Livsmedelsverket
Swedish Food Agency

European Union Reference Laboratory
for Foodborne Viruses

EURL – FOODBORNE VIRUSES

Final report

Proficiency testing scheme EFV 12, 2024

Detection of norovirus and hepatitis A virus on surfaces

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Summary

This report describes the performance evaluation of National Reference Laboratories (NRLs) in the proficiency testing (PT) EFV12 organised by the EURL for Foodborne Viruses. Distribution of samples occurred on April 22nd, 2024 to 22 laboratories registered for the PT. The PT aimed to quantitatively detect hepatitis A virus (HAV) and norovirus genogroups I (GI) and II (GII) on surface of three samples of ceramic tile.

The participating laboratories were instructed to analyse the samples using their routine method, though the EURL recommended following ISO 15216-2 method. Alongside PT samples, laboratories requesting in advance received external control (EC) RNA 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 ceramic tiles artificially contaminated on the surface with characterised norovirus GI and GII sourced from human faecal material, as well as HAV from cell culture supernatant. 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 12

Viruses	Origin	Strain ID/genotype
Hepatitis A virus	Cell culture supernatant	ATCC® VR-1402™ (HM 175/18f)
Norovirus genogroup I	Faecal material	GI.4 (capsid sequence)
Norovirus genogroup II	Faecal material	GII.6 (capsid sequence)

Table 2: Spiking of PT EFV 12 samples

Sample	Norovirus GI	Norovirus GII	HAV
24EFV12 A	–	–	–
24EFV12 B	≈10^4*	–	≈10^4*
24EFV12 C	–	≈10^4*	–

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

Preparation of samples

Approximately 200 ceramic tiles, each measuring8 23,4 cm^2 were purchased from a retailer in Sweden. The tiles underwent a preparation process involving soaking in chlorine, rinsing with water, and drying on sterile paper sheets. Following this, each tile was glued in a petri dish and subsequently spiked with the target viruses. The dishes were then sealed and stored at 4° C for approximately one hour prior to dispatch.

Distribution of the proficiency testing items

Samples were dispatched in refrigerated conditions by courier on April 22nd, following IATA packing instructions 650 for UN3373. Each of 22 laboratories received three ceramic tile samples on dry ice. Additionally, laboratories that requested it were provided with EC RNA and/or process control virus (mengovirus). An instruction sheet and results form were emailed to the designated contact persons at each laboratory. All laboratories were instructed to perform the extraction within 24 hours of receiving the samples and to report the results no later than May 13th.

Quality control

The ceramic tiles used to produce the test items were tested negative for HAV, norovirus GI and norovirus GII. Spiked samples were examined for homogeneity and stability, with results indicating acceptable inhibition and extraction efficiency for all the samples used in these tests.

Stability levels in ceramic tile samples

A study was conducted to investigate the stability of spiked viruses in samples stored in fridge. This investigation was conducted on two different occasions (before and after dispatch) using two distinct sets of samples. Preliminary tests demonstrated that virus levels remained stable for up to three days. However, participants were instructed to perform virus extraction within 24 hours of delivery to account for potential transport delays. The procedures and results of the stability tests conducted after dispatch, along with the homogeneity tests, are detailed in the reference samples section.

Reference results- Homogeneity and stability of virus levels in ceramic tile samples

The homogeneity of dispatched samples as well as stability of virus levels were evaluated using ten random samples each of 24EFV12B and 24EFV12C simulating realistic shipping and storage conditions at participating laboratories. Samples were refrigerated upon dispatch and analyzed on days 1 and 3. Testing followed EURL SOP based on ISO 15216-1 for quantifying target viruses. The results for both days are presented in Tables 3, 4 and Annex A, with homogeneity tests illustrated in Graph 1 using box and whisker plots (10 samples each). Inhibition and extraction efficiency calculations were performed for all reference samples, confirming PT samples' homogeneity for all target viruses and for trial 12 purposes.

Table 3: Qualitative results for reference samples for PT EFV 12

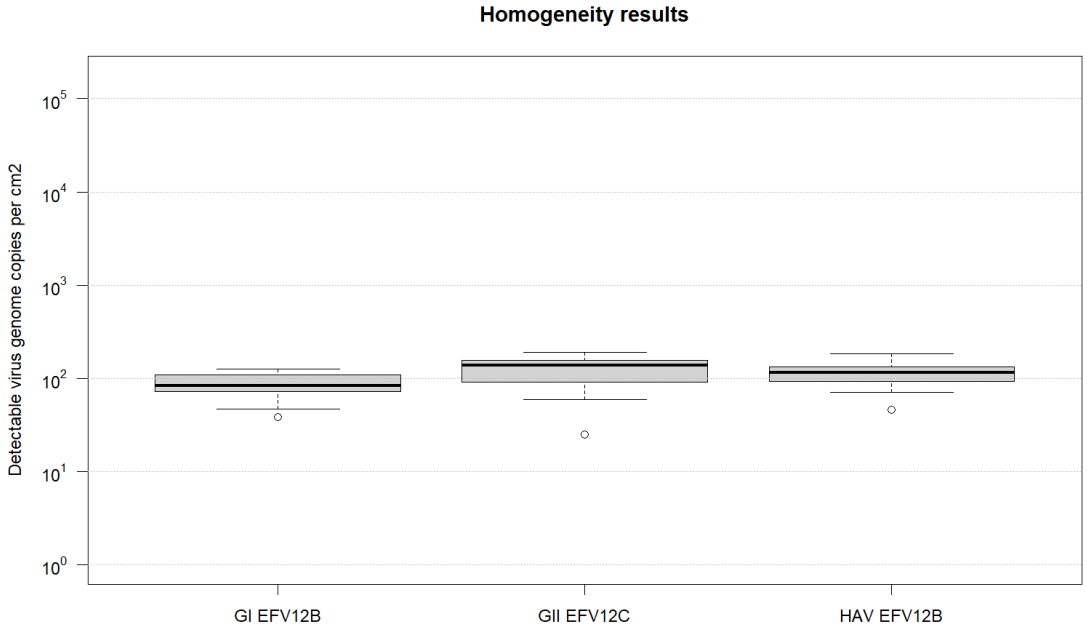
Sample	Norovirus GI	Norovirus GII	HAV
24EFV12 A	Not detected	Not detected	Not detected
24EFV12 B	Detected	Not detected	Detected
24EFV12 C	Not detected	Detected	Not detected

Table 4: Quantitative results for ten reference samples for PT EFV 12
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
24EFV12 A	Not detected	Not detected	Not detected
24EFV12 B	$4.4 \times 10^1 – 1.61 \times 10^2$*	Not detected	$5.6 \times 10^1 – 2.2 \times 10^2$*
24EFV12 C	Not detected	$4.4 \times 10^1 – 2.98 \times 10^2$*	Not detected

**detectable virus genome copies per cm²*

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]



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 check of homogeneity against criteria is performed by use of the theoretic standard deviation (SD). These values were used to determine

[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/.

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 EFV12B	0.4	**yes**	No
	0.5	**yes**	**Yes**
GII EFV12C	0.3	**yes**	No
	0.5	**yes**	**Yes**
HAV EFV12B	0.1	yes	no
	0.5	**yes**	**yes**

Results and discussion

Samples were distributed to 22 laboratories, including 20 NRLs and one undergoing designation. All 22 laboratories returned their results. However, NRL 119, 123 and 130 submitted their results after the deadline. The EURL retained the intended results until these NRLs submitted theirs, allowing them an opportunity to demonstrate their competence. Unfortunately, their results were excluded because they did not meet the deadline criteria for reporting PT results.

All laboratories, except two, received the samples on April 23th, one day after dispatch. One participant received the samples on April 24th, and another on April 26th. The majority of laboratories conducted their analyses within 24 to 48 hours following dispatch.

Nearly all 19 laboratories reported true results for each sample and agent, with two exceptions: Laboratory 116 reported a false positive result for norovirus GII in sample A, while Laboratory 120 did not report detected/not detected results for Hepatitis A virus across all three samples.

Furthermore, number of none valid negative results is due to the fact that in four cases no results for inhibition and or extraction efficiency were reported. Overview of results is demonstrated 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 24EFV12 A, B and C

Target viruses	N*	24EFV12 A				24EFV12 B				24EFV12 C			
		T	FP	FN	NV	T	FP	FN	NV	T	FP	FN	NV
Norovirus GI	19	19	0	-	3	19	-	0	-	19	0	-	3
Norovirus GII	19	18	1	-	3	19	0	-	3	19	-	0	-
Hepatitis A virus	19	18^{nr}	0	-	4	18^{nr}	-	0	-	18^{nr}	0	-	4

N: Number of laboratories that reported results for the analysis, ^{nr}: number of true results affected by the fact that one laboratory did not report detected/not detected results for Hepatitis A virus across all three samples, 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

The maximum achievable score for each laboratory, per target virus and based on the results from all three samples, is 6 points (see Table 7).

Table 7: Calculated data used for scoring assessment

Presence/absence			
Lab ID	GI	GII	HAV
101*	6 out of 6	6 out of 6	6 out of 6
103**	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
105*	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
109*	6 out of 6	6 out of 6	6 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	6 out of 6	6 out of 6
116*	6 out of 6	4 out of 6^{FP}	6 out of 6
118*	6 out of 6	6 out of 6	6 out of 6
120*	6 out of 6	6 out of 6	0 out of 6^{NR}
121*	6 out of 6	6 out of 6	6 out of 6
131*	6 out of 6^{ei}	6 out of 6^{ei}	6 out of 6^{ei}
133*	6 out of 6^{i}	6 out of 6^{i}	6 out of 6^{i}
134*	6 out of 6	6 out of 6	6 out of 6
Excluded			
119*	4 out of 6^{FN,e}	4 out of 6^{FN,e}	4 out of 6^{FN,e}
123*	6 out of 6	6 out of 6	4 out of 6^{FP}
130*	6 out of 6	6 out of 6	4 out of 6^{FP}

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

^{e}: unacceptable extraction efficiency, ^{fn}: false negative, ^{fp}: false positive, ^{i}: unacceptable inhibition, ^{NR}:no results

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.

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 area of sample tested) cm^2.

All qualitative results reported as detected for norovirus GII in sample C and norovirus GI and HAV 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

Eight 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 EFV12, organized in April 2024 by EURL for Foodborne Viruses, was to assess the NRLs' ability for quantitative detection of HAV, norovirus GI and norovirus GII on the surface of ceramic tile samples.

Nineteen laboratories submitted their results within the given time for this PT and 89 % of the participating laboratories obtained fully satisfactory results.

Annex A

Participant's results

with EURL standards with own standards false results

	24EFV12 A			24EFV12 B					24EFV12 C			
	GI	GII	HAV	GI[t]	GI[t]	GII	HAV[t]	HAV[t]	GI	GII[t]	GII[t]	HAV
Lab ID	Cq	Cq	Cq	Cq	gc/cm^2	Cq	Cq	gc/cm^2	Cq	Cq	gc/cm^2	Cq
101*	ND	ND	ND	33,29	9,79E+02	ND	31,08	1,10E+01	ND	34,02	2,76E+02	ND
103**	ND	ND	ND	27,49		ND	31,20		ND	28,07		ND
104*	ND	ND	ND	29,42		ND	27,96		ND	30,58		ND
105*	ND	ND	ND	32,47		ND	32,22		ND	28,28		ND
107*	ND	ND	ND	28,59		ND	28,26		ND	29,73		ND
109*	ND	ND	ND	30,21	3,83E+02	ND	28,98	1,268E+03	ND	29,03	5,23E+02	ND
110*	ND	ND	ND	29,00	1,02E+02	ND	29,22	4,14E+02	ND	29,73	1,68E+02	ND
111*	ND	ND	ND	35,140	3,45E+00	ND	36,28	3,74E+00	ND	38,27	4,60E-01	ND
112*	ND	ND	ND	30,91		ND	29,17		ND	30,66		ND
113*	ND	ND	ND	32,55		ND	31,39		ND	32,60		ND
114*	ND	ND	ND	30,84	8,40E+01	ND	29,135	3,20E+02	ND	29,23	1,90E+02	ND
115	ND	ND	ND	33,93		ND	32,03		ND	34,58		ND
116*	ND	D	ND	36,0		ND	34,0		ND	34,8		ND
118*	ND	ND	ND	31,66		ND	31,81		ND	33,94		ND
120*	ND	ND	NR	32,42	2,12E+02	ND	NR		ND	32,32	1,39E+02	NR
121*	ND	ND	ND	34,50		ND	32,14		ND	35,38		ND

**Designated EU/EFTA member state NRL, ** in designation process, D: reported as detected, ND: reported as not detected, NR: not reported, [t]: target virus*

	24EFV12 A			24EFV12 B					24EFV12 C			
	GI	GII	HAV	GI^{t}	GI^{t}	GII	HAV^{t}	HAV^{t}	GI	GII^{t}	GII^{t}	HAV
Lab ID	Cq	Cq	Cq	Cq	gc/cm^2	Cq	Cq	gc/cm^2	Cq	Cq	gc/cm^2	Cq
131*	ND	ND	ND	NR		ND			ND	NR		ND
133*	ND	ND	ND	28,86		ND			ND	30,53		ND
134*	ND	ND	ND	31,74		ND			ND	31,50		ND
Ref.	ND	ND	ND	31,46	1,26E+02	ND	32,91	1,17E+02	ND	29,08	1,46E+02	ND
Excluded												
119*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND
123*	ND	ND	ND	32,3		ND		30,6	ND	30,6		D
130*	ND	ND	ND	30,28		ND		29,15	ND	32,99		D

**Designated EU/EFTA member state NRL, ** in designation process, Ref.: Reference results from day 2, D: reported as detected, ND: reported as not detected, ^{t}: target virus*

Annex B

Inhibition and extraction efficiency results

Inhibition and extraction efficiency results for sample 24EFV12 A

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII	HAV		GI	GII	HAV
101*	A	A	A	A	V	V	V
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
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	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
118*	A	A	A	A	V	V	V
120*	A	A	NR	A	V	V	NV
121*	A	A	A	A	V	V	V
131*	NR	NR	NR	NR	NV	NV	NV
133*	NR	NR	NR	A	NV	NV	NV
134*	A	A	A	A	V	V	V
Excluded							
129*	A	A	A	U	V	V	V
123*	A	A	FN	A	V	V	FN
130*	A	A	A	A	V	V	V

** Designated EU/EFTA member state NRL*

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

Inhibition and extraction efficiency results for sample 24EFV12 B

	Inhibition			Efficiency	Results		
Lab. ID	GI[t]	GII	HAV[t]		GI[t]	GII	HAV[t]
101*	A	A	A	A	V	V	V
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
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
120*	A	A	NR	A	V	V	V
121*	A	A	A	A	V	V	V
131*	NR	NR	NR	NR	V	NV	V
133*	A	NR	A	A	V	NV	V
134*	A	A	A	A	V	V	V
Excluded							
119*	FN	A	FN	U	FN	NV	FN
123*	A	A	A	A	V	V	V
130*	A	A	A	A	V	V	V

** Designated EU/EFTA member state NRL*

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

Inhibition and extraction efficiency results for sample 24EFV12 C

	Inhibition			Efficiency	Results		
Lab. ID	GI	GII[t]	HAV		GI	GII[t]	HAV
101*	A	A	A	A	V	V	V
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
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
120*	A	A	NR	A	V	V	NR
121*	A	A	A	A	V	V	V
131*	NR	NR	NR	NR	NV	V	NV
133*	NR	A	NR	A	NV	V	NV
134*	A	A	A	A	V	V	V
Excluded							
119*	A	FN	A	A	V	FN	V
123*	A	A	FP	A	V	V	FP
130*	A	A	FP	A	V	V	FP

** 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*	B	D	H	J	R		W	Nylon
103**	A	D	H	N	R		X	Polyester
104*	B		H	N	R	UV	Xx	Wipe
105*	A	D	H	J	R	UV	Wi	Cotton
107*	A	D	H	P	R	UV	Za	Cotton
109*	A	D	H	K	R		Ys	Cotton
110*	A	E	H	M	R	UV	W	Polyester
111*	A	D	H	N	R		Y	Cotton
112*	A	G	H	J	R		Zq	Cotton
113*	B	D	H	L	T		W	Cotton
114*	A	D	H	J	R		Z	Wipe
115	A	D	H	J	R		Zb	Polyester
116	A	D	H	J	R		W	Cotton
118*	A	G	H	J	R	UV	Wii	Nylon
120*	A	D	H	N	R		X	Cotton
121*	A	D	H	J	R	UV	Zq	Cotton
131*	A	D	H	M	R	UV	Zq	Cotton
133*	A	F	H	O	R		Yr	Cotton
134*	A	Ff	H	J	R	UV	Za	Nylon
119*	A	Gg	H	J	R		W	Cotton
123*	A	D	H	J	R		X	Cotton
130*	A	Ee	H	J	R	UV	W	

** Designated EU/EFTA member state NRL*

Key to method codes

1. Virus isolation and concentration method	
A	ISO 15216-2
B	Internal method
2. RNA extraction methods/reagents	
D	NucliSens® (BioMérieux)
E	NucliSens® (BioMérieux), alternative robot system QuikPick Tool
F	QIAamp Viral RNA Mini Kit (Qiagen)
G	TANBead Nucleic Acid Extraction Kit
Ff	Syngen Viral Mini Kit PLUS
Gg	PureLink™ Viral RNA/DNA Mini Kit (Invitrogen)
Ee	MagPurix® Viral Nucleic Acid Extraction Kit
3. PCR method RT-PCR	
H	One step
4. RT-PCR reagents	
J	RNA UltraSense™ One-Step Quantitative RT-PCR System
K	Luna® Universal Probe One-Step RT-qPCR 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	AgPath-ID™ One-Step RT-PCR Reagents
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)*

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	CFX Opus Real-Time PCR (Biorad)
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
Ys	Applied Biosystems™ 7900 Real-Time PCR System
Za	Rotor-Gene Q (Qiagen)
Zb	Stratagene MX3005P® QPCR System
Zq	Applied Biosystems™ QuantStudio™ 5

European Union Reference Laboratory
for Foodborne Viruses