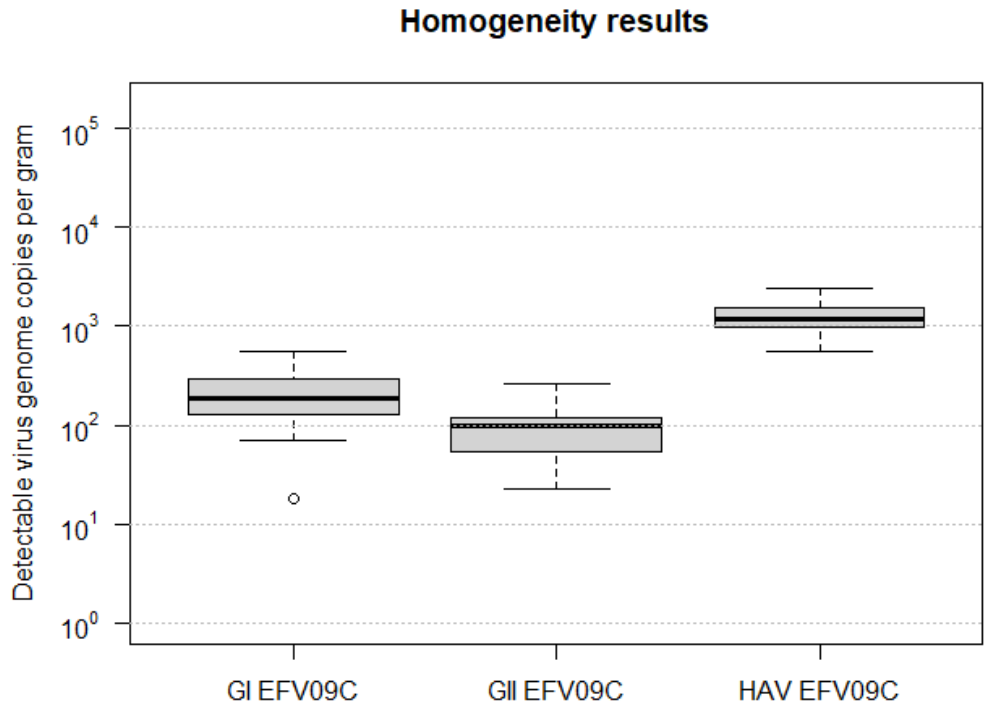
Sample	Norovirus GI	Norovirus GII	HAV
22EFV09 A	Not detected	Not detected	Not detected
22EFV09 B	Not detected	Not detected	Not detected
22EFV09 C	Detected	Detected	Detected

Table 4: Quantitative results for ten reference samples for PT EFV 09
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
22EFV09 A	Not detected	Not detected	Not detected
22EFV09 B	Not detected	Not detected	Not detected
22EFV09 C	$3.51 \times 10^1 – 8.28 \times 10^2$*	$1.86 \times 10^1 – 3.50 \times 10^2$*	$5.75 \times 10^2 – 2.59 \times 10^3$*

**detectable virus genome copies per gram sample*

Graph 1: Box and whisker plots for homogeneity test of samples 22EFV09 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 (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

[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/.

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 0.539 for Norovirus GI, 0.513 for Norovirus GII and 0.389 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 EFV09C	0.1	**yes**	No
	0.4	**yes**	No
	0.5	**yes**	**Yes**
GII EFV09C	**0.1**	**yes**	**Yes**
HAV EFV09C	0.1	no	No
	0.3	**yes**	no
	0.5	**yes**	**yes**

Results and discussion

Samples were sent to 23 laboratories (including 19 NRLs and one in designation process) and 22 laboratories returned their results. All laboratories except two (which received the samples on November 16th and 18th) received the samples on November 15th (one day after dispatch) and the majority of the participants analysed the samples within the first few days upon arrival.

All laboratories reported true results for sample B and the majority reported true results for sample A. However, some false negative results were reported for sample C.

One NRL did not report any qualitative and quantitative results for any samples and only reported inhibition and extraction efficiency results.

Furthermore, the number of none valid negative results was on average around five in each sample types and for all agents. Overview of results is demonstrated in Table 6.

Detailed information about the participating laboratories results can be found in Annex A. The results of references samples analysed at day 2 are presented as Ref.

Table 6: Overview of participants´ results for samples 22EFV09 A, B and C

Target viruses	N	22EFV09 A				22EFV09 B				22EFV09 C			
		T*	FP	FN	NV	T*	FP	FN	NV	T*	FP	FN	NV
Norovirus GI	22	21	0	-	2	21	0	-	1	20	-	1	-
Norovirus GII	22	20	1	-	2	21	0	-	2	17	-	4	-
Hepatitis A virus	22	21	0	-	1	21	0	-	1	20	-	1	-

**: 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 the results were 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 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	22EFV09 C GI	22EFV09 C GII	22EFV09 C HAV
Average	3.063	2.395	3.950
SD	0.539	0.513	0.389

-Values in log10 copies/g

- The results of references samples analysed at day 2 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.

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

- 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 included in the score calculations and are presented as Ref.

Scoring results are shown 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	4 out of 6[fn]	6 out of 6	6 out of 6	4[fn] 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	6 out of 6	6 out of 6	4 out of 6
108*	6 out of 6	6 out of 6	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	6 out of 6	6 out of 6
110*	6 out of 6	4 out of 6[fn]	4 out of 6[fn]	6 out of 6	4[fn] out of 6	4[fn] out of 6
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	4 out of 6[fp]	6 out of 6	6 out of 6	6 out of 6	6 out of 6
119*	4 out of 6[fn]	4 out of 6[fn]	6 out of 6	4[fn] out of 6	4[fn] out of 6	5 out of 6
120*	6 out of 6[ei]	6 out of 6[ei]	6 out of 6[ei]	6 out of 6	6 out of 6	6 out of 6
122*	0 out of 6[ei]	0 out of 6[ei]	0 out of 6[ei]	0[nr] out of 6	0[nr] out of 6	0[nr] 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[i]	6 out of 6[i]	6 out of 6[i]	5 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	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
132*	6 out of 6	4 out of 6[fn]	6 out of 6	6 out of 6	4[fn] out of 6	6 out of 6

** Designated EU/EFTA member state NRL*

[e]: unacceptable extraction efficiency, [fn]: false negative, [fp]: false positive, [i]: unacceptable inhibition

Table 9: Differences between participants' results and the participants' mean presented in terms of SD.

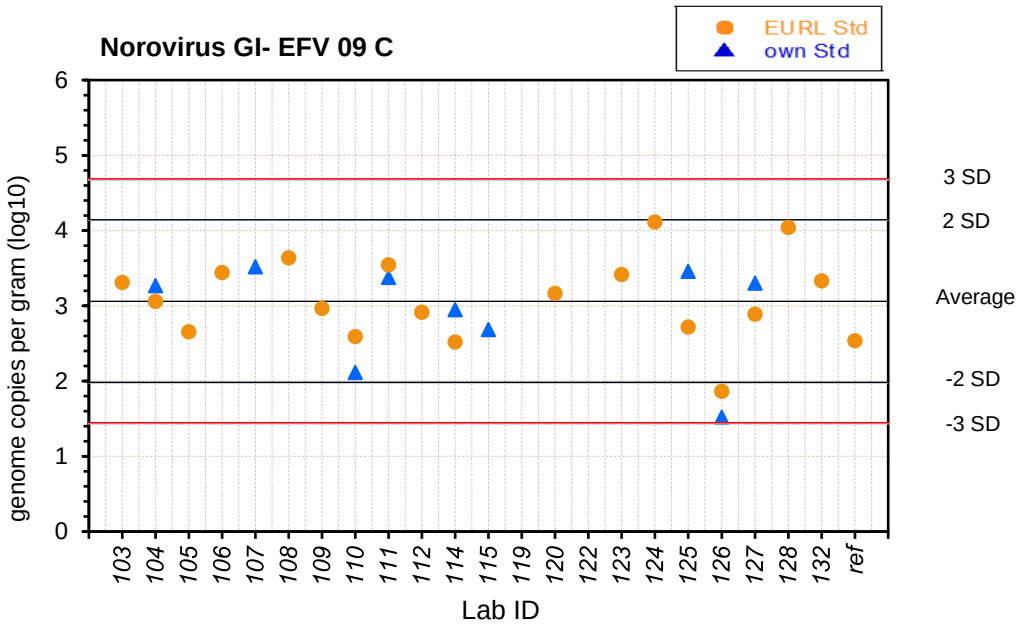
All the laboratories received EURL quantification standards together with PT materials, therefore some participants provided two sets of results determined by both EURL and their own standards. In such cases, only the results using their own standards were considered for performance scoring. However,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

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

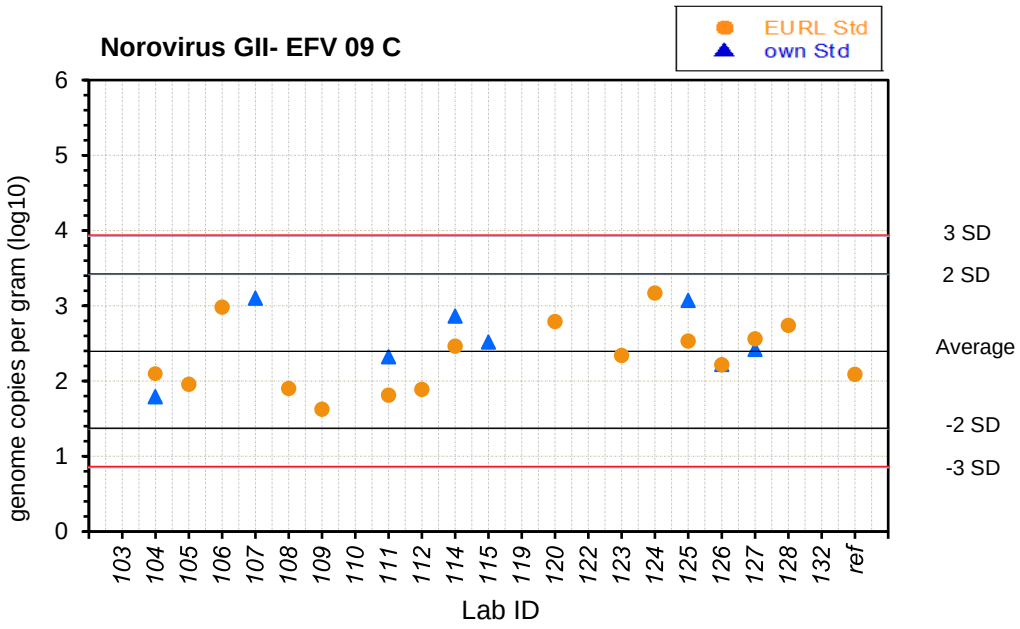
Lab ID	**GI** 22EFV09 C		**GII** 22EFV09 C		**HAV** 22EFV09 C	
	EURL STD	*Own STD*	*EURL STD*	*Own STD*	*EURL STD*	*Own STD*
103	-0.247		FN		-0.091	
104*	-0.002	0.206	-0.298	-0.606	0.042	0.026
105*	-0.409		-0.436		-0.381	
106*	0.378		0.590		0.236	
107*		0.455		0.709		-1.483
108*	0.576		-0.493		0.024	
109*	-0.096		-0.770		0.021	
110*	-0.472	-0.949	FN	FN	FN	FN
111*	0.481	0.317	-0.582	-0.073	0.050	-0.021
112*	-0.146		-0.503		0.536	
114*	-0.545	-0.114	0.067	0.468	-0.307	0.254
115		-0.377		0.123		-0.049
119*	FN		FN		-0.950	
120*	0.102		0.395		-0.195	
122*	NR		NR		NR	
123*	0.353		-0.055		-0.010	
124*	1.050		0.777		0.618	
125	-0.345	0.395	0.136	0.678	-0.337	0.273
126*	-1.200	-1.545	-0.178	-0.175	NR[1]	NR[1]
127*	-0.175	0.238	0.166	0.026	0.925	0.460
128*	0.978		0.345		0.129	
132*	0.267		FN		-0.075	
Ref.	-0.527		-0.306		-0.708	

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

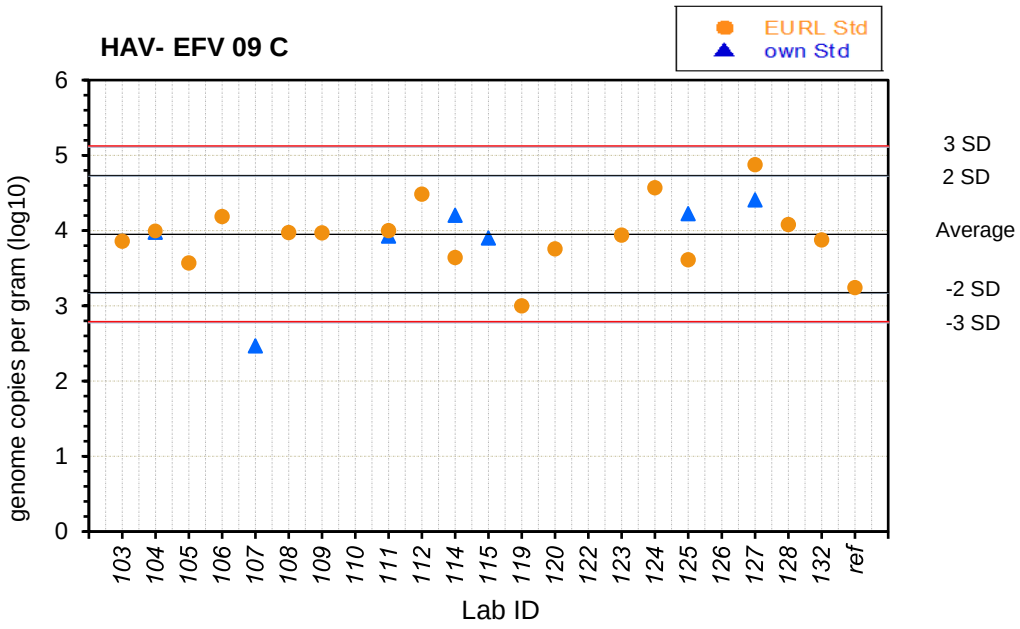
Graph 2: Distribution of results for norovirus GI in 22EFV09 C



Graph 3: Distribution of results for norovirus GII in 22EFV09 C



Graph 4: Distribution of results for HAV in 22EFV09 C



Inhibition and efficiency results

The results were also evaluated based on inhibition and extraction efficiency outcomes.

All laboratories except two, reported acceptable extraction efficiency in all samples. One NRL (Lab ID: 122) reported unacceptable extraction efficiency in all samples and reported unacceptable inhibition results for norovirus GII for sample B and for norovirus GI in sample A and B.

Another NRL (Lab ID: 120) did not report extraction efficiency and inhibition results for samples A and B. One NRL (Lab ID: 126) did not report inhibition results for hepatitis A virus in any samples.

Since it was not possible to provide the laboratories with a retest option, this evaluation is not a part of performance assessment and scoring for qualitative results. Performance assessment and scoring for qualitative results follows the same path except for true positive results, which were not quantifiable due to unacceptable inhibition and/or extraction efficiency).

According to ISO 15216-1 and 2, negative results are not valid in absence of inhibition or/and extraction efficiency values as well as in case of unacceptable inhibition (>2 Ct values or >75%) or/and extraction efficiency results (<1%) 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 qualitative results reported as detected for norovirus GI, norovirus GII and HAV in sample C, are valid regardless the inhibition and extraction efficiency values, since EURL does not provide extra samples. Results are presented in Annex B.

Methods used by the participants

Nine laboratories were accredited according to ISO/IEC 17025 for quantitative detection of norovirus GI, norovirus GII and seven for HAV. All the laboratories followed ISO 15216-1 with exception of one laboratory which does not perform quantitative detection of HAV. Detailed information on the methodologies used is shown in Appendix C.

Conclusion

The aim of PT EFV09 organized in November 2022 by 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.

Twenty-two laboratories submitted their results for this PT. Furthermore, 73 % and 68% of the participating laboratories obtained full satisfactory results for qualitative and quantitative analysis respectively.

Annex A

Participant's results

with EURL standards with own standards false results

Lab. ID No.	22EFV09 A			22EFV09 B			22EFV09 C					
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)	HAV (Cq)	HAV (c/g)
103	ND	ND	ND	ND	ND	ND	32.49	2.04E+03	ND	ND < 40	31.89	7.23E+03
104*	ND	ND	ND	ND	ND	ND	35.47	1.15E+03	39.45	1.25E+02	30.9	9.82E+03
104*	ND	ND	ND	ND	ND	ND	35.47	1.86E+03	39.45	6.16E+01	30.9	9.46E+03
105*	ND	ND	ND	ND	ND	ND	35.77	4.51E+02	35.71	9.10E+01	34.25	3.71E+03
106*	ND	ND	ND	ND	ND	ND	35.08	2.76E+03	36.12	9.66E+02	32.82	1.54E+04
107*	ND	ND	ND	ND	ND	ND	34.47	3.30E+03	36.61	1.27E+03	33.24	2.93E+02
108*	ND	ND	ND	ND	ND	ND	32.23	4.36E+03	35.23	7.98E+01	30.6	9.42E+03
109*	ND	ND	ND	ND	ND	ND	35.19	9.27E+02	36.1	4.22E+01	33.46	9.35E+03
110*	ND	ND	ND	ND	ND	ND	35.37	3.90E+02	ND	ND	ND	ND
110*	ND	ND	ND	ND	ND	ND	35.37	1.30E+02	ND	ND	ND	ND
111*	ND	ND	ND	ND	ND	ND	34.44	3.50E+03	39.11	6.50E+01	32.09	1.00E+04
111*	ND	ND	ND	ND	ND	ND	34.44	2.40E+03	39.11	2.10E+02	32.09	8.50E+03
112*	ND	ND	ND	ND	ND	ND	36.44	8.26E+02	38.82	7.80E+01	29.72	3.06E+04
114*	ND (45)	ND (45)	ND (45)	ND (45)	ND (45)	ND (45)	36.95	3.30E+02	34.51	2.90E+02	32.205	4.40E+03
114*	ND (45)	ND (45)	ND (45)	ND (45)	ND (45)	ND (45)	36.95	8.90E+02	34.51	7.30E+02	32.205	1.60E+04
115	ND	D	ND	ND	ND	ND	36.62	4.86E+02	37.38	3.30E+02	32.96	7.98E+03
119*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	39.91	1.00E+03
120*	ND	ND	ND	ND	ND	ND	36.4	1.46E+03	35.35	6.17E+02	34.63	5.69E+03
122*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
123*	ND	ND	ND	ND	ND	ND	33	2.61E+03	34.9	2.19E+02	32.4	8.72E+03

** Designated EU/EFTA member state NRL, ** Reference results from day 2, D: reported as detected, ND: reported as not detected*

Lab. ID No.	22EFV09 A			22EFV09 B			22EFV09 C					
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GI (c/g)	GII (Cq)	GII (c/g)	HAV (Cq)	HAV (c/g)
124*	ND	ND	ND	ND	ND	ND	33.01	1.30E+04	35.35	1,49E+03	30.89	3.70E+04
125	ND	ND	ND	ND	ND	ND	35.32	5.23E+02	36.53	3.40E+02	32.24	4.10E+03
125	ND	ND	ND	ND	ND	ND	35.32	2.87E+03	36.53	1.18E+03	32.24	1.67E+04
126*	ND	ND	ND	ND	ND	ND	40.78	7.30E+01	38.86	1.65E+02	33.2	NR[1]
126*	ND	ND	ND	ND	ND	ND	40.78	3.30E+01	38.86	1.66E+02	33.2	NR[1]
127*	ND	ND	ND	ND	ND	ND	34.24	7.73E+02	35.68	3.64E+02	30.02	7.50E+04
127*	ND	ND	ND	ND	ND	ND	34.24	2.00E+03	35.68	2.64E+02	30.02	2.57E+04
128*	ND	ND	ND	ND	ND	ND	36.06	1.1E+04	40.73	5.5E+02	35.52	1.2E+04
132*	ND	ND	ND	ND	ND	ND	33.26	2.14E+03	ND	ND	33.37	7.50E+03
Ref.**	ND	ND	ND	ND	ND	ND	35.87	3.44E+02	35.83	1.23E+02	35.01	1.75E+03

** Designated EU/EFTA member state NRL, ** 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.*

Annex B

Inhibition and extraction efficiency results

Inhibition and extraction efficiency results for sample 22EFV09 A

	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
106*	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	FP	A	A	V	FP	V
119*	A	A	A	V	V	V	V
120*	NR	NR	NR	NR	NV	NV	NV
122*	NV	NV	A	NV	NV	NV	NV
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	NR	A	V	V	NV
127*	A	A	A	A	V	V	V
128*	A	A	A	A	V	V	V
132*	A	A	A	A	V	V	V

** Designated EU/EFTA member state NRL*

A: Acceptable, FN: false negative, FP: false positive, NR: not reported, NV: not valid, U: Unacceptable, V: valid results

Inhibition and extraction efficiency results for sample 22EFV09 B

	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
106*	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	A	V
119*	A	A	A	V	V	V	V
120*	NR	NR	NR	NR	NV	NV	NV
122*	A	NV	A	NV	NV	NV	NV
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	NR	A	V	V	NV
127*	A	A	A	A	V	V	V
128*	A	A	A	A	V	V	V
132*	A	A	A	A	V	V	V

** Designated EU/EFTA member state NRL*
A: Acceptable, FN: false negative, FP: false positive, NR: not reported, NV: not valid, U: Unacceptable, V: valid results

Inhibition and extraction efficiency results for sample 22EFV09 C

	Inhibition			Efficiency	Results		
Lab. ID	GI*	GII*	HAV*		GI*	GII*	HAV*
103	A	FN	A	A	V	FN	V
104*	A	A	A	A	V	V	V
105*	A	A	A	A	V	V	V
106*	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	FN	FN	A	V	FN	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	A	V
119*	FN	FN	A	V	FN	FN	V
120*	A	A	A	V	V	V	V
122*	A	A	A	NV	NV	NV	NV
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	NR	A	V	V	NV
127*	A	A	A	A	V	V	V
128*	A	A	A	A	V	V	V
132*	A	FN	A	A	V	FN	V

** Designated EU/EFTA member state NRL*

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	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		Yr
107*	A	E	H	P	S	UV	Za
108*	A	D	H	L	T		X
109*	A	G	H	O	R		V
110*	A	F	H	M	R TM9		W
111*	A	D	H	N	R		Yr
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	Ff	H	J	R		zzqq
120*	A	D	H	J	R TM9		X
122*	A	Gg	H	M	R		Xx
123*	A	D	H	J	R		X
124*	A	D	H	J	R TM9		Wii
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
128*	A, C	D	H	J	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
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	VIRSeek
Gg	QIAamp Viral RNA Mini Kit
Ff	MagMAX™
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	QuantiNova™Probe RT-PCR Kit (Qiagen)
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
T	CeeramTools®

6. Accreditation	
U	Norovirus
V	HAV
7. PCR system	
V	Qtower³ Analytik Jena
W	CFX96™ Real-Time PCR Detection System (Biorad)
Wi	LightCycler® 96 System (Roche)
Wii	LightCycler® 480 System (Roche)
X	AriaMx Real-time PCR System
Xx	AriaDx Real-time PCR System
Xa	Mx3000P qPCR Systems
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
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