Livsmedelsverket
National Food Agency

European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

FINAL REPORT

PROFICIENCY TESTING SCHEME EFV 02, 2019

Detection of norovirus and hepatitis A virus in raspberries

Final Report – Version 1 (03/09/2019)

National Food Agency Reference: 2019/02376

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CONTENTS

INTRODUCTION

This report describes the performance of NRLs to detect viral contamination of raspberries in proficiency testing (PT) scheme EFV02, organised by the European Union Reference Laboratory (EURL) for Foodborne Viruses to support the official controls on foodborne viruses in line with Regulation (EU) 2017/625.

Distribution was made 6th of May 2019 to nineteen laboratories that signed up to take part in the PT and was designed for the detection of hepatitis A virus (HAV) and norovirus genogroup I (GI) and genogroup II (GII) in three samples of frozen raspberries.

The participating laboratories were requested to examine the samples using their routine method, however the EURL recommended to analyse the samples according to ISO/TS 15216-2. A Standard Operating Procedure (SOP) for qualitative detection of norovirus and hepatitis A virus in soft fruit, based on ISO/TS 15216-2, was therefore provided.

External control (EC) RNA and process control virus were distributed together with PT samples, upon request.

LEGISLATION

The Swedish National Food Agency has been appointed EURL for Foodborne Viruses according to Regulation (EU) 2017/625, since 2018. Under Article 94, the EURL is responsible to organise 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.

Due to the temporary legislation to control norovirus in imported frozen raspberries from Serbia (EU 669/2009, EU 2298/2017), PT EFV 02 prioritised the identification of HAV, norovirus GI and norovirus GII in soft fruits, in conjunction with implementing the EURL SOP, based on ISO/TS 15216-2, to contribute to method harmonisation (EU 2017/625).

SAMPLES

Materials dispatched consisted of artificially contaminated frozen raspberries 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 02

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.6 (capsid sequence)

For each virus, sample A and B were inoculated with approximately 10^5 virus genome copies per 25 gram raspberries and sample C was spiked with approximately 10^4 virus genome copies per 25 gram raspberries. Intended results are shown in Table 2.

Table 2: Intended results of PT EFV 02
Results are presented as detected/ not detected in 25 gram sample

Sample	Norovirus GI	Norovirus GII	HAV	Spiked virus genome copies/25 gram raspberries
19EFV02 A	detected	detected	detected	10^5
19EFV02 B	not detected	not detected	detected	10^5
19EFV02 C	not detected	detected	not detected	10^4

DISTRIBUTION OF THE PROFIENCY TEST ITEMS

Samples were inoculated and dispatched on dry ice by courier in accordance with IATA packing instructions 650 for UN3373, on May 6th. All nineteen laboratories received three frozen raspberry samples and the ones that so requested also received EC RNA and/or process control virus (mengovirus). 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 24th.

CONFIDENTIALITY

In order to ensure confidentiality, all participants are assigned a unique laboratory identification number. Only staff within the proficiency testing 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 (long-term) performance assessment.

QUALITY CONTROL

Frozen raspberries used to produce the test items were purchased from a retail and tested negative for HAV, norovirus GI and norovirus GII. Inoculated material was also examined for homogeneity of samples, as well as stability of inoculated viruses.

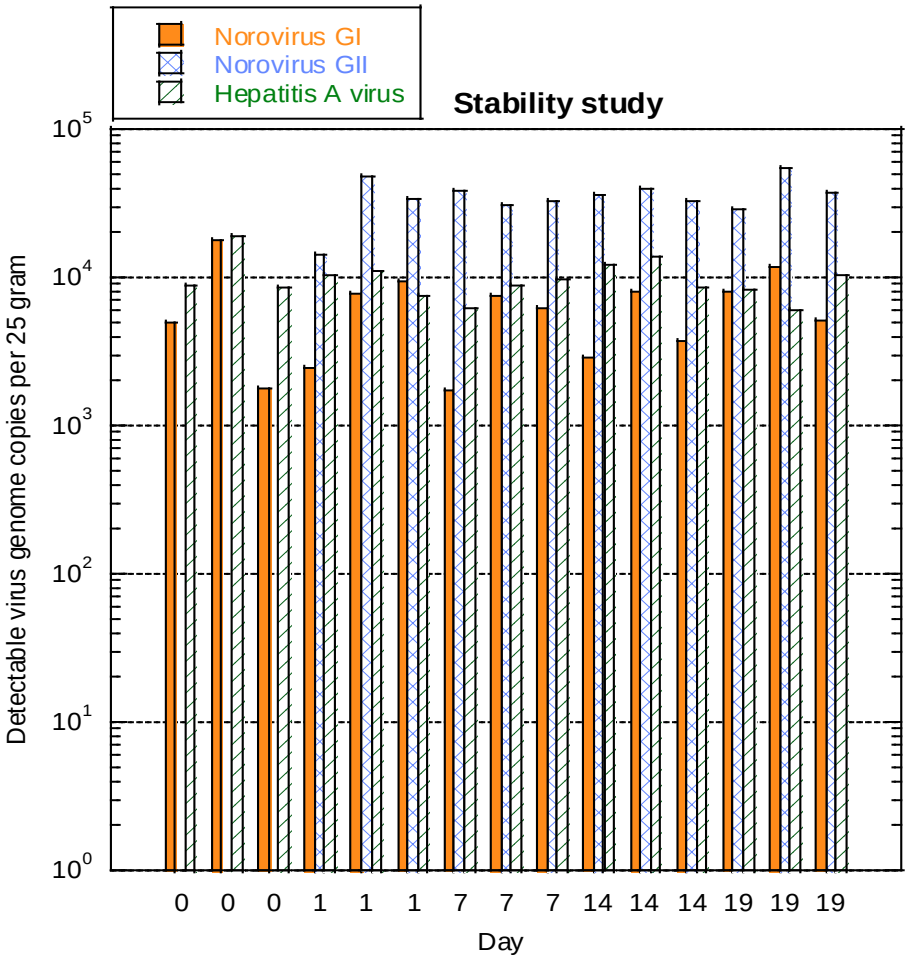
STABILITY OF VIRUS LEVELS IN RASPBERRY SAMPLES

In order to investigate the stability of inoculated viruses in raspberry samples stored in freezer, a stability study was conducted prior to dispatch. Twelve samples were inoculated with the target viruses and kept on dry ice overnight. Three samples were tested the day after (day 0), and the rest of samples were stored in -20 °C and analysed at day 1, 7, 14 and 19.

Qualitative analyses were performed according to EURL SOP based on ISO/TS 15216-2 and quantitative analyses according to ISO 15216-1.

The results indicated that the level of detectable virus genome copies after 24 hours storage on dry ice was reduced approximately by one $\log_{10}$ compared to virus genome copies spiked to the sample. After day 0, virus levels were considered stable up to 19 days when stored at -20 °C. Therefore, the participants were given a period of approximately 19 days to perform the analyses and report their results. Stability results are demonstrated in graph 1.

Graph 1: Stability study of frozen raspberries inoculated with the target viruses used for PT EFV 02.
Results for norovirus GII day 0 are not included due to problems during centrifugation.



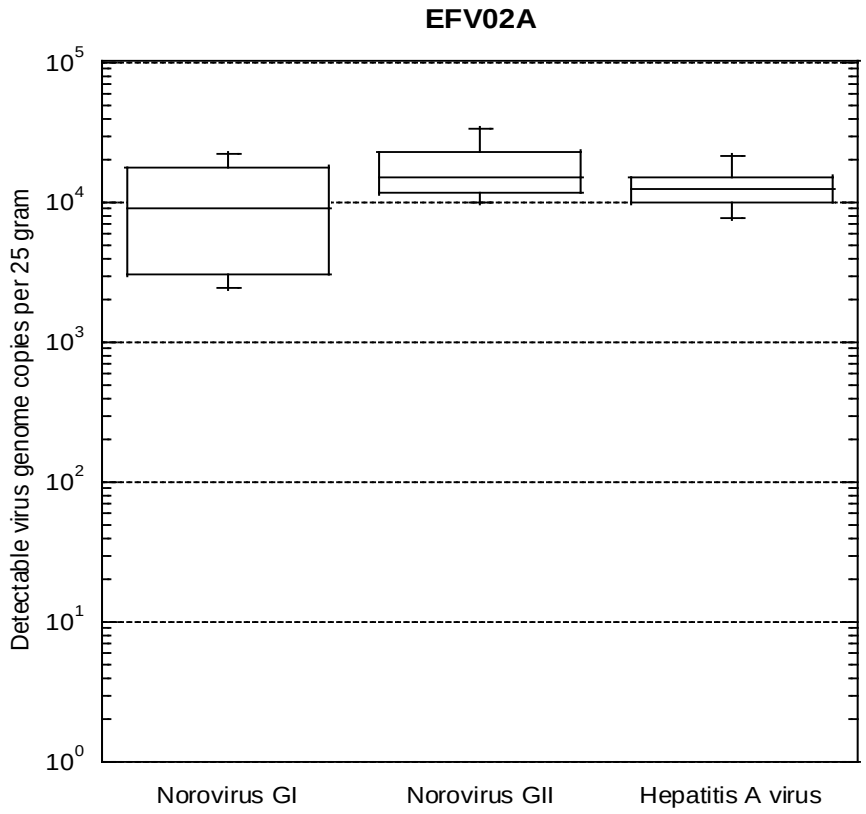
REFERENCE RESULTS AND HOMOGENEITY OF VIRUS LEVELS IN RASPBERRY SAMPLES

In order to mimic realistic shipping conditions as well storage conditions at the participating laboratories, ten samples each of 19EFV02A, 19EFV02B and 19EFV02C were stored on dry ice on the dispatch date (May 6th 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 we analysed according to EURL SOP based on ISO/TS 15216-2 and ISO 15216-1 for qualitative and quantitative detection of target viruses respectively. The results are shown in Table 3, with box and whisker plots included in graphs 2, 3 and 4. Inhibition and extraction efficiency were calculated for all the samples during both stability and homogeneity test. All the results were within the criteria recommended by ISO 15216 and therefore considered valid.

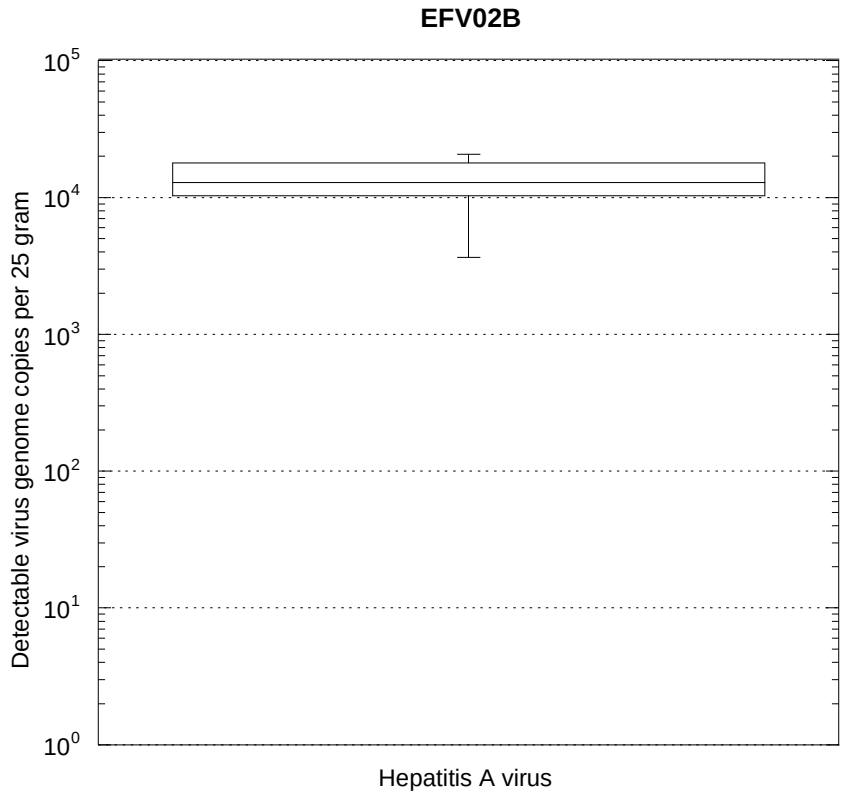
Table 3: Qualitative results for reference samples for PT EFV 02
Results are presented as detected/ not detected in 25 gram sample

Sample	Norovirus genogroup I	Norovirus genogroup II	Hepatitis A Virus
19EFV02 A	detected	detected	detected
19EFV02 B	not detected	not detected	detected
19EFV02 C	not detected	detected	not detected

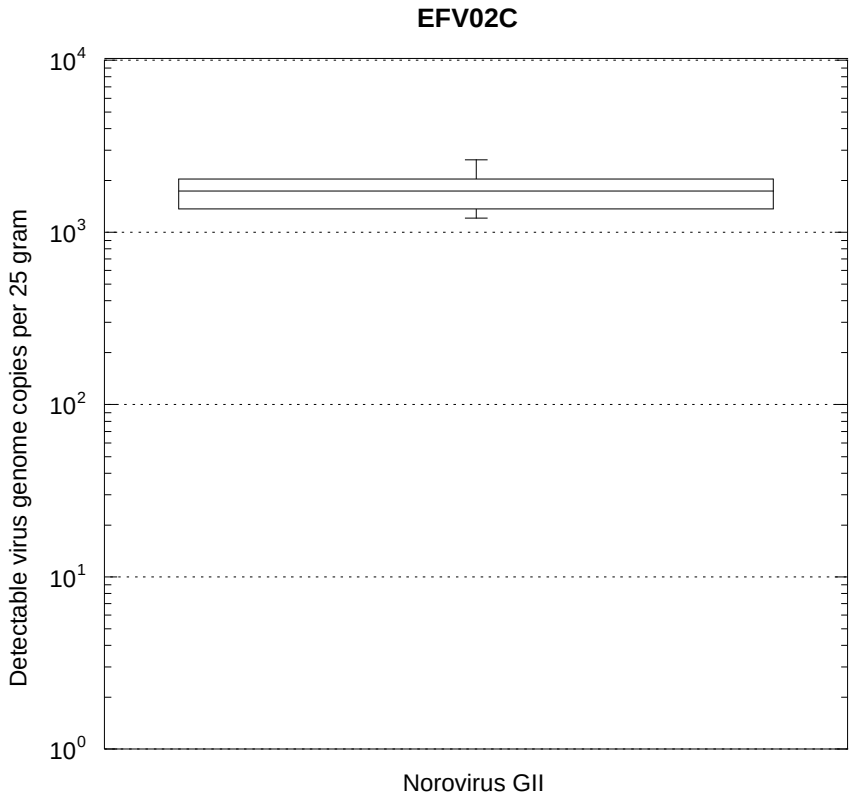
Graph 2: Box and whisker plots for homogeneity test of sample EFV02A



Graph 3: Box and whisker plots for homogeneity test of sample EFV02B



Graph 4: Box and whisker plots for homogeneity test of sample EFV01C



The box includes 50 % of the results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines outside the box.

PERFORMANCE ASSESSMENT

Performance was assessed according to three criteria:

- Relative specificity (SP)
  Percentage relative specificity = SP (%) = [TN/(TN+FP)]*100
- Relative sensitivity (SE
  Percentage relative sensitivity = SE (%) = [TP/(TP+FN)]*100
- Relative accuracy (AC)
  Percentage relative accuracy = AC (%) = [(TP+TN)/N]*100

Where:
TN: True negatives
FP: False positives
TP: True positives
FN: False negatives
N: Total number of tests

The results were also evaluated based on inhibition and extraction efficiency. The fundament for this is not performance assessment since retesting of not valid results is not possible for the participating laboratories. The evaluation should rather be looked upon as a guidance to laboratories in applying ISO/TS 15216-2 for valid reporting in official control.

RESULTS

Nineteen laboratories, including 18 NRLs participated in the current PT and all returned their results. Information provided by laboratories showed that all received the samples the day after dispatch (May 7th), with only one laboratory analysing the material on arrival. Nine laboratories analysed the samples within the first three days after the arrival, two laboratories after a week, five laboratories after eight days and two laboratories within the last three days before the deadline (May 24th).

In total, three false negative and one false positive results were reported by the laboratories. However, some of the true negative results were not valid due to unacceptable inhibition and/or extraction efficiency. Overview of results are demonstrated in table 4.

Despite the fact that the current PT was dedicated to qualitative detection of HAV, norovirus GI and norovirus GII, four laboratories reported quantitative results as well. These results are shown in Annex C. Detailed information about the participating laboratories quantification cycle (Cq) values can be found in Annex A and B.

Table 4: Overview of participants´ results

Target viruses	N	Sample 19EFV02A				Sample 19EFV02B				Sample 19EFV02C			
		SF	FP	FN	NV	SF	FP	FN	NV	SF	FP	FN	NV
Norovirus GI	19	19	0	0	0	10	1	0	7	11	0	0	8
Norovirus GII	19	19	0	0	0	11	0	0	7	18	0	1	0
Hepatitis A virus	19	18	0	1	0	18	0	1	0	12	0	0	7

N: Number of laboratories that reported results for the analysis, SF: Number of laboratories with satisfactory result
FP: False positive, FN: False negative, NV: Not valid negative results

METHODS USED BY THE PARTICIPANTS

Nine laboratories were accredited according to ISO/IEC 17025 for detection of norovirus GI, norovirus GII and HAV and the majority (17 out on 19) followed ISO 15216-2. One laboratory used an in house method adapted from ISO/TS 15216-2 and one laboratory applied a modified version of ISO 15216-1. Detailed information on the methodologies used is shown in Annex A.

PERFORMANCE ASSESMENT

All the results were assessed as presence–absence data in concordance with intended results as percentage relative accuracy, specificity and sensitivity as previously described in this report and are presented in Table 5.

Table 5: Participants´ results for PT 19EFV02

	Norovirus genogroup I			Norovirus genogroup II			Hepatitis A virus		
Lab. ID	AC (%)	SP (%)	SE (%)	AC (%)	SP (%)	SE (%)	AC (%)	SP (%)	SE (%)
101	100	100	100	100	100	100	100	100	100
104	100	100	100	100	100	100	100	100	100
105	67	50	100	100	100	100	100	100	100
107	100	100	100	100	100	100	100	100	100
108	100	100	100	100	100	100	67	100	50
109	100	100	100	100	100	100	100	100	100
110	100	100	100	100	100	100	100	100	100
111	100	100	100	100	100	100	100	100	100
112	100	100	100	100	100	100	67	100	50
113	100	100	100	100	100	100	100	100	100
114	100	100	100	100	100	100	100	100	100
115	100	100	100	100	100	100	100	100	100
116	100	100	100	100	100	100	100	100	100
117	100	100	100	100	100	100	100	100	100
119	100	100	100	100	100	100	100	100	100
120	100	100	100	67	100	50	100	100	100
121	100	100	100	100	100	100	100	100	100
122	100	100	100	100	100	100	100	100	100
123	100	100	100	100	100	100	100	100	100

AC: Relative accuracy, SP: Relative specificity, SE: Relative sensitivity

INHIBITION and EFFICIENCY RESULTS

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

In total, 9 inhibition results was expected to be reported by the participants. However, five laboratories (26 %) reported incomplete inhibition results. One laboratory reported unacceptable inhibition for norovirus GI in sample C, and another laboratory reported unacceptable inhibition for norovirus GI in sample B.

Two laboratories did not report inhibition results at all. One of those laboratories described Ceeramtools real time RT-PCR kits as the reason and the other one explained it as a result of not having access to EC RNA.

One laboratory reported ISO/TS 15216-2 as their used method, but reported inhibition in percentage and did not provide any quantitative results.

Two laboratories reported Cq value of (sample RNA + EC RNA) as Cq inhibition. Both laboratories were contacted by the EURL and one of those laboratories resubmitted their inhibition results.

The majority of laboratories, 15 out of 19 (79 %) reported acceptable extraction efficiency values. One laboratory reported unacceptable values in sample A and C and no value for sample B. Another laboratory reported unacceptable values in sample B and C.

One laboratory did not report any value for sample A. One laboratory did not have the process control virus and therefore was unable to calculate the extraction efficiency, and thus was not able to provide any extraction efficiency results.

In total, 7 out of 19 laboratories (37 %) reported acceptable results for both inhibition and efficiency values.

According to ISO 15216, true positive results could be considered valid despite unacceptable inhibition and extraction efficiency results. Therefore, all the results reported for sample 19EFV02A are valid regardless the inhibition and extraction efficiency values, since it was positive for all the target viruses. Results are presented in table 6.

Table 6: Inhibition and efficiency results for sample 19EFV02A

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV		GI	GII	HAV
101	A	A	A	A	V	V	V
104	NR	NR	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	U	V	V	V
109	A	A	A	A	V	V	V
110	A	NR	NR	A	V	V	V
111	A	A	A	A	V	V	V
112	A	A	A*	NR	V	V	V
113	NR	NR	NR	A	V	V	V
114	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
116	A	A	A	A	V	V	V
117	A	A	A	A	V	V	V
119	A	A	A	A	V	V	V
120	A	A	A	A	V	V	V
121	A	A	A	A	V	V	V
122	NR	NR	NR	NR	V	V	V
123	A	A	A	A	V	V	V

A: Acceptable, U: Unacceptable, NR: not reported, *: false results

V: valid results

Based on ISO 15216, 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. Therefore, some reported results are not valid for norovirus GI and norovirus GII in 19EFV02B (8 laboratories) and for norovirus GI and hepatitis A virus in 19EFV02C (8 laboratories). Results are presented in tables 7 and 8.

Table 7: Inhibition and efficiency results for sample 19EFV02B

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV		GI	GII	HAV
101	A	A	A	A	V	V	V
104	NR	NR	A	A	IVi	IVi	V
105	A*	A	A	A	V	V	V
107	A	A	A	A	V	V	V
108	A	A	A*	NR	IVe	IVe	V
109	A	A	A	A	V	V	V
110	NR	NR	A	A	IVi	IVi	V
111	A	A	A	U	IVe	IVe	V
112	A	A	A	A	V	V	V
113	NR	NR	NR	A	IVi	IVi	V
114	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
116	A	A	A	A	V	V	V
117	A	A	A	A	V	V	V
119	NR	NR	A	A	IVi	IVi	V
120	A	A	A	A	V	V	V
121	U	A	A	A	IVi	V	V
122	NR	NR	NR	NR	IVie	IVie	V
123	A	A	A	A	V	V	V

A: Acceptable, U: Unacceptable, NR: not reported, *: false results

V: valid results, IVi: invalid negative results, unacceptable/not reported inhibition, IVe: invalid negative results, unacceptable/not reported extraction efficiency

Table 8: Inhibition and efficiency results for sample 19EFV02C

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV		GI	GII	HAV
101	A	A	A	A	V	V	V
104	NR	NR	A	A	IVi	V	IVi
105	A	A	A	A	V	V	V
107	A	A	A	A	V	V	V
108	A	A	A	U	IVe	V	IVe
109	A	A	A	A	V	V	V
110	NR	A	NR	A	IVi	V	IVi
111	A	A	A	U	IVe	V	IVe
112	A	A	A	A	V	V	V
113	NR	NR	NR	A	IVi	V	IVi
114	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
116	U	A	A	A	IVi	V	V
117	A	A	A	A	V	V	V
119	NR	A	NR	A	IVi	V	IVi
120	A	A*	A	A	V	V	V
121	A	A	A	A	V	V	V
122	NR	NR	NR	NR	IVie	V	IVie
123	A	A	A	A	V	V	V

A: Acceptable, U: Unacceptable, NR: not reported, *: false results

V: valid results, IVi: invalid negative results, unacceptable/not reported inhibition, IVe: invalid negative results, unacceptable/not reported extraction efficiency

DISCUSSION

The majority of the participating laboratories obtained intended results and analysed the samples according to ISO/TS 15216-2. The laboratories overall accuracies for all reported results were 98 % for norovirus GI and norovirus GII and 97 % for HAV. However, some negative results were not valid due to unacceptable inhibition and/or efficiency results. Furthermore, it was observed that some laboratories had some problems regarding the calculating and reporting of inhibition and extraction efficiency results.

Based on ISO/TS 15216-2, external control (EC) RNA should serve as a control for RT-PCR inhibition. Inhibition is calculated as Cq value (sample RNA + EC RNA) − Cq value (water + EC RNA). When the ΔCq in undiluted sample is ≥2, the calculation shall be repeated for diluted (1:10) samples. If ΔCq in diluted samples still is ≥2, negative results are not valid. Some laboratories performed quantitative analyses and calculated inhibition by using both ΔCq and m (slope of the dsDNA standard curve), $(1 - 10^{(\Delta Cq/m)}) \times 100$ %. According to ISO 15216-1, when the inhibition in undiluted samples is >75 %, calculation shall be repeated for diluted samples and if it still is >75 %, negative results are not valid.

According to ISO 15216, process control virus (for instance mengovirus) must be added to the samples prior to virus extraction. A process control virus standard curve is produced in order to estimate extraction efficiency. Extraction efficiency is calculated as $10^{(\Delta Cq/m)} \times 100$ %, where ΔCq is the Cq value for process control virus in sample RNA − Cq value for undiluted process control virus RNA (the first point in the process control virus RNA standard curve) and m is the slope of the process control virus RNA standard curve. If the extraction efficiency is <1 %, negative results are not valid. If 10−1 sample RNA results are used, multiply by 10 to correct for the dilution factor.

CONCLUSION

PT EFV02 organized by EURL for Foodborne Viruses in 2019, aimed at assessing the NRLs abilities to qualitatively detect HAV, norovirus GI and norovirus GII in frozen raspberries. Nineteen laboratories participated in the PT and the majority of reporting laboratories followed the standard method ISO/TS 15216-2. In a few cases, the quantitative method ISO 15216-1 was used and quantitative results were reported in addition to Cq values. The performance of the participating laboratories was satisfactory with overall accuracies of 98 % for norovirus GI and GII and 97 % for HAV for all reported results.

Reporting inhibition and extraction efficiency turned out to be complicated for several laboratories. A clarification has been included under the discussion point in this report.

APPENDIX A

Results and methods used for PT EFA 02. *For key to method codes see next page.*

Lab. ID No.	Sample EFA02A			Sample EFA02B			Sample EFA02C			1	2	3	4	5	6
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)						
101	29,53	31,33	30,61			30,34		35,54		A	D	H	J	P	S
104[a]	27,9	27,48	28,65			29,22		NV		A	D	H	J	P	T
105[a]	29,2	25,8	29,9	37[f]		30		28,9		A	D	H	J	P	T
107[a]	27,52	25,37	26,19			25,9		29,25		A	E	I	K	Q	S
108[a]	31,22	31,5	33,74			NR[f]		NV		A	D	H	L	R	S
109[a]	29,17	27,61	28,8			29,24		30,48		A	D	H	J	P	T
110	31,68	27,12	29,18			30,38		31,95		A	F	H	M	P	T
111	33,041	29,815	30,312			NV (29,88)		NV		A	D	H	N	P	S
112[a]	38,77	33,39	NR[f]			36,25		32,43		A	D	H	J	P	T
113[a]	32,11[b]	31,22[b]	33,52[b]			NV (33,87[b])		NV (35,4[b])		B	D	H	L	R	S
114[a]	27,2	26,48	29,34	45	45	30,82	45	30,24	45	A	D	H	J	P	S
115	31,302	29,191	28,577			29,101		34,153		A	D	H	J	P	T
116	32,71	31,56	35,91			36,95		35,8		A	D	I	J	P	T
117	30,37	28,5	27,78			28,84		32,46		C	G	H	J	P	?
119	28,24	27,21	30,04			29,99		30,82		A	D	H	J	P	T
120	37,74[b]	35,5[b]	36,53[b]			35,48[b]		NR[f]		A	D	H	J	P	S
121[a]	34,64	29,24	32,04			31,87		32,85		A	D	H	J	P	S
122	NR	NR	NR	NR	NR	NR	NR	NR	NR	A	D	H	O	P	S
123	29,36	30,54	29,76			29,42		35,4		A	D	H	J	p	U

NR: No results returned, NA: Not valid

a: Accredited for detection of norovirus and HAV in soft fruit, b: RNA extract diluted 10^{-1}, f: false positive and or negative results

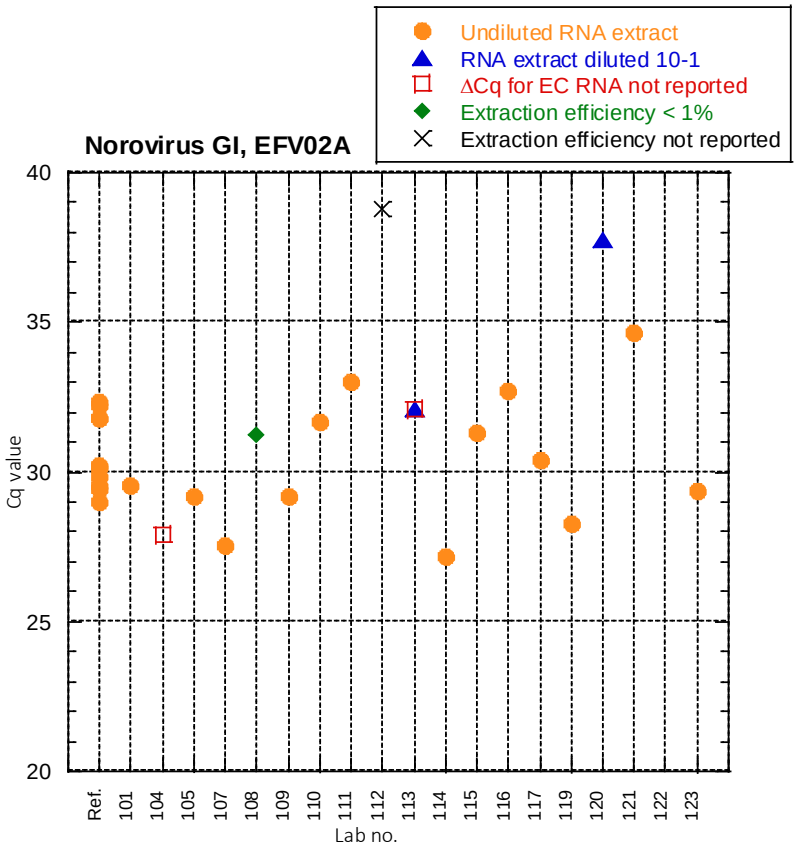
Key to method codes

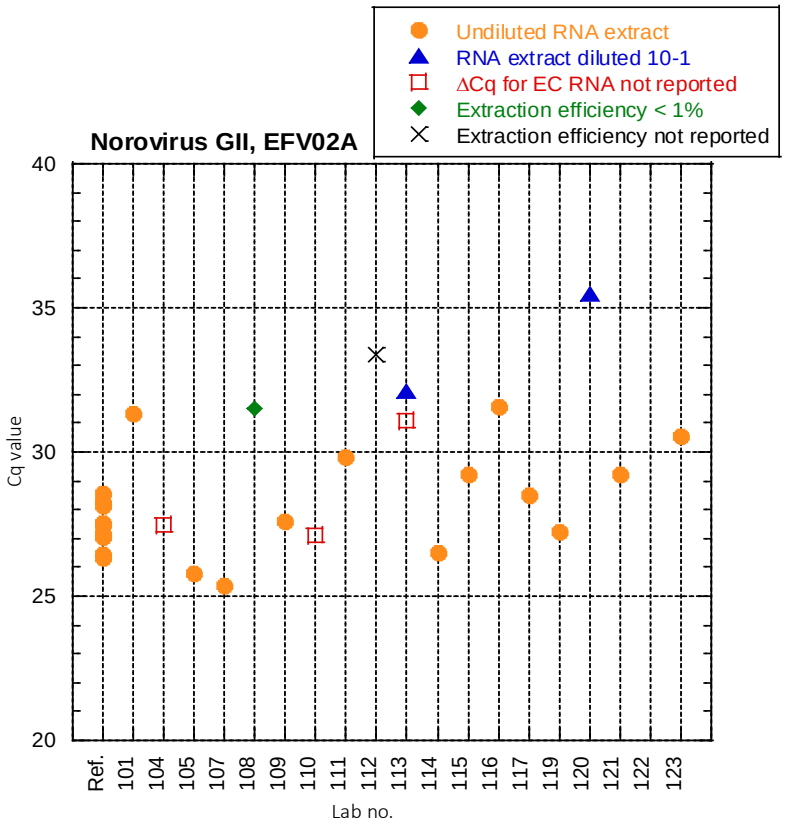
	1. Virus isolation method
A	ISO/TS 15216-2
B	Modified ISO/TS 15216-2
C	Modified ISO 15216-1
	2. RNA extraction reagents
D	NucliSens® (BioMérieux)
E	QIAamp® Airal RNA Mini Kit (Qiagen)
F	NucliSens® (BioMérieux), alternative robot system
G	Ambion® Plant RNA Isolation Aid (Life technologies)
	3. RNA extraction 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
	5. Primers and probes
P	ISO 15216 *(The probe, NAGG1p or TM9, for norovirus GI was not asked to be specified)*
Q	ISO 15216, with different fluorophores & quenchers
R	CeeramTools®

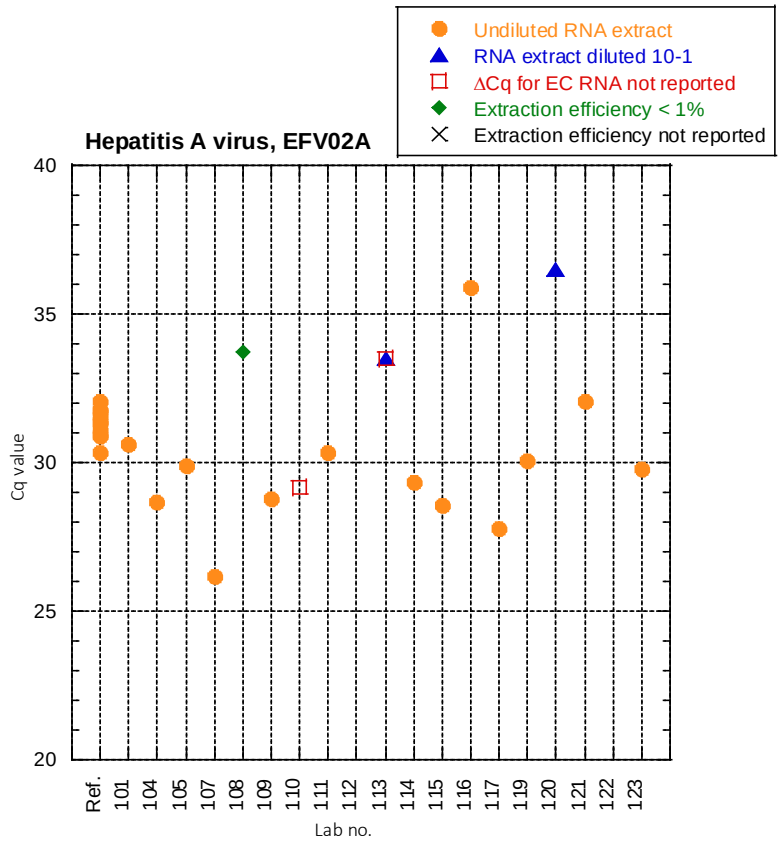
	6. Inhibition removal method
S	None
T	Zymo research OneStep™ PCR Inhibitor Removal Kit
U	MobiSpin S-400

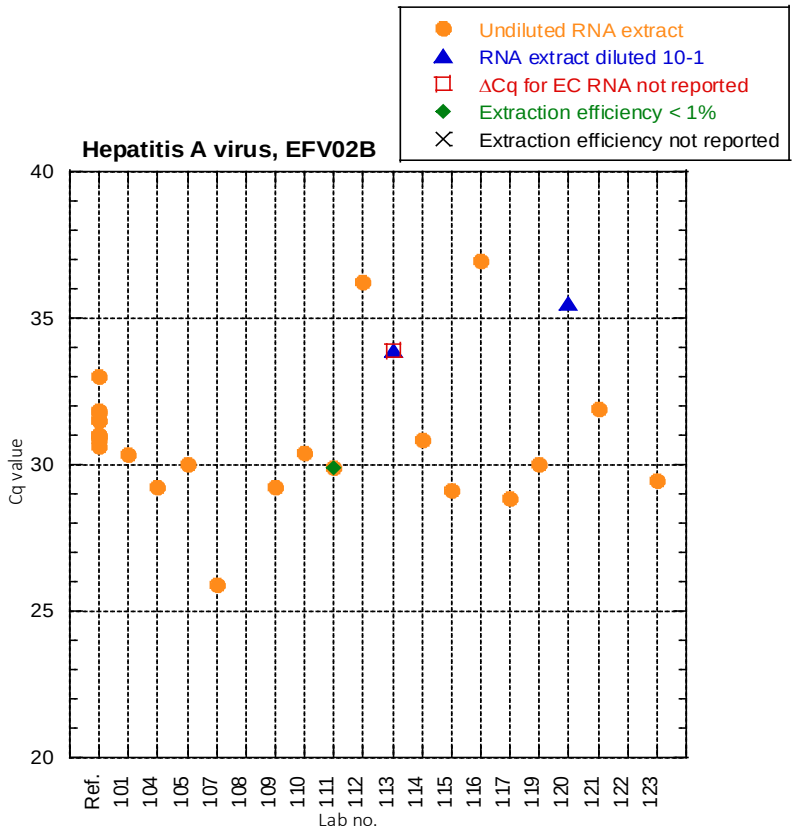
APPENDIX B

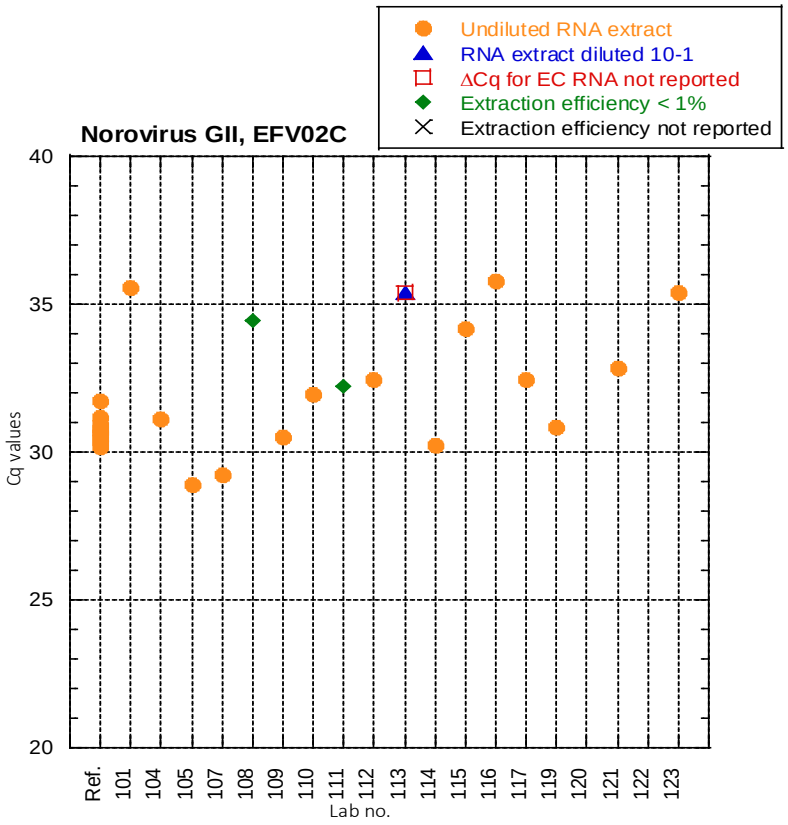
The participating laboratories Cq values compared with reference results.





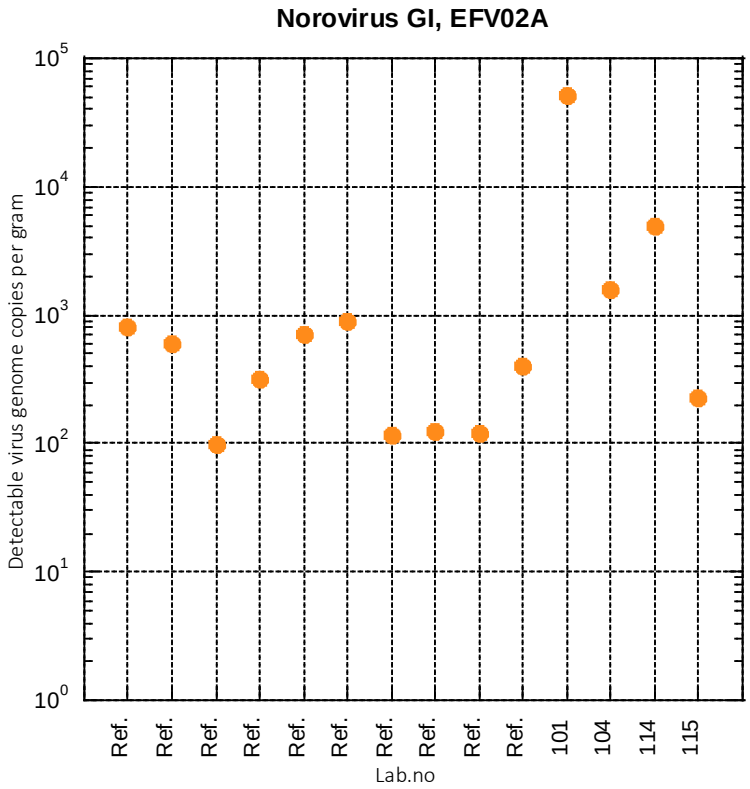

Undiluted RNA extract
RNA extract diluted 10-1
ΔCq for EC RNA not reported
Extraction efficiency < 1%
Extraction efficiency not reported
Hepatitis A virus, EFV02A
Cq value
40
35
30
25
20
Ref.
101
104
105
107
108
109
110
111
112
113
114
115
116
117
119
120
121
122
123
Lab no.

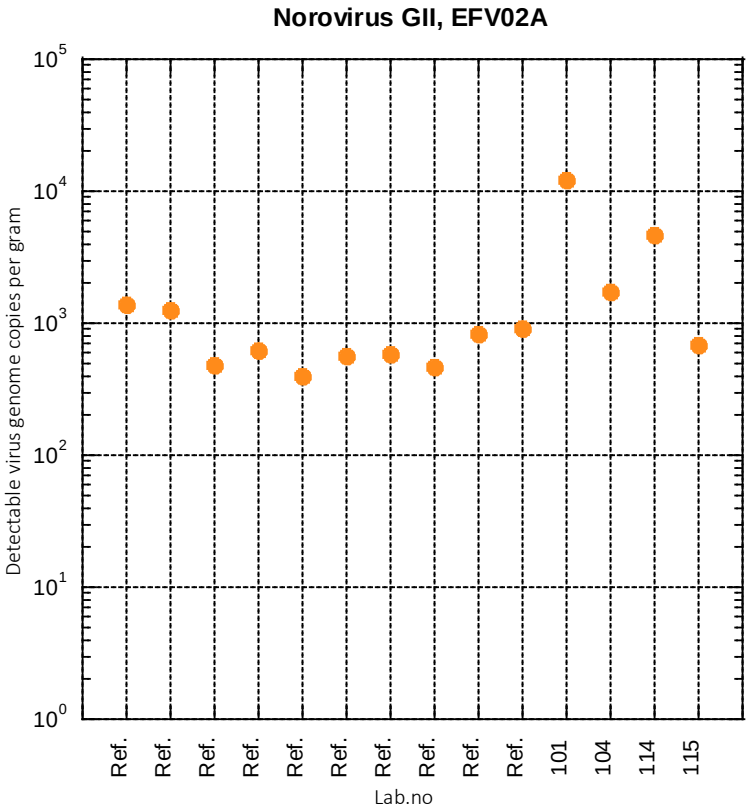

Undiluted RNA extract
RNA extract diluted 10-1
ΔCq for EC RNA not reported
Extraction efficiency < 1%
Extraction efficiency not reported
Hepatitis A virus, EFV02B
Cq value
40
35
30
25
20
Ref.
101
104
105
107
108
109
110
111
112
113
114
115
116
117
119
120
121
122
123
Lab no.

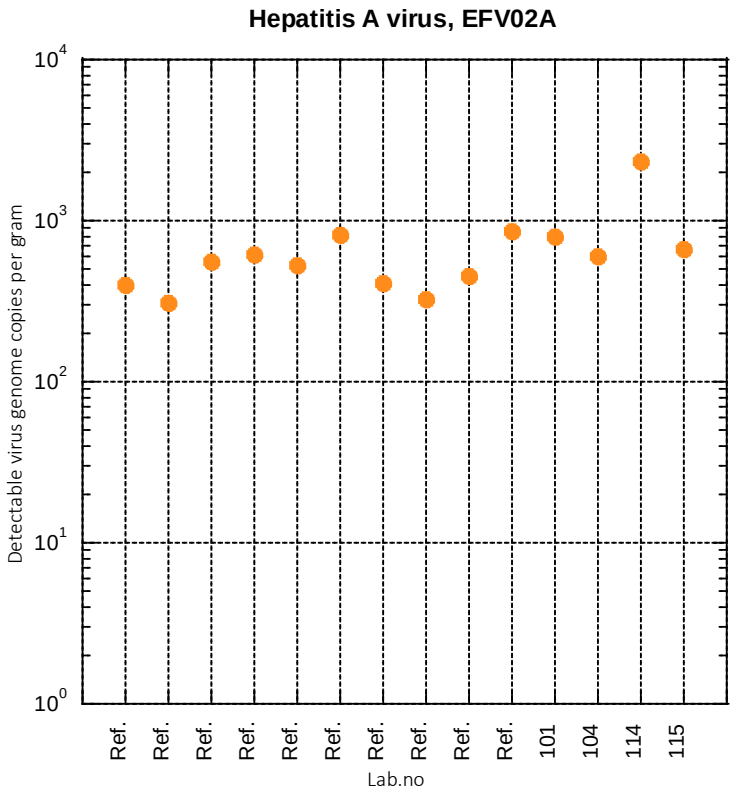

Undiluted RNA extract
RNA extract diluted 10-1
ΔCq for EC RNA not reported
Extraction efficiency < 1%
Extraction efficiency not reported
Norovirus GII, EFV02C
Cq values
40
35
30
25
20
Ref.
101
104
105
107
108
109
110
111
112
113
114
115
116
117
119
120
121
122
123
Lab no.

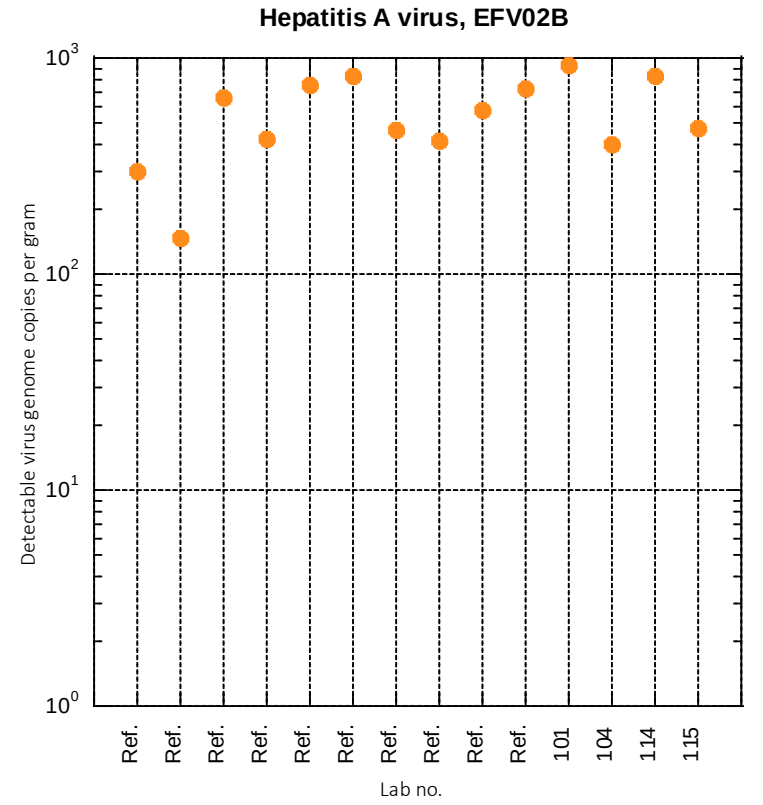
APPENDIX C

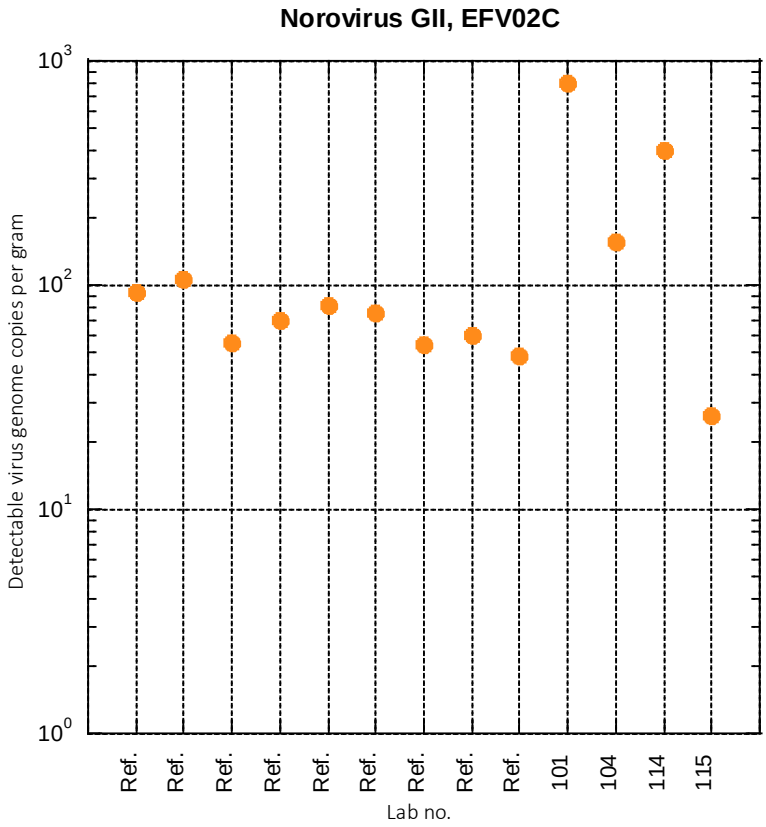
The participating laboratories quantitative results compared with reference results.




Norovirus GII, EFV02A
Detectable virus genome copies per gram
10^5
10^4
10^3
10^2
10^1
10^0
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
101
104
114
115
Lab.no


Hepatitis A virus, EFV02A
Detectable virus genome copies per gram
10^4
10^3
10^2
10^1
10^0
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
101
104
114
115
Lab.no


Hepatitis A virus, EFV02B
Detectable virus genome copies per gram
10^3
10^2
10^1
10^0
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
101
104
114
115
Lab no.


Norovirus GII, EFV02C
Detectable virus genome copies per gram
10^3
10^2
10^1
10^0
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
Ref.
101
104
114
115
Lab no.