Livsmedelsverket
Swedish Food Agency

European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

Final report

Proficiency testing scheme EFV 07, 2021

Detection of norovirus and hepatitis A virus in Strawberries

Final report- Version 1 (2023.03.14)

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Summary

This report describes the performance of NRLs for detection of viral contamination of strawberries in PT scheme EFV07, organised by the EURL for Foodborne Viruses. Distribution was made 13th of September 2021 to 24 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 strawberries.

The participating laboratories were requested to examine the samples using their routine method, however the EURL recommended to analyse the samples according to ISO 15216-2. A Standard Operating Procedure (SOP) for detection of norovirus and hepatitis A virus in soft fruit, based on ISO 15216-2, 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 strawberries 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 07

Viruses	Origin	Strain ID/genotype
Hepatitis A virus	Cell culture supernatant	ATCC® VR-1402™ (HM 175/18f)
Norovirus genogroup I	Faecal material	GI.7 (capsid sequence)
Norovirus genogroup II	Faecal material	GII.4 Sydney (capsid sequence)

Sample A was inoculated with Hepatitis A virus, and sample C was inoculated with norovirus GI and GII. Concentration values are shown in Table 2.

Table 2: Spiking of PT EFV 07 samples

Sample	Norovirus GI	Norovirus GII	HAV
21EFV07 A	–	–	$\approx 10^5$*
21EFV07 B	–	–	–
21EFV07 C	$\approx 5 \times 10^4$*	$\approx 5 \times 10^4$*	–

**Detectable virus genome copies inoculated to each sample*

Preparation of samples

Approximately 2.5 kg frozen strawberries of the same batch were purchased from a retail in Sweden. A homogenous mixture was prepared by mixing all the strawberries together. The material was then divided into 25 grams, transferred to plastic bags, spiked with the target viruses, sealed and stored in 4° C for approximately one hour before dispatching.

Distribution of the proficiency testing items

Samples were dispatched on dry ice by courier in accordance with IATA packing instructions 650 for UN3373, on September 13th. All 24 laboratories received three frozen samples and the ones that requested in advance 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 performing the extraction and submitting the results were September 28th and October 6th respectively.

Quality control

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

Stability levels in strawberry samples

In order to investigate the stability of spiked viruses in samples stored in freezer, a study was conducted before and after dispatch. The preliminary test showed that the virus levels have a tendency to decrease after 6 days and therefore the participants were asked to perform the virus extraction within the first 5 days after the dispatch date. The procedure and results of the stability test done after dispatch are presented in the reference samples section together with the homogeneity test.

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

In order to mimic realistic shipping conditions, storage conditions at the participating laboratories, stability of virus levels as well testing the homogeneity, nineteen random samples each of 21EFV07A, and 21EFV07C were tested. Three samples of each were tested immediately after the inoculation on the dispatch date (September 13th, day 0), and the rest of samples were transferred to dry ice container on the dispatch date and stored for 24 hours. Three samples of each were tested after 24 hours storage on dry ice and the rest of samples were stored in -20 °C. Three samples of each were tested after 24 hours storage in -20 °C (day 1) and the rest of samples were tested at day 4, 7, 12 and 14. Samples were analysed according to EURL SOP based on ISO 15216-1 for the quantification of target viruses respectively. The results (d0-d14, 16 samples each of 21EFV07A, and 21EFV07C) are shown in Table 3 and 4, with box and whisker plots for stability test (10 samples of each) 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 all the target viruses and for trial 07 purposes.

Table 3: Qualitative results for reference samples for PT EFV 07

Sample	Norovirus GI	Norovirus GII	HAV
21EFV07 A	Not detected	Not detected	Detected
21EFV07 B	Not detected	Not detected	Not detected
21EFV07 C	Detected	Detected	Not detected

Table 4: Quantitative results for ten reference samples for PT EFV 07

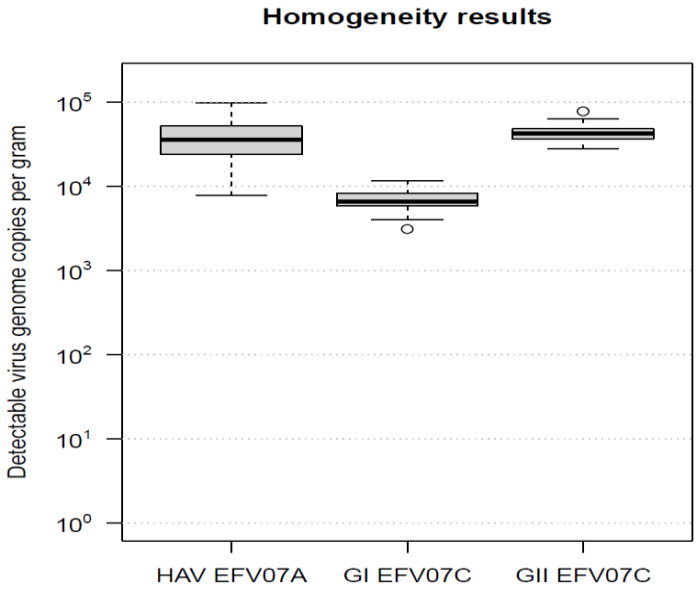
Ranges based on a 95 % confidence limit determined as two geometric standard deviations above and below the geometric mean

Sample	Norovirus GI	Norovirus GII	HAV
21EFV07 A	Not detected	Not detected	$1.27 \times 10^4 – 1.03 \times 10^5$*
21EFV07 B	Not detected	Not detected	Not detected
21EFV07 C	$3.78 \times 10^3 – 1.78 \times 10^4$*	$1.59 \times 10^4 – 1.24 \times 10^5$*	Not detected

**detectable virus genome copies per gram sample*

Graph 1: Box and whisker plots for homogeneity test of samples 21EFV07 A and C

The box includes 50 % of the results from 10 samples of each A and 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]



[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/.

Results and discussion

Samples were sent to 24 laboratories (including 20 NRLs and one in the process of becoming NRL) and 23 laboratories returned their results. The majority of laboratories received the samples the day after dispatch (on September 14th) and about half of the participants analysed the samples within the first week after the dispatch date.

In total, number of true positive and negative results were high for each sample and each agent. However, five false negative results was reported for norovirus GI in sample C which is relatively higher than other false results reported for other agents in general. Sample C was inoculated with the lowest copies of norovirus GI comparing to Hepatitis A virus in sample A and norovirus GII in sample C. Furthermore, Number of none valid negative results was on average around seven in all sample types and for all agents. Overview of results is demonstrated in Table 5.

Detailed information about the participating laboratories results can be found in Annex A. The results of references samples analysed at day 4 are presented as Ref.

Table 5: Overview of participants´ results for samples 21EFV07 A, B and C

Target viruses		Sample 21EFV07 A				Sample 21EFV07 B				Sample 21EFV07 C			
	N	T	FP	FN	NV	T	FP	FN	NV	T	FP	FN	NV
Norovirus GI	23	21	2	-	6	21	2	-	8	18	-	5	-
Norovirus GII	23	22	1	-	7	20	3	-	8	21	-	2	-
Hepatitis A virus	23	22	-	1	-	22	1	-	8	22	1	-	8

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

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

- 0 points: Incorrect results for each target virus

The maximum score for each laboratory (for each target virus), taking into account the results of all three samples is therefore six (Table 6).

Table 5: Calculated data used for scoring assessment

Presence/absence			
Lab ID	GI	GII	HAV
103	6 out of 6[i]	4 out of 6[i]	6 out of 6[i]
104*	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
107*	4 out of 6	6 out of 6	6 out of 6
108*	6 out of 6	6 out of 6	6 out of 6
109*	6 out of 6	2 out of 6	4 out of 6
110*	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
112*	6 out of 6	6 out of 6	6 out of 6
113*	6 out of 6[i]	6 out of 6[i]	6 out of 6[i]
114*	6 out of 6	6 out of 6	6 out of 6
115	6 out of 6[i]	6 out of 6	6 out of 6
116	6 out of 6	6 out of 6	6 out of 6
119*	4 out of 6[ei]	6 out of 6[e]	6 out of 6[e]
120	6 out of 6[e]	6 out of 6[e]	6 out of 6[e]
121*	2 out of 6	6 out of 6	6 out of 6
122*	2 out of 6[i]	4 out of 6[i]	2 out of 6[i]
123*	6 out of 6	6 out of 6	6 out of 6
129*	4 out of 6[e]	6 out of 6[e]	6 out of 6[e]
130*	4 out of 6	6 out of 6	6 out of 6
131*	4 out of 6[e]	6 out of 6[e]	6 out of 6[e]
133*	6 out of 6[i]	6 out of 6[i]	6 out of 6[i]
134*	6 out of 6[i]	4 out of 6[i]	6 out of 6[i]

** Designated EU/EFTA member state NRL*

[e]: unacceptable efficiency, [i]: unacceptable inhibition

Inhibition and efficiency results

The results were also evaluated based on inhibition and extraction efficiency outcomes. One laboratory reported unacceptable extraction efficiency in all samples. Another participant didn't report any extraction results and one laboratory reported unacceptable extraction efficiency results for samples B and C. In case of inhibition results, few laboratories didn't report any inhibition results and two laboratories reported unacceptable inhibition results.

Since it was not possible to provide the laboratories with a retest option, this evaluation is not a part of performance assessment and scoring. However, it can provide a guidance for valid reporting in official control according to ISO 15216-2.

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.

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 EURL doesn't provide extra samples. Results are presented in Annex B.

Methods used by the participants

Fifteen laboratories were accredited according to ISO/IEC 17025 for detection of norovirus GI, norovirus GII and sixteen for HAV. All the laboratories followed ISO 15216-2/1 with exception of one laboratory adopted an internal method. Detailed information on the methodologies used is shown in Appendix C.

Conclusion

The aim of PT EFV07 organized in September 2021 by EURL for Foodborne Viruses was to assess the NRLs ability to qualitatively detect HAV, norovirus GI and norovirus GII in frozen strawberry samples.

Twenty-three laboratories submitted their results for this PT and 48 % of the participating laboratories obtained full satisfactory results.

Annex A

Participant's results

with EURL standards with own standards false results

Lab. ID No.	21EFA07 A			21EFA07 B			21EFA07 C		
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)
103	ND	ND	33.65	ND	32.81	ND	34.88	29.46	ND
104*	ND	ND	30.18 (1.77×10^{4})	ND	ND	ND	36.1 5 (7.95×10^{2})	33.43 (5.4×10^{3})	ND
104*	ND	ND	30.18 (4.40×10^{3})	ND	ND	ND	36.15 (1.79×10^{2})	33.43 (2.51×10^{3})	ND
105*	ND	ND	32.64	ND	ND	ND	34.70	30.47	ND
107*	ND	ND	33.60	34.73	ND	ND	34.93	33.46	ND
108*	ND	ND	29.05	ND	ND	ND	35.56	33.41	ND
109*	ND	38.53	30.04	ND	33.31	38.31	36.18	36.60	ND
110*	ND	ND	32.08	ND	ND	ND	37.09	30.07	ND
111*	ND	ND	32.38 (3.24×10^{1})	ND	ND	ND	35.30 (3.53×10^{1})	31.08 (3.11×10^{1})	ND
112*	ND	ND	30.75	ND	ND	ND	36.79	28.51	ND
113*	ND	ND	35.14	ND	ND	ND	39.41	33.19	ND
113*	ND	ND	36.43	ND	ND	ND	39.98	36.43	ND
114*	ND	ND	30.89 (2.20×10^{2})	ND	ND	ND	40.98 (3.9)	33.76 (1.80×10^{1})	ND
114*	ND	ND	29.89 (1.40×10^{3})	ND	ND	ND	39.92 (1.10×10^{1})	31.95 (1.50×10^{2})	ND
115	0	0	33.95 (8.6×10^{1})	0	0	0	38.34 (7.5)	39.12 (3.8)	0
115	0	0	33.95 (6.6×10^{1})	0	0	0	38.34 (2.5)	39.12 (1.7)	0
116	0	0	37.3	0	42.14	0	40.44	40.04	0

** Designated EU/EFTA member state NRL*

D: reported as detected, ND: reported as not detected, NR: Cq not reported

Lab. ID No.	21EFA07 A			21EFA07 A			21EFA07 A		
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)
119*	ND	ND	35.84	ND	ND	ND	ND	35.20	ND
120	ND	ND	29.9	ND	ND	ND	36.40	33.20	ND
121*	D 42	ND	37.125	D 41.11	ND	ND	40.27	36.33	ND
122*	D 33.52	ND	ND	ND	ND	ND	ND	ND	D 43.39
123*	ND	ND	32.7 (1:10)	ND	ND	ND	35.6 (1:1)	34.6 (1:1)	ND
129*	ND	ND	34.29	ND	ND	ND	ND	33.06	ND
130*	ND	ND	33.28	ND	ND	ND	ND	38.45	ND
131*	ND	ND	35.81	ND	ND	ND	ND	D, NR	ND
133*	ND	ND	34	ND	ND	ND	33	30	ND
134*	ND	ND	ND, NR	ND	ND	ND	D, NR	ND	ND
118*	No results								
EURL**	ND	ND	28.89 (7.34×10^3)	ND	ND	ND	30.98 (7.34×10^3)	26.88 (4.29×10^4)	ND

** Designated EU/EFTA member state NRL*

*** Reference results from day 4*

D: reported as detected, ND: reported as not detected, NR: Cq not reported

Annex B

Inhibition and extraction efficiency results

Inhibition and extraction efficiency results for sample 21EFV07 A

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV^t		GI	GII	HAV^t
103	NR	NR	NR	A	NV	NV	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	FP	A	A	V	FP	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
113*	NR	NR	NR	A	NV	NV	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
119*	NR	A	A	U	NV	NV	V
120	A	A	A	A	V	V	V
121*	FP	A	A	A	FP	V	V
122*	FP	U	A	A	FP	NV	V
123*	A	A	A	A	V	V	V
129*	A	A	A	A	V	V	V
130*	A	A	A	A	V	V	V
131*	A	A	A	U	NV	NV	V
133*	NR	NR	NR	A	NV	NV	V
134*	NR	NR	NR	A	NV	NV	V

** Designated EU/EFTA member state NRL*

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

Inhibition and extraction efficiency results for sample 21EFV07 B

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV		GI	GII	HAV
103	NR	FP	NR	A	NV	FP	NV
104*	A	A	A	A	V	V	V
105*	A	A	A	A	V	V	V
107*	FP	A	A	A	FP	V	V
108*	A	A	A	A	V	V	V
109*	A	FP	FP	A	V	FP	FP
110*	A	A	A	A	V	V	V
111*	A	A	A	A	V	V	V
112*	A	A	A	A	V	V	V
113*	NR	NR	NR	A	NV	NV	NV
114*	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
116	A	FP	A	A	V	FP	V
119*	NR	A	A	U	NV	NV	NV
120	A	A	A	NR	NV	NV	NV
121*	FP	A	A	A	FP	V	V
122*	A	U	A	A	V	NV	V
123*	A	A	A	A	V	V	V
129*	A	A	A	U	NV	NV	NV
130*	A	A	A	A	V	V	V
131*	A	A	A	NR	NV	NV	NV
133*	NR	NR	NR	A	NV	NV	NV
134*	NR	NR	NR	A	NV	NV	NV

* Designated EU/EFTA member state NRL

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

Inhibition and extraction efficiency results for sample 21EFV07 C

	Inhibition			Efficiency	Results		
Lab. ID	GI[t]	GII[t]	HAV		GI[t]	GII[t]	HAV
103	NR	NR	NR	A	V	V	NV
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
113*	NR	NR	NR	A	V	V	NV
114*	A	A	A	A	V	V	V
115	A	A	A	A	V	V	V
116	A	A	A	A	V	V	V
119*	FN	A	A	U	FN	V	NV
120	A	A	A	A	V	V	V
121*	A	A	A	A	V	V	V
122*	FN	FN	FP	A	FN	FN	FP
123*	A	A	A	A	V	V	V
129*	FN	A	A	U	FN	V	NV
130*	FN	A	A	A	FN	V	V
131*	FN	A	A	U	FN	V	NV
133*	NR	NR	NR	A	V	V	NV
134*	NR	FN	NR	A	V	FN	NV

* Designated EU/EFTA member state NRL

A: Acceptable, FN: false negative, FP: false positive, NR: not reported, [t]: target virus, NV: not valid, 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
107*	A	F	H	P	R	UV	Za
108*	A	D	H	L	T	UV	X
109*	A	D	H	J	R	UV	Xx
110*	A	E	H	M	R TM9	UV	W
111*	A	D	H	N	R		Y
112*	A	D	H	J	R	UV	Zq
113*	B	D	H	L	T	UV	W
114*	C	D	H	J	R TM9	UV	Z
115	C	D	H	J	R TM9		Zb
116	A	D	H	J	R	V	X
119*	A	D	H	J	R	UV	Zzqq
120	A	D	H	J	R TM9, MNV		X
121*	A	D	H	J	R	UV	Zq
122*	A	D	H	M	R		X
123*	A	D	H	J	R		X
129*	A	D	H	L	T		W
130*	C	D	H	J	R	UV	W
131*	A	D	H	M	T	UV	Yr
133*	A	F	H	O	?		Yr
134*	A	G	H	J	R	UV	Za

** Designated EU/EFTA member state NRL*

Key to method codes

1. Virus isolation and concentration method	
A	ISO 15216-2
B	Internal method
C	ISO 15216-1
2. RNA extraction methods/reagents	
D	NucliSens® (BioMérieux)
E	NucliSens® (BioMérieux), alternative robot system QuikPick Tool
F	QIAamp Viral RNA Mini Kit (Qiagen)
G	Syngen Viral Mini Kit PLUS
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	Genesig Advanced Kit Norovirus Genogroups 1 and 2, Hepatitis A Virus 5'NCRGenesig Advanced 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
T	CeeramTools®

6. Accreditation	
U	Norovirus
V	HAV
7. PCR system	
W	CFX96™ Real-Time PCR Detection System (Biorad)
X	AriaMx Real-time PCR System
Xx	Analytic Jena qTower3G
Y	Applied Biosystems™ 7500 Fast Real-Time PCR System
Z	Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System
Wi	LightCycler® 96 System (Roche)
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

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