

# EURL – FOODBORNE VIRUSES

## Draft final report

Proficiency testing scheme EFV 10, 2023

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Detection of norovirus and hepatitis A virus in raspberries

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# Contents

Summary .....	5
Background.....	6
Samples .....	7
Preparation of samples .....	7
Distribution of the proficiency testing items .....	7
Quality control.....	8
Reference results- Homogeneity and stability of virus levels in raspberry samples .....	8
Results and discussion.....	11
Performance assessment .....	11
Presence- Absence .....	11
Inhibition and efficiency results .....	13
Methods used by the participants .....	13
Conclusion .....	14
Annex A .....	15
Participants' results.....	15
Annex B.....	17
Inhibition and extraction efficiency results.....	17
Annex C.....	20
General information on methods .....	20
Key to method codes.....	21



# Summary

This report describes the performance of NRLs for detection of viral contamination in raspberries in PT scheme EFV10, organised by the EURL for Foodborne Viruses. Distribution was made on 11<sup>th</sup> of September 2023 to 26 laboratories that signed up to take part in the PT which 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 15216-2. A Standard Operating Procedure (SOP) for detection of norovirus and hepatitis A virus in soft fruits, based on ISO 15216-2, is therefore available at EURL homepage. External control (EC) RNA and process control virus were distributed together with PT samples to the participants who 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 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 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 10

Viruses	Origin	Strain ID/genotype
Hepatitis A virus	Cell culture supernatant	ATCC® VR-1402™ (HM 175/18f)
Norovirus genogroup I	Faecal material	GI.3 + GI.7 (capsid sequence)
Norovirus genogroup II	Faecal material	GII.4 Sydney (capsid sequence)

Table 2: Spiking of PT EFV 10 samples

Sample	Norovirus GI	Norovirus GII	HAV
23EFV10 A	$\approx 4 \times 10^2^*$	$\approx 4 \times 10^3^*$	$\approx 4 \times 10^3^*$
23EFV10 B	–	–	–
23EFV10 C	–	–	–

\*Detectable virus genome copies inoculated to each sample

## Preparation of samples

Approximately 2.5 kg frozen raspberries of the same batch were purchased from a retail in Sweden. A homogenous mixture was prepared by mixing all the raspberries together. The material was then divided into 25 grams, transferred to plastic bags, spiked with the target viruses, sealed and stored in  $-20^\circ\text{C}$  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 11<sup>th</sup>. All 26 laboratories received three frozen samples and the ones that requested in advance received EC RNA and 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 22<sup>nd</sup> and October 6<sup>th</sup> respectively.

# Quality control

Frozen raspberries 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 raspberry samples

In order to investigate the stability of spiked viruses in samples stored in freezer, a thorough study was conducted previously along with other raspberry PTs and the deadline for extraction was set based on those studies.

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 of 23EFV10A were tested. All ten reference samples were stored on dry ice after inoculation on the dispatch date (September 11<sup>th</sup>), and five samples were tested after 24 hours and the rest of samples were stored in -20 °C and tested after 48 hours.

Samples were analysed according to EURL SOP based on ISO 15216-1 for the quantification of target viruses respectively. The results are shown in Table 3 and 4, with box and whisker plots included in Graph 1. The results of one reference sample from day 3 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 all the target viruses and for trial 10 purposes.

*Table 3: Qualitative results for reference samples for PT EFV 10*

Sample	Norovirus GI	Norovirus GII	HAV
23EFV10 A	Detected	Detected	Detected
23EFV10 B	Not detected	Not detected	Not detected
23EFV10 C	Not detected	Not detected	Not detected



Table 4: Quantitative results for ten reference samples for PT EFV 10

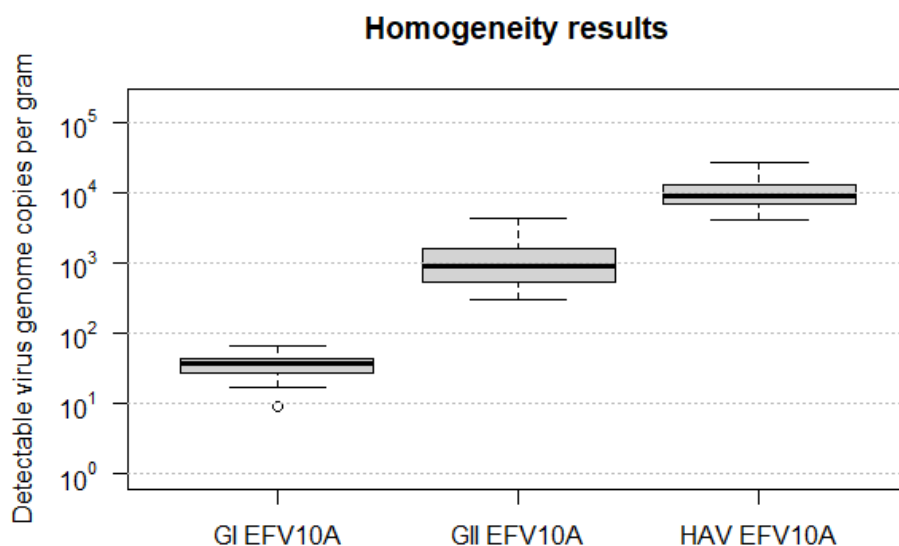
Ranges based on a 95 % confidence limit determined as two geometric standard deviations above and below the geometric mean

Sample	Norovirus GI	Norovirus GII	HAV
23EFV10 A	$1.21 \times 10^1 - 9.00 \times 10^1^*$	$2.24 \times 10^2 - 3.93 \times 10^3^*$	$3.34 \times 10^3 - 2.59 \times 10^4^*$
23EFV10 B	Not detected	Not detected	Not detected
23EFV10 C	Not detected	Not detected	Not detected

\*detectable virus genome copies per gram sample

Graph 1: Box and whisker plots for homogeneity test of samples 23EFV10 A

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.<sup>1</sup>



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 one occasion and at the same time.

As there are not enough previous values of standard deviation for proficiency assessment ( $\sigma_{pt}$ ) 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. Theoretic SD values are used as tentative values of  $\sigma_{pt}$ , to

<sup>1</sup> 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/>.

be compared to values in coming PT schemes. 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  $\sigma_{pt}$  values, at least according to criterion 2. Other values of  $\sigma_{pt}$  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.  $\sigma_{pt}$  (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

$\sigma_{pt}$ : 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; yellow indicate homogeneity according to one criterion and green fields indicate homogeneity of the samples according to both criteria.

Virus type	$\sigma_{pt}$	Homogenous?	Homogenous?
		$s_s < 0.3 * \sigma_{pt}$	$s_s < \sqrt{c}$
GI EFV010A	0.2	no	yes
	0.4	no	yes
	0.5	yes	yes
GII EFV10B	0.4	no	yes
	0.6	no	yes
	0.7	yes	yes
HAV EFV10C	0.3	no	yes
	0.4	no	yes
	0.5	yes	yes

# Results and discussion

Samples were sent to 26 laboratories (including 23 NRLs and one in designation process) and 25 laboratories returned their results. All laboratories except three (which received the samples on September 13<sup>th</sup> and 15<sup>th</sup>) received the samples on September 12<sup>th</sup> (one day after dispatch) and half of the participants analysed the samples within the first week of dispatching (September 13<sup>th</sup>- 14<sup>th</sup>) and half of participants analysed the samples within the second week (September 19<sup>th</sup>- 21<sup>st</sup>).

The majority of laboratories reported true results. However, some false negative results were reported. Furthermore, the number of non-valid negative results for all agents were six and seven for samples B and C respectively. 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 3 are presented as Ref.

Table 6: Overview of participants' results for samples 23EFV10 A, B and C

Target viruses	N	23EFV10 A				23EFV10 B				23EFV10 C			
		T	FP	FN	NV	T	FP	FN	NV	T	FP	FN	NV
Norovirus GI	25	24	-	1	-	25	0	-	6*	25	0	-	7*
Norovirus GII	25	25	-	0	-	24	1	-	6*	23	2	-	7*
Hepatitis A virus	25	25	-	0	-	24	1	-	6*	24	1	-	7*

\*: one NRL did not provide any inhibition results due to the method. N: Number of laboratories that reported results for the analysis, T: true results, FP: False positive, FN: False negative, NV: Non-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).

Table 7: scoring assessment

Lab ID	Presence/absence		
	GI	GII	HAV
101*	6 out of 6 <sup>e</sup>	6 out of 6 <sup>e</sup>	4 out of 6 <sup>fp,e</sup>
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
108*	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 <sup>i1</sup>	6 out of 6 <sup>i1</sup>	6 out of 6 <sup>i1</sup>
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	6 out of 6	6 out of 6
118*	6 out of 6	6 out of 6	6 out of 6
119*	6 out of 6	4 out of 6 <sup>fp</sup>	6 out of 6
120*	6 out of 6	6 out of 6	6 out of 6
121*	6 out of 6	6 out of 6	6 out of 6
122*	6 out of 6 <sup>i</sup>	6 out of 6 <sup>i</sup>	6 out of 6 <sup>i</sup>
123*	6 out of 6	6 out of 6	4 out of 6 <sup>fp</sup>
129*	4 out of 6 <sup>fn,e</sup>	2 out of 6 <sup>fp,e</sup>	6 out of 6 <sup>e</sup>
130*	6 out of 6	6 out of 6	6 out of 6
131*	6 out of 6 <sup>ei</sup>	6 out of 6 <sup>ei</sup>	6 out of 6 <sup>ei</sup>
133*	6 out of 6 <sup>ei</sup>	6 out of 6 <sup>ei</sup>	6 out of 6 <sup>ei</sup>
134*	6 out of 6 <sup>i</sup>	6 out of 6 <sup>i</sup>	6 out of 6 <sup>i</sup>

\* Designated EU/EFTA member state NRL

<sup>e</sup>: unacceptable extraction efficiency, <sup>fn</sup>: false negative, <sup>fp</sup>: false positive, <sup>i</sup>: unacceptable inhibition, <sup>i1</sup>: no inhibition results due to the method

## Inhibition and efficiency results

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

NRL 131 did not report any inhibition and extraction efficiency results. NRL 133 did not report any extraction efficiency results and NRL 134 did not report any inhibition results. NRL 123 reported acceptable inhibition results for 1:10 dilution for all three samples and all the agents. NRL 113 did not report any inhibition results due to characteristics of the method used by this NRL. NRL 114 reported acceptable inhibition results for 1:10 dilution for GI in samples A and B, GII and HAV in sample B. NRL 119 reported acceptable inhibition results for 1:10 dilution for GI, GII and HAV 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 for qualitative results.

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.

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

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

# Conclusion

The aim of PT EFV10 organized in September 2023 by EURL for Foodborne Viruses was to assess the NRLs ability for detection of HAV, norovirus GI and norovirus GII in frozen raspberry samples.

Twenty-five laboratories submitted their results for this PT and 88 % of the participating laboratories obtained full satisfactory results.

# Annex A

## Participants' results

with EURL standards 
  with own standards 
  false results

Lab. ID No.	23EFV10 A			23EFV10 B			23EFV10 C		
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)
101*	32.55	28.70	24.24	ND	ND	ND	ND	ND	<b>D 33.5</b>
103	30.15	24.99	25.95	ND	ND	ND	ND	ND	ND
104*	31.68	24.98	23.35	ND	ND	ND	ND	ND	ND
104*	31.68	24.98	23.35	ND	ND	ND	ND	ND	ND
105*	31.72	23.37	24.44	ND	ND	ND	ND	ND	ND
107*	28.72	22.81	23.13	ND	ND	ND	ND	ND	ND
108*	37.5	32.44	29.45	ND	ND	ND	ND	ND	ND
109*	35.22	25.94	25.15	ND	ND	ND	ND	ND	ND
110*	31.28	25.58	25.34	ND	ND	ND	ND	ND	ND
111*	33.47	27.55	25.97	ND	ND	ND	ND	ND	ND
111*	33.47	27.55	25.97	ND	ND	ND	ND	ND	ND
112*	37.88	31.41	28.92	ND	ND	ND	ND	ND	ND
113*	34.11	33.67	29.02	ND	ND	ND	ND	ND	ND
114*	31.31	26.81	26.92	ND	ND	ND	ND	ND	ND
114*	31.31	26.81	26.92	ND	ND	ND	ND	ND	ND
115	35.11	31.06	26.29	ND	ND	ND	ND	ND	ND
116*	37.2	31.1	29.1	ND	ND	ND	ND	ND	ND
118*	36.29	29.61	29.53	ND	ND	ND	ND	ND	ND
119*	36.56	27.82	26.36	ND	ND	ND	ND	<b>D 33.76</b>	ND

\* Designated EU/EFTA member state NRL, D: reported as detected, ND: reported as not detected

Lab. ID No.	23EFV10 A			23EFV10 B			23EFV10 C		
	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)	GI (Cq)	GII (Cq)	HAV (Cq)
120*	33.9	26.3	25.8	ND	ND	ND	ND	ND	ND
121*	35.57	28.96	26.14	ND	ND	ND	ND	ND	ND
122*	37.77 (1:10)	31.21	32.2	ND	ND	ND	ND	ND	ND
123*	34.7 (1:10)	29.3 (1:10)	26.8 (1:10)	ND	ND	<b>D 39.4 (1:10)</b>	ND	ND	ND
129*	<b>ND</b>	30.24	28.69	ND	<b>D 30.4</b>	ND	ND	<b>D 36.19</b>	ND
130*	30.58 (1:10)	31.83 (1:10)	28.34 (1:10)	ND	ND	ND	ND	ND	ND
131*	33.5	36.58	28.97	ND	ND	ND	ND	ND	ND
133*	31.1	24	33.6	ND	ND	ND	ND	ND	ND
133*	39.4	27.3	35.1	ND	ND	ND	ND	ND	ND
134*	<b>D NR</b>	<b>D NR</b>	<b>D NR</b>	<b>ND NR</b>	<b>ND NR</b>	<b>ND NR</b>	<b>ND NR</b>	<b>ND NR</b>	<b>ND NR</b>
Ref**	<u>32.52 (35.5)<sup>vg</sup></u>	<u>25.4 (1699.2)<sup>vg</sup></u>	<u>25.8 (8920)<sup>vg</sup></u>	ND	ND	ND	ND	ND	ND

\* Designated EU/EFTA member state NRL, \*\* Reference results from day 3, D: reported as detected, ND: reported as not detected, <sup>vg</sup>: Virus genome/g



# Annex B

## Inhibition and extraction efficiency results

*Inhibition and extraction efficiency results for sample 23EFV10 A*

Lab. ID	Inhibition			Efficiency	Results		
	GI <sup>t</sup>	GII <sup>t</sup>	HAV <sup>t</sup>		GI <sup>t</sup>	GII <sup>t</sup>	HAV <sup>t</sup>
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
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	V
114*	A (1:10)	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*	A (1:10)	A (1:10)	A (1:10)	A	V	V	V
120*	A	A	A	A	V	V	V
121*	A	A	A	A	V	V	V
122*	NR	NR	NR	A	V	V	V
123*	A (1:10)	A (1:10)	A (1:10)	A	V	V	V
129*	FN	A	A	FN (GI), U (GII, HAV)	FN	V	V
130*	A	A	A	A	V	V	V
131*	NR	NR	NR	NR	V	V	V
133*	A	A	A	NR	V	V	V
134*	NR	NR	NR	A	V	V	V

\* Designated EU/EFTA member state NRL

A: Acceptable, FN: false negative, FP: false positive, NR: not reported, NV: non-valid, †: target virus, U: Unacceptable, V: valid results

*Inhibition and extraction efficiency results for sample 23EFV10 B*

Lab. ID	Inhibition			Efficiency	Results		
	GI	GII <sup>†</sup>	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
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	NV	NV
114*	A (1:10)	A (1:10)	A (1:10)	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*	A	A	A	A	V	V	V
120*	A	A	A	A	V	V	V
121*	A (1:10)	A (1:10)	A (1:10)	A	V	V	V
122*	NR	NR	NR	A	NV	NV	NV
123*	A (1:10)	A (1:10)	FP	A (GI, GII), FP (HAV)	V	V	FP
129*	A	FP	A	U (GI, HAV), FP GII	NV	FP	NV
130*	A	A	A	A	A	A	A
131*	NR	NR	NR	NR	NV	NV	NV
133*	NR	NR	NR	NR	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, NV: non-valid, U: Unacceptable, V: valid results

*Inhibition and extraction efficiency results for sample 23EFV10 C*

Lab. ID	Inhibition			Efficiency	Results		
	GI	GII	HAV		GI	GII	HAV
101*	A	A	FP	U	NV	NV	FP
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
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	NV	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*	A	FP	A	A	V	FP	V
120*	A	A	A	A	V	V	V
121*	A (1:10)	A (1:10)	A (1:10)	A	V	V	V
122*	NR	NR	NR	A	NV	NV	NV
123*	A (1:10)	A (1:10)	A (1:10)	A	V	V	V
129*	A	FP	A	U	NV	FP	NV
130*	A	A	A	A	V	V	V
131*	NR	NR	NR	NR	NV	NV	NV
133*	NR	NR	NR	NR	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, NV: non-valid, U: Unacceptable, V: valid results

# Annex C

## General information on methods

Lab. ID	1	2	3	4	5	6	7
101*	A	D	H	J	R		W
103	A	D	H	N	R	UV	Xm
104*	A	D	H	J	R	UV	W
105*	A	D	H	J	R TM9	UV	Wi
107*	A	E	H	P	S	UV	Za
108*	A	D	H	L	T	UV	Xm
109*	A	G	H	Q	R	UV	V
110*	A	F	H	M	R TM9	UV	W
111*	A	D	H	N	R		Y
112*	A	1	H	J	R	UV	Zq
113*	A, B	D	H	L	T	UV	W
114*	A	D	H	J	R TM9	UV	Z
115	C	D	H	J	R TM9		Zb
116*	A	D	H	J	R TM9	V	W
118*	A	2	H	J	R	UV	Wr
119*	A	3	H	J	R	UV	Wii
120*	A	D	H	J	Tt (Gl), R		Xm
121*	A	D	H	J	R	UV	Zq
122*	A	D	H	1	R		Xd
123*	A	D	H	J	R		X
129*	A	D	H	L	T		W
130*	A	4	H	J	R	UV	W
131*	A	D	H	M	R	UV	Zq
133*	A	1	H	2	R	UV	Y
134*	A	5	H	J	R	UV	Za

\* Designated EU/EFTA member state NRL

## Key to method codes

<b>1. Virus isolation and concentration method</b>	
A	ISO 15216-2
B	Internal method
C	ISO 15216-1
<b>2. RNA extraction methods/reagents</b>	
D	NucliSens® (BioMérieux)
E	NucliSens® (BioMérieux), TANBead Maelstrom™
F	NucliSens® (BioMérieux), alternative robot system QuikPick Tool
G	VIRSeek
1	QIAamp Viral RNA Mini Kit
2	GeneAll® Ribospin™ vRD
3	PureLink™ Viral RNA/DNA Mini Kit
4	MagPurix® Viral RNA Extraction Kit
5	Syngen Viral Mini spin Kit
<b>3. PCR method RT-PCR</b>	
H	One step
<b>4. RT-PCR reagents</b>	
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

<b>RT-PCR reagents</b>	
Q	Luna® Universal One-Step RT-qPCR Kit
1	SensiFAST™ Probe Hi-ROX One-Step Kit
2	AgPath-ID™ One-Step RT-PCR
<b>5. Primers and probes</b>	
R	ISO 15216 ( <i>The probe, NVGG1p or TM9 for norovirus GI was not asked to be specified</i> )
S	Modified ISO 15216
T	CeeramTools®
Tt	Other
<b>6. Accreditation</b>	
U	Norovirus
V	HAV
<b>7. PCR system</b>	
V	Qtower <sup>3</sup> Analytik Jena
W	CFX96™ Real-Time PCR Detection System (Biorad)
Wi	LightCycler® 96 System (Roche)
Wii	ViiA 7 Real-Time PCR System
Wr	LightCycler® 480 System (Roche)
X	Aria Real-Time PCR
Xd	AriaDx Real-time PCR System
Xm	AriaMx Real-time PCR System
Y	Applied Biosystems™ 7500 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

