Public Health England

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Cefas

Summary of Results

Norovirus and Hepatitis A virus Scheme

External Quality Assessment for Microbiology

Distribution Number: NHV00[illegible]
Sample Numbers: NHV0007 & NHV0008

Distribution Date:	**1 October 2018**
Results Due:	**2 November 2018**
Report Date:	**12 November 2018**
Samples prepared and quality control tested by:	**Justin Avant (Cefas)**
Data analysed by:	**Nita Patel** **Manchari Rajkumar**
Report compiled by:	**Nita Patel** **Manchari Rajkumar**
Authorised by:	**Nita Patel**

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Lab no:

The data in FEPTU reports is confidential

Overview:
This Scheme provides external quality assessment samples for laboratories that examine food products or waters for hepatitis A virus and norovirus using the reverse-transcription polymerase chain reaction (RT-PCR). Regulation (EC) No 669/2009 sets out specifications for an increased level of official controls on imports of certain feed and food of non-animal origin and via Implementing Regulation (EU) 2016/2107 currently applies to frozen raspberries imported from Serbia.

According to the European Food Safety Authority (EFSA) foodborne viruses are the second most common cause of foodborne outbreaks in the European Union (EU) after *Salmonella* https://www.food.gov.uk/science/microbiology/norovirus/norovirus-eu. EFSA has identified that removal of viral contamination is extremely difficult. It therefore recommends that the focus on control of viruses in the food chain needs to be based on preventing contamination and cross-contamination of food. Viruses cannot be cultured therefore molecular techniques are the methods of choice for the detection, identification and quantification of foodborne viruses.

ISO 15216-1:2017 Microbiology of the food chain -- Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR -- Part 1: Method for quantification is used by many laboratories. This ISO method covers pre-treatment steps to elute viruses from the different matrices.

This proficiency testing scheme challenges laboratories in detection and quantification (copies per sample) of hepatitis A virus (HAV) and Norovirus GI and GII. It has been organised in collaboration with Cefas[i] as the European Union Reference Laboratory (EURL) for monitoring bacteriological and viral contamination of bivalve molluscs.

FEPTU Quality Control:
The samples were prepared and quality controlled at Cefas.

To demonstrate homogeneity of the sample 20 LENTICULE® discs selected randomly from a batch were examined.

To demonstrate stability of the sample a minimum of Ten LENTICULE discs, selected randomly from a batch, were examined throughout the distribution period.

Cefas used a qRT-PCR using the RNA UltraSense™ One-Step Quantitative RT-PCR System (ThermoFisher), on a Stratagene Mx3005P real-time PCR machine.

The intended results letters provide guidance for participants regarding the intended result.

Guidelines and general advise:
If you experience difficulties with any of the examinations please refer to section 17.0 of the Scheme Guide
https://www.gov.uk/government/publications/food-and-water-proficiency-testing-schemes-scheme-guide

Please contact FEPTU staff for advice and information:

Repeat samples	Carmen Grimes or Kermin Daruwalla	**Tel: +44 (0)20 8327 7119**
Data analysis	Nita Patel or Manchari Rajkumar	**Fax: +44 (0)20 8200 8264**
Microbiological advice	Nita Patel	**E-mail: foodeqa@phe.gov.uk**
General comments and complaints	Nita Patel	**FEPTU's website**
Scheme Advisors	Justin Avant[i] & James Lowther[i]	
Scheme Co-ordinator	Nita Patel	

Accreditation:
PHE will be applying for this scheme to be accredited with the United Kingdom Accreditation Service (UKAS) to ISO/IEC 17043:2010

[i]The Centre for Environment, Fisheries and Aquaculture Science, European Union Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs, Weymouth Laboratory, Dorset, DT4 8UB, United Kingdom

Total number of participants sent distribution NHV004	**36**
Number of laboratories not returning a result for NHV004	**1**
Number of laboratories not examining any samples in NHV004	**1**

Sample: NHV0007

Sample type: LENTICULE® discs prepared with known levels of norovirus GII from human faeces and known levels of Hepatitis A virus (HAV) cell culture supernatant.

Request:
Examine for the presence of viruses (norovirus GI and GII, hepatitis A virus)
Quantify these viruses in the sample (if routinely done)

Contents and summary of results:

Examination		Expected result	Your Result	PHE Score	Z-score
Virus	Norovirus GI	Positive			
	Norovirus GII	Positive			
	HAV	Negative			

Quantification results

Examination		Expected range	Your Result	PHE Score	Z-score
Virus	Norovirus GI	60 – $2.5x10^3$			
	Norovirus GII	58 – $3.6x10^3$			
	HAV	-			

Norovirus GI (graph of results shown on page 4)

Total participants reporting for Norovirus GI	32
Participants reporting correctly a detected result	30 (94%)
Number of laboratories reporting a result copies per sample	15
Expected range as copies per sample	60 – $2.5x10^3$ (1.78 $\log_{10}$ – 3.39 $\log_{10}$)
Assigned value (participants' median)	$3.8x10^2$ copies per sample (2.58 $\log_{10}$)
Uncertainty of assigned value ($U(X_{pt})$ = $\log_{10}$)	0.13
No. of outlying counts	1 (1 high)
Participants mean	$3.6x10^3$ copies per sample (3.55 $\log_{10}$)
Standard deviation of participants results **	0.40 copies per sample $\log_{10}$
FEPTU's QC median	$1.70x10^2$ copies per sample (2.23 $\log_{10}$)

Norovirus GII (graph of results shown on page 4)

Total participants reporting for Norovirus GII	32
Participants reporting correctly a detected result	28 (88%)
Number of laboratories reporting a result copies per sample	13
Expected range as copies per sample	58 – $3.6x10^3$ (1.76 $\log_{10}$ – 3.56 $\log_{10}$)
Assigned value (participants' median)	$4.6x10^2$ copies per sample (2.66 $\log_{10}$)
Uncertainty of assigned value ($U(X_{pt})$ = $\log_{10}$)	0.16
Participants mean	$5.51x10^2$ copies per sample (2.74 $\log_{10}$)
Standard deviation of participants results **	0.45 copies per sample $\log_{10}$
FEPTU's QC median	$4.5x10^2$ copies per sample (2.65 $\log_{10}$)

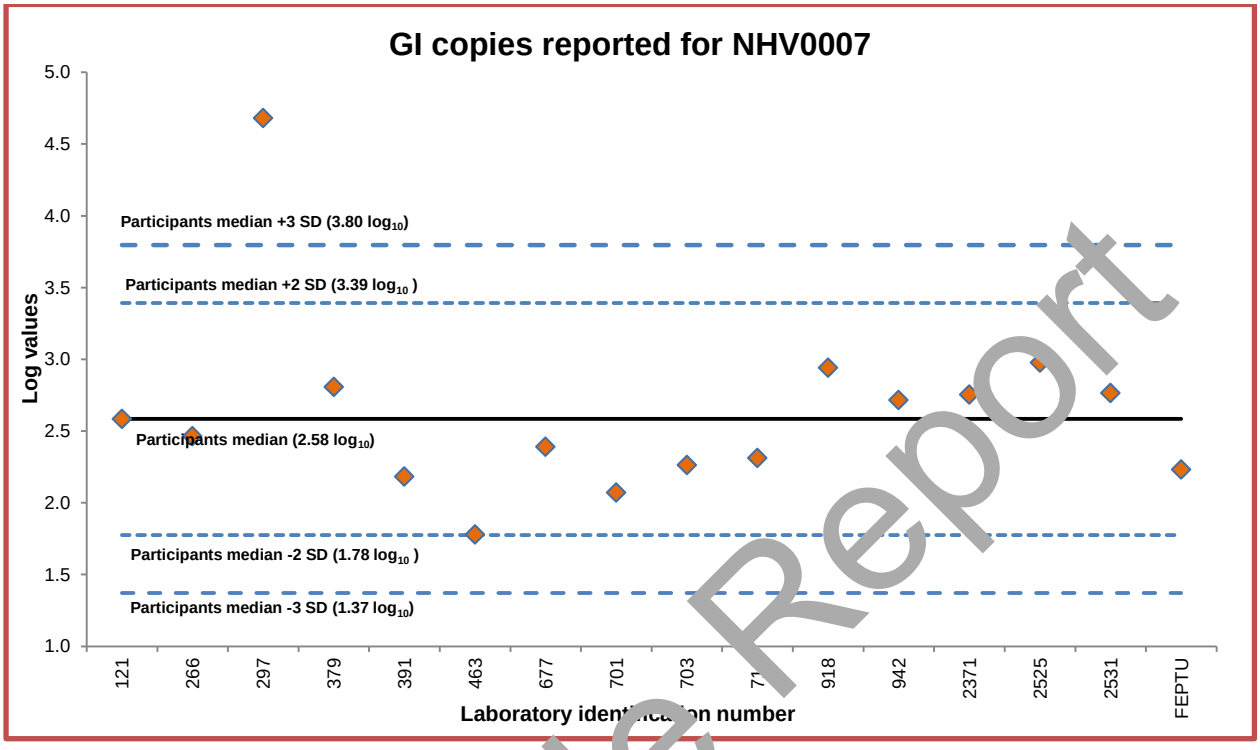
** Robust SD based on median absolute deviation about the participants' median (*MADe*)

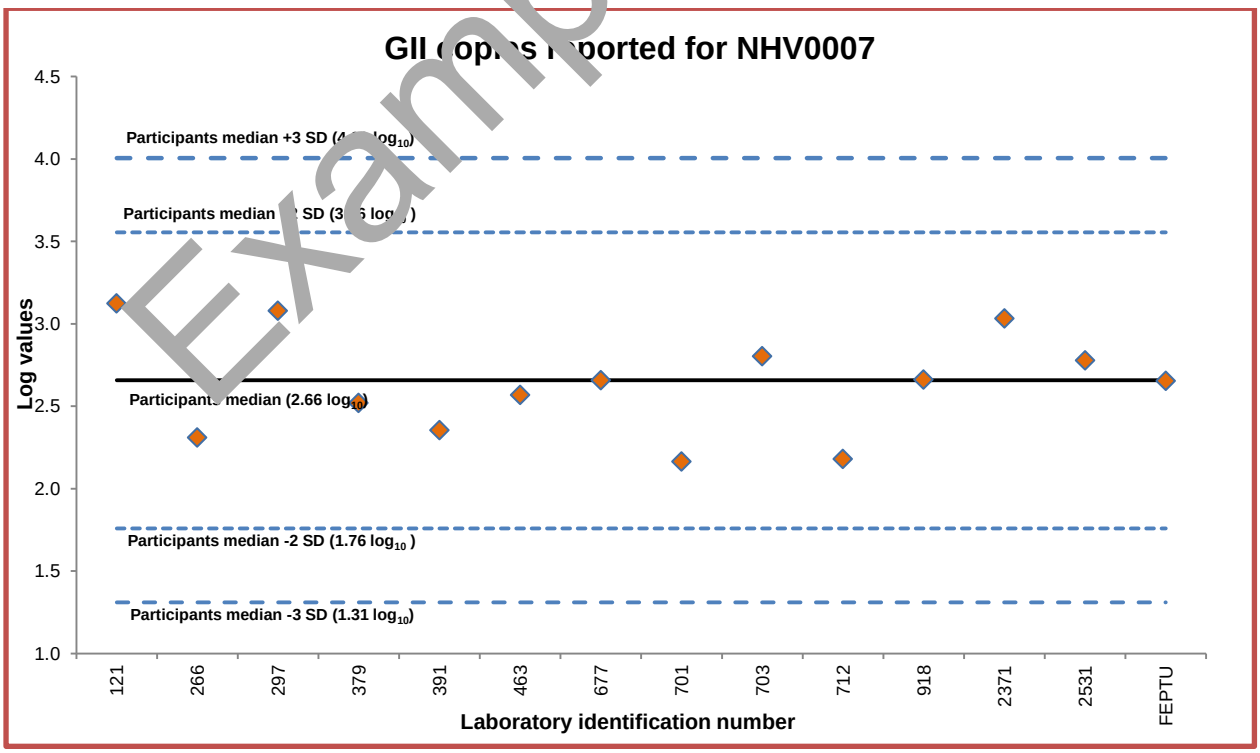
HAV

Total participants reporting for HAV	33
Participants reporting correctly reporting a not detected result	32 (97%)

Sample specific comment

Two laboratories reported a false negative result for Norovirus GI, four laboratories reported a false negative result for GII and one laboratory reported a false positive result for HAV.





Sample: NHV0008

Sample type: LENTICULE® discs prepared with known levels of norovirus GII from human faeces and known levels of Hepatitis A virus (HAV) cell culture supernatant.

Request:
Examine for the presence of viruses (norovirus GI and GII, hepatitis A virus)
Quantify these viruses in the sample (if routinely done)

Contents and summary of results:

Examination		Expected result	Your Result	PHE Score	Z-score
Virus	Norovirus GI	Negative			
	Norovirus GII	Negative			
	HAV	Positive			

Quantification results

Examination		Expected range	Your Result	PHE Score	Z-score
Virus	Norovirus GI	-			
	Norovirus GII	-			
	HAV	$1.3x10^2 – 4.6x10^4$			

Norovirus GI

Total participants reporting for Norovirus GI	32
Participants reporting correctly a not detected result	31 (97%)

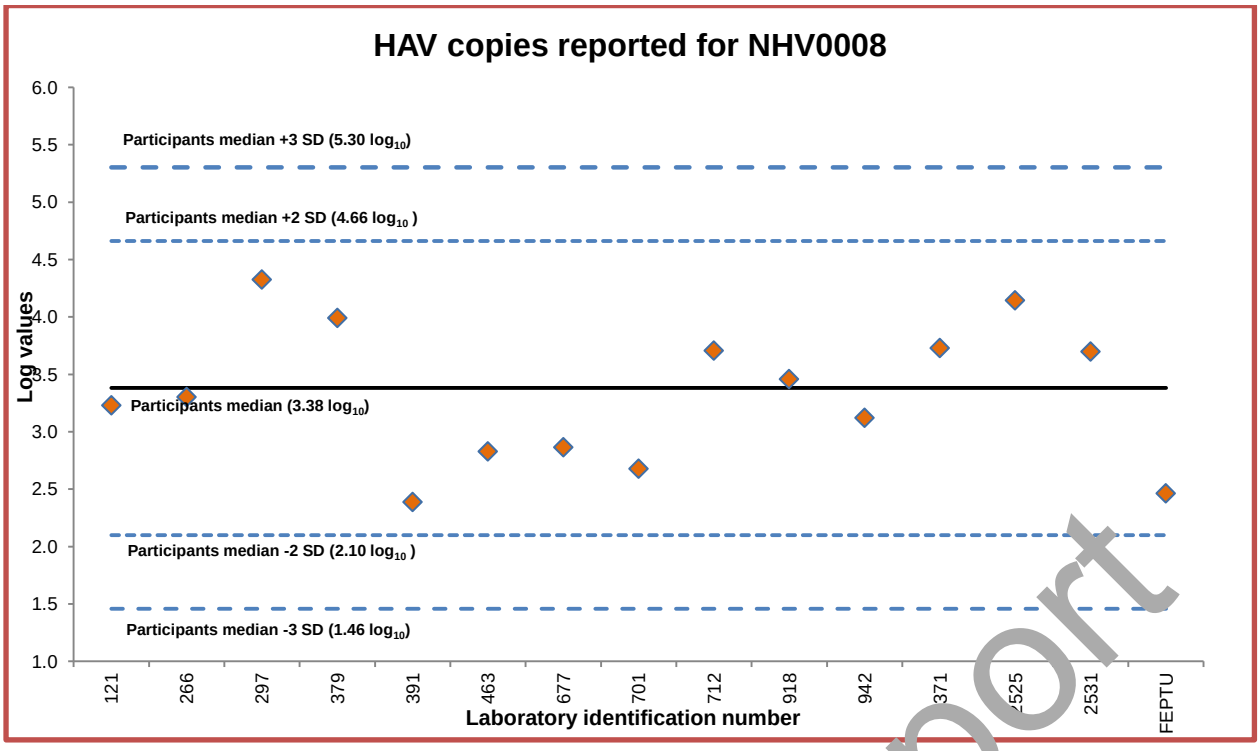
Norovirus GII

Total participants reporting for Norovirus GII	32
Participants reporting correctly a not detected result	31 (97%)

HAV (graph of results shown on page 6)

Total participants reporting for HAV	33
Participants reporting correctly reporting detected result	32 (97%)
Number of laboratories reporting a result copies per sample	14
Expected range as copies per sample	$1.3x10^2$ - $4.6x10^4$ (2.10 $\log_{10}$ – 4.66 $\log_{10}$)
Assigned value (participants' median)	$2.4x10^3$ copies per sample (3.38 $\log_{10}$)
Uncertainty of assigned value ($U(X_{pt})$ = $\log_{10}$)	0.21
Participants mean	$5.0x10^3$ copies per sample (3.70 $\log_{10}$)
Standard deviation of participants results **	0.64 copies per sample $\log_{10}$
FEPTU's QC median	$2.90x10^2$ copies per sample (2.46 $\log_{10}$)

** Robust SD based on median absolute deviation about the participants' median (*MADe*)



General comments for this distribution

This is the fourth distribution of the Norovirus and Hepatitis A virus scheme since it was launched in April 2017.

The results data suggests that one participant has either swapped their samples during testing or entered their results incorrectly.

Scoring applied

The samples in this distribution have been scored using the below scoring criteria.

Presence/absence results
Participants' correct results for detection are allocated scores up to a maximum of two points as follows:

Fully correct result for the intended result	2
False positive / false negative result	0

Quantification results
The expected range for each copies per sample result reported is calculated using the median absolute deviation from the median (*MADe***) values (see * below) which are determined from the median result reported by participants' and take into account the following criteria:

(1) median ± 2 *MADeS**
(2) median ± 3 *MADeS**
(3) median ± 0.5 $\log_{10}$ units

If the ranges in (1) and/or (2) are less than the value of the median ± 0.5 $\log_{10}$ units then the expected range is extended as described in (3).

	Score
Expected range within the range according to criteria (1)	2
Outlying results (1) within the range of criteria (2) but not within criteria (1)	1
Outlying results (2) outside the range of criteria (2)	0

Non-return of results
Participants who do not return a result by the specified date are allocated a PHE score of zero for all tests.

*Statistics applied for copies per sample

The median absolute deviation from the median (*MADe*) values is used to identify outlying counts when there are less than 50 data sets, i.e. when less than 50 participants return results for copies per sample. The use of MAD values provides a statistically robust method for calculating the acceptable range using an analysis that requires calculation of the median difference from the median for every participant's result. This is then multiplied by a constant (1.4826) to obtain a robust estimate of the standard deviation (MAD value). The results are initially interpreted as follows:

Results in range of participants' median ±2 MADSD	satisfactory
Results between ±2 MAD and ±3MADSD	questionable
Results >±3 MADSD	unsatisfactory

Statistical evaluation for quantification results

Participants are advised that for a robust statistical evaluation for quantification data at least 20 results are required for each examination. When statistical calculation is based on 10 – 19 results, they should be interpreted with caution as they may be overly influenced by outlying results. When there are fewer than 10 reported results, the statistics are not considered robust to enable the examination to be scored.

Summary of participants results NHV0007 (any incorrect results are shown in red)							
Lab	Norovirus GI		Norovirus GII		HAV		RNA extraction RT PCR reagent
	Detection	Copies	Detection	Copies	Detection	Copies	
	+	384	+	1326	-	0	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+		+		-		BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	+		+		-		Qiagen QIAamp® Viral RNA CeeramTools® GI, GII and HAV detection kits
	+	291	+	204	-		BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	+		+		-		In house CeeramTools® GI, GII and HAV detection kits
	+	48000	+	1200	-		BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	+		+		-		CONGEN SureFast® Prep DNA/RNA virus CONGEN SureFast® Norovirus/Hepatitis A 3plex kit
	+		+		-		KingFisher™ MagMAX™ CORE Nucleic Acid Purification Kit Applied Biosystems® TaqMan® Universal PCR Master Mix
	+	640		330	-	<20	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	152	+	226	-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+		+		-		BioMérieux NucliSENS® miniMag®

Summary of participants results NHV0007 (any incorrect results are shown in red)							
Lab	Norovirus GI		Norovirus GII		HAV		RNA extraction RT PCR reagent
	Detection	Copies	Detection	Copies	Detection	Copies	
							QIAGEN® QuantiTect® Probe RT-PCR kit
	+		+		-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	60	+	369	-	-	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+		+				Stratec INVISORB Spin Virus RNA Mini Kit Altona DIAGNOSTICS RealStar® Norovirus RT-PCR Kit 2.0
	+	245	+	454	-	Not detected	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	118	+	146	-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	183	+	636	-	Not detected	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	205		151	-		QIAGEN QIAsymphony DSP virus/pathogen mini kit Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	872	+	457	-	-	BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	+	519	+	<LOQ	-		Roche High Pure Viral RNA kit Applied Biosystems™ TaqMan™ Fast Virus One-Step Master Mix

Summary of participants results NHV0007 (any incorrect results are shown in red)							
Lab	**Norovirus GI**		**Norovirus GII**		**HAV**		**RNA extraction** **RT PCR reagent**
	Detection	**Copies**	**Detection**	**Copies**	**Detection**	**Copies**	
	+		+				QIAGEN QIAamp® Viral RNA CeeramTools® GI, GII detection kits
	+		+		-		CONGEN SureFast® Prep DNA/RNA virus
	Not examined		Not examined		-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+		+		-		QIAGEN RNeasy kit CeeramTools® GI, GII and HAV detection kits
	Non-return of results						
	+		+		-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+		+		-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+		-		-		BioMérieux NucliSENS® miniMag® Applied Biosystems™ AgPath-ID™ One-Step PCR Reagents
	+	568		1075	-		BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	-	0	-	0	+	3210	MACHEREY-NAGEL NucleoSpin® RNA Virus ISO 15216-1:2017MasterMix Invitrogen
	+		+		-		CONGEN SureFast® Prep DNA/RNA virus CONGEN SureFast® Norovirus/Hepatitis A 3plex kit

Summary of participants results NHV0007 (any incorrect results are shown in red)							
Lab	**Norovirus GI**		**Norovirus GII**		**HAV**		**RNA extraction** **RT PCR reagent**
	Detection	**Copies**	**Detection**	**Copies**	**Detection**	**Copies**	
					-		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	+	947	-		-		QIAGEN QIAamp® Viral RNA QIAGEN OneStep RT-PCR kit
	+	580	+	600	-		BIOTECON Diagnostics Extraction Kit
	-		-		Not examined		CONGEN SureFast® Prep DNA/RNA virus CONGEN SureFast® Norovirus PLUS
	Not examined				-		BIOTECON Diagnostics Extraction Kit

Summary of participants results NHV0008 (any incorrect results are shown in red)							
Lab	Norovirus GI		Norovirus GII		HAV		RNA extraction RT PCR reagent
	Detection	Copies	Detection	Copies	Detection	Copies	
	-	0	-	0	+	1702	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+		BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	-		-		+		Qiagen QIAamp® Viral RNA CeeramTools® GI, GII and HAV detection kits
	-		-		+	2008	BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	-		-		+	NE	In house CeeramTools® GI, GII and HAV detection kits
	-		-		+	21200	BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	-	-	-		+		CONGEN SureFast® Prep DNA/RNA virus CONGEN SureFast® Norovirus/Hepatitis A 3plex kit
	-		-		+		KingFisher™ MagMAX™ CORE Nucleic Acid Purification Kit Applied Biosystems® TaqMan® Universal PCR Master Mix
	-	<20		<20	+	9800	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+	244	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+		BioMérieux NucliSENS® miniMag®

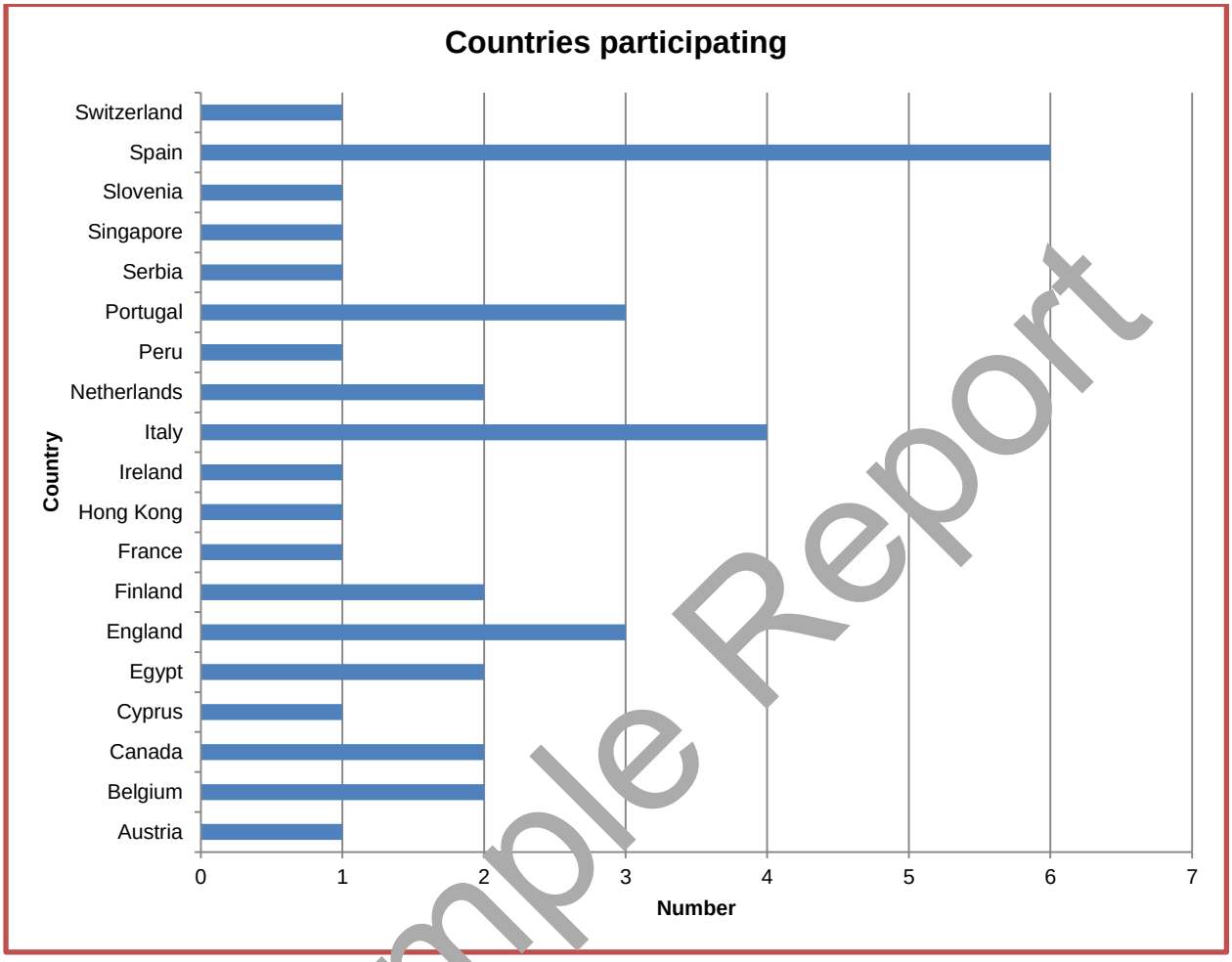
Summary of participants results NHV0008 (any incorrect results are shown in red)							
Lab	Norovirus GI		Norovirus GII		HAV		RNA extraction RT PCR reagent
	Detection	Copies	Detection	Copies	Detection	Copies	
							QIAGEN® QuantiTect® Probe RT-PCR kit
	-		-		+		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+	674	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-				Stratec INVISORB Spin Virus RNA Mini Kit Altona DIAGNOSTICS RealStar® Norovirus RT-PCR Kit 2.0
	-	Not detected	-	Not detected	+	733	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+	478	BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-	Not detected	-	Not detected	+		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-				+	5080	QIAGEN QIAsymphony DSP virus/pathogen mini kit Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-	-	-	-	+	2880	BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	-		-		+	1322	Roche High Pure Viral RNA kit Applied Biosystems™ TaqMan™ Fast Virus One-Step Master Mix

Summary of participants results NHV0008 (any incorrect results are shown in red)							
Lab	**Norovirus GI**		**Norovirus GII**		**HAV**		**RNA extraction** **RT PCR reagent**
	Detection	**Copies**	**Detection**	**Copies**	**Detection**	**Copies**	
	-		-				QIAGEN QIAamp® Viral RNA CeeramTools® GI, GII detection kits
	-		-		+		CONGEN SureFast® Prep DNA/RNA virus
	Not examined		Not examined		+		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+		QIAGEN RNeasy kit CeeramTools® GI, GII and HAV detection kits
	Non-return of results						
	-		-		+		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+		BioMérieux NucliSENS® miniMag® Applied Biosystems™ AgPath-ID™ One-Step PCR Reagents
	-				+	5361	BioMérieux NucliSENS® miniMag® CeeramTools® GI, GII and HAV detection kits
	+	946	+	891	-	0	MACHEREY-NAGEL NucleoSpin® RNA Virus ISO 15216-1:2017MasterMix Invitrogen
	-		-		+		CONGEN SureFast® Prep DNA/RNA virus CONGEN SureFast® Norovirus/Hepatitis A 3plex kit

Summary of participants results NHV0008 (any incorrect results are shown in red)							
Lab	**Norovirus GI**		**Norovirus GII**		**HAV**		**RNA extraction** **RT PCR reagent**
	Detection	**Copies**	**Detection**	**Copies**	**Detection**	**Copies**	
					+		BioMérieux NucliSENS® miniMag® Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System
	-		-		+	13936 [illegible]	QIAGEN QIAamp® Viral RNA QIAGEN OneStep RT-PCR kit
	-		-		+	[illegible]000	BIOTECON Diagnostics Extraction Kit
	-		-		Not exa[illegible]		CONGEN SureFast® Prep DNA/RNA virus CONGEN SureFast® Norovirus PLUS
					+		BIOTECON Diagnostics Extraction Kit

Questionnaire results:
Please note that not all participants provided the relevant information. The data analysed does not evaluate or associate the results with a failure with PT to a method/process used nor does it attempt to compare performance of the various molecular kits/processes with each other.

The graph below shows the participants that took part in this distribution from each country.



The RNA extractions used is shown in the table below:

	No of users	% of users
BioMérieux NucliSENS® miniMag®	19	54
BIOTECON Diagnostics Extraction Kit	2	6
CONGEN SureSens® Prep DNA/RNA virus	4	11
In-house	1	3
KingFisher™ MagMAX™ CORE Nucleic Acid Purification Kit	1	3
MACHEREY-NAGEL NucleoSpin® RNA Virus	1	3
QIAGEN QIAamp® Viral RNA	3	9
QIAGEN QIAsymphony DSP virus/pathogen mini kit	1	3
QIAGEN RNeasy kit	1	3
Roche High Pure Viral RNA kit	1	3
Stratec INVISORB Spin Virus RNA Mini Kit	1	3

The PCR reagents used is shown in the table below:

	No of users	% of users
Altona DIAGNOSTICS RealStar® Norovirus RT-PCR Kit 2.0	1	3
Applied Biosystems® TaqMan® Universal PCR Master Mix	1	3
Applied Biosystems™ AgPath-ID™ One-Step PCR Reagents	1	3
Applied Biosystems™ TaqMan™ Fast Virus One-Step Master Mix	1	3
CeeramTools® GI, GII and HAV detection kits	9	28
CONGEN SureFast® Norovirus PLUS	1	3
CONGEN SureFast® Norovirus/Hepatitis A 3plex kit	2	6
Invitrogen™ RNA UltraSense™ One-Step Quantitative RT-PCR System	13	41
ISO 15216-1:2017 MasterMix Invitrogen	1	3
QIAGEN OneStep RT-PCR kit	1	3
QIAGEN® QuantiTect® Probe RT-PCR kit	1	3

End of report