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European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

Final report

Proficiency testing scheme EFV 08, 2022

Detection of norovirus and hepatitis A virus on food surfaces

Final report- Version 1 (2023.06.26)

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Contents

Summary

This report describes the performance of NRLs for detection of viral contamination on food surfaces in PT scheme EFV08, organised by the EURL for Foodborne Viruses. Distribution was made second of May 2022 to 22 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) on surface of two samples of bell pepper.

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. 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 fresh bell peppers artificially contaminated on the surface 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 08

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)

Surface of sample A was inoculated with Hepatitis A virus and norovirus GI, and surface of sample B was inoculated with norovirus GII. Concentration values are shown in Table 2.

Table 2: Spiking of PT EFV 08 samples

Sample	Norovirus GI	Norovirus GII	HAV
22EFV08 A	≈10^4*	–	≈10^4*
22EFV08 B	–	≈10^4*	–

Detectable virus genome copies inoculated to surface of each sample

Preparation of samples

Approximately 52 bell peppers of the same batch were purchased from a retailer in Sweden. They were sliced into 20 cm^2 pieces and each piece was placed in a container and secured by sealing foam. The surface of each sample was then 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 in refrigerated condition by courier in accordance with IATA packing instructions 650 for UN3373, on May 2nd. All 22 laboratories received two refrigerated bell pepper 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 performing the extraction and submitting the results were May 3rd and May 10th respectively.

Quality control

Bell peppers 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 bell pepper samples

In order to investigate the stability of spiked viruses in samples stored in refrigerator, a study was conducted before and after dispatch. The preliminary test showed that the virus levels were stable up to 6 days. However, the participants were asked to perform the virus extraction within 24 hours upon the delivery since the samples were fresh. 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, ten random samples each of 22EFV08A, and 22EFV08B were tested. Samples were stored in refrigerator on the dispatch date and tested at days 1 and 2. Samples were analysed according to EURL SOP based on ISO 15216-1 for the quantification of target viruses respectively. The results (day 1 and day 2) samples each of 22EFV08A, and 22EFV08B 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 1 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 08 purposes.

Table 3: Qualitative results for reference samples for PT EFV 08

Sample	Norovirus GI	Norovirus GII	HAV
22EFV08 A	Detected	Not detected	Detected
22EFV08 B	Not detected	Detected	Not detected

Table 4: Quantitative results for ten reference samples for PT EFV 08
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
22EFV08 A	$8.03 \times 10^1 – 2.82 \times 10^2$*	Not detected	$2.01 \times 10^2 – 1.05 \times 10^3$*
22EFV08 B	Not detected	$1.20 \times 10^2 – 4.22 \times 10^2$*	Not detected

detectable virus genome copies per cm^2

Graph 1: Box and whisker plots for homogeneity test of samples 22EFV08 A and B
The box includes 50 % of the results from 10 samples of each A and B. 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.[1]

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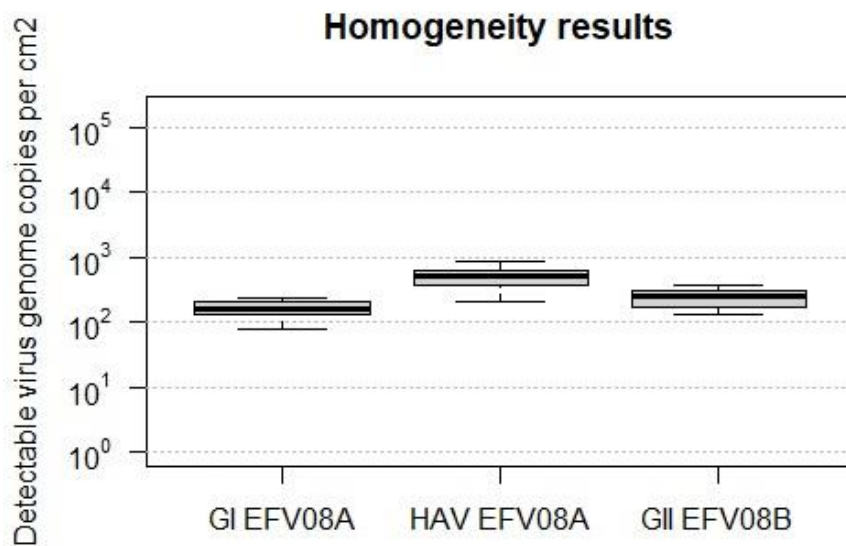
[1] R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Results and discussion

Samples were sent to 22 laboratories (including 18 NRLs) and 20 laboratories returned their results. All laboratories except one (which received the samples on May 4th) received the samples on May 3rd (one day after dispatch) and the majority of the participants analysed the samples on May 4th.

In total, 18 out 20 participants reported true results for each sample and each agent and therefore number of true positive and negative results were high. One laboratory reported false negative results for Hepatitis A virus in sample A and another laboratory reported false positive results for norovirus GII in sample A, false negative results for Hepatitis A virus in sample A and false negative results for norovirus GII in sample B.

Furthermore, number of none valid negative results was in total ten 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 1 are presented as Ref.

Table 5: Overview of participants´ results for samples 22EFV08 A and B

Target viruses	N	Sample 22EFV08 A				Sample 22EFV08 B			
		T	FP	FN	NV	T	FP	FN	NV
Norovirus GI	20	20	-	0	0	20	0	-	4
Norovirus GII	20	19	1	-	2	19	-	1	0
Hepatitis A virus	20	18	-	2	0	20	0	-	4

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 both samples is therefore four (Table 6).

Table 6: Calculated data used for scoring assessment

Presence/absence			
Lab ID	GI	GII	HAV
101	4 out of 4	4 out of 4	4 out of 4
104*	4 out of 4	4 out of 4	4 out of 4
105*	4 out of 4	4 out of 4	4 out of 4
107*	4 out of 4	4 out of 4	4 out of 4
108*	4 out of 4	4 out of 4	4 out of 4
109*	4 out of 4	4 out of 4	4 out of 4
110*	4 out of 4	4 out of 4	4 out of 4
111*	4 out of 4	4 out of 4	4 out of 4
112*	4 out of 4	4 out of 4	4 out of 4
113*	4 out of 4[i]	4 out of 4[i]	4 out of 4[i]
114*	4 out of 4	4 out of 4	4 out of 4
115	4 out of 4	4 out of 4	4 out of 4
116	4 out of 4	4 out of 4	4 out of 4
118*	4 out of 4	4 out of 4	4 out of 4
119*	4 out of 4[i]	4 out of 4	4 out of 4[i]
121*	4 out of 4	4 out of 4	4 out of 4
128*	4 out of 4	4 out of 4	4 out of 4
129*	4 out of 4	4 out of 4	2 out of 4
130*	4 out of 4	4 out of 4	4 out of 4
133*	4 out of 4[i]	0 out of 4	2 out of 4[i]

* Designated EU/EFTA member state NRL

[i]: unacceptable inhibition

Inhibition and efficiency results

The results were also evaluated based on inhibition and extraction efficiency outcomes. All laboratories reported acceptable extraction efficiency in all samples. In case of inhibition results, two laboratories didn't report any inhibition results. Another laboratory didn't report inhabitation results for norovirus GI and Hepatitis A virus. One laboratory didn't report any inhibition results for sample B or inhibition results for Hepatitis A virus in sample A.

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 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 area of sample tested) cm^2.

All the results reported as detected for norovirus GII in sample B, norovirus GI and HAV in sample A, 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

Four laboratories were accredited according to ISO/IEC 17025 for detection of norovirus GI, norovirus GII and HAV on surfaces. The majority of laboratories followed ISO 15216-2/1 with exception of three laboratories that adopted an internal method. Detailed information on the methodologies used is shown in Appendix C.

Conclusion

The aim of PT EFV08 organized in May 2022 by EURL for Foodborne Viruses was to assess the NRLs ability to detect HAV, norovirus GI and norovirus GII on the surface of bell pepper samples by swabbing method.

Twenty laboratories submitted their results for this PT and 90 % of the participating laboratories obtained full satisfactory results.

Annex A

Participant's results

 with EURL standards　 with own standards　 false results

Lab. ID No.	22EFA08 A			22EFA08 B		
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)
	gc/cm²	gc/cm²	gc/cm²	gc/cm²	gc/cm²	gc/cm²
101	29.53 (1.49 ×10²)	ND	29.04 (3.61×10²)	ND	30.0 (1.85×10²)	ND
104*	35.2 (2×10¹)	ND	30.25 (1.62×10²)	ND	33.24 (1.2×10²)	ND
104*	35.2 (1.7×10¹)	ND	30.25 (1.38×10²)	ND	33.8 (2.8×10¹)	ND
105*	32.3 (9.1×10¹)	ND	30.5 (2.89×10²)	ND	28.9 (3.93×10²)	ND
107*	31.38	ND	31.26	ND	29.01	ND
108*	30.98	ND	30.96	ND	29.71	ND
109*	36.08 (5.5×10²)	ND	31.55 (5.1×10²)	ND	30.92 (1.6×10²)	ND
110*	30.06 (1.57×10²)	ND	29.84 (2.03×10²)	ND	30.23 (1.19×10²)	ND
111*	33.08 (1.83×10²)	ND	28.31 (2.66×10³)	ND	31.36 (2.48×10²)	ND
111*	33.08 (3.1×10¹)	ND	28.31 (5.71×10²)	ND	31.36 (1.63×10²)	ND
112*	33.26	ND	31.23	ND	33.62	ND
113*	32.95	ND	31.64	ND	32.17	ND
114*	31.07 (3.4×10²)	ND	27.78 (1.5×10³)	ND	31.19 (4.2×10²)	ND
114*	31.07 (7.6×10²)	ND	27.78 (8.2×10³)	ND	31.19 (1×10³)	ND
115	33.82 (1.34×10²)	ND	32.87 (3.76×10²)	ND	33.53 (1.5×10²)	ND
116*	37.7 (4.29×10¹)	ND	35.2 (1.14×10²)	ND	35.7 (3.57×10¹)	ND
116*	37.7 (1×10¹)	ND	35.2 (1.81×10¹)	ND	37.86 (2.26×10¹)	ND
118*	30.69	ND	30.16	ND	30.27	ND
119*	40.27	ND	30.35	ND	38.4	ND
121*	33.25 (5.2×10⁰)	ND	32.11 (3.76×10¹)	ND	31.59 (1.86×10¹)	ND
128*	33.72 (1.3×10²)	ND	32.08 (1.84×10²)	ND	36.27 (3×10¹)	ND
129*	32.93	ND	ND	ND	31.55	ND
130*	33.72	ND	28.85	ND	33.43	ND
133*	27	D	ND	ND	ND	ND
EURL**	31.48 (1.33×10²)	ND	30.75 (5.65×10²)	ND	30.26 (1.5×10²)	ND

Designated EU/EFTA member state NRL

** *Reference results from day 2*

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

Annex B

Inhibition and extraction efficiency results

Inhibition and extraction efficiency results for sample 22EFV08 A

Lab. ID	Inhibition			Efficiency	Results		
	GI^t	GII	HAV^t		GI^t	GII	HAV^t
101	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
113*	NR	NR	NR	A	V	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
118*	A	A	A	A	V	V	V
119*	NR	A	NR	A	V	V	V
121*	A	A	A	A	V	V	V
128*	A	A	A	A	V	V	V
129*	A	A	FN	A	V	V	FN
130*	A	A	A	A	V	V	V
133*	A	FP	FN	A	V	FP	FN

* 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 22EFV08 B

Lab. ID	Inhibition			Efficiency	Results		
	GI	GII^t	HAV		GI	GII^t	HAV
101	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
113*	NR	NR	NR	A	NV	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
118*	A	A	A	A	V	V	V
119*	NR	A	NR	A	NV	V	NV
121*	A	A	A	A	V	V	V
128*	A	A	A	A	V	V	V
129*	A	A	A	A	V	V	V
130*	A	A	A	A	V	V	V
133*	A	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	Swab
101	A, C	F	H	J	R		W	Cotton
104*	B	D	H	J	R	UV	W	Cotton
105*	C	D	H	J	R TM9		Wi	Cotton
107*	A	F	H	P	R		Za	Cotton
108*	A	D	H	L	T		X	Cotton
109*	A, C	D	H	J	R		Xx	Cotton
110*	A	E	H	M	R TM9	UV	W	Polyester
111*	A	D	H	N	R		Y	Cotton
112*	A	F	H	J	R		Zq	Plain swab, plastic/vegetal proteins
113*	B	D	H	L	T		W	Deltalab
114*	A, C	D	H	J	R TM9		Z	Texwipe CleanFoam
115	C	D	H	J	R TM9		Zb	Cotton
116	A	D	H	J	R		W	Cotton
118*	A	G	H	J	R		Wii	FLOQ (Copan)
119*	A	D	H	J	R		Zzqq	Cotton
121*	A, C	D	H	J	R	UV	Zq	Cotton
128*	B, C	D	H	J	R, S		Y	Flocked swab (Copan)
129*	A	D	H	L	T		W	Cotton
130*	A	D	H	J	R	UV	Zq	Cotton
133*	A	Ff	H	O	-		Yr	Cotton

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	GeneAll® Ribospin™ vRD
Ff	RNeasy Micro Kit (Qiagen)
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®

Key to method codes cont.

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)
Wii	LightCycler® 480 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