



European Union Reference Laboratory (EURL) and Public Health England (PHE)

Norovirus and Hepatitis A Virus Scheme

EURL PT reference number: PT 77

Final report

02.01.19

7 pages

Contract Reference: Cefas ref (C7707A)

Document approved by:	C6927 Project Manager – James Lowther	Review date:	Not applicable
Document checked by:	Louise Stockley	Classification:	Official
Document prepared by:	James Lowther	Location	EURL drive

Contents	Page number
Introduction	2
Schemes	2
Samples	2
NRL participation	2
Results	3
References	4
Appendices	5

Introduction

This report describes the performance of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs in virus LENTICULE[®] proficiency testing (PT) schemes jointly organised by the EURL and Public Health England (PHE) during 2018. Test samples were prepared and assessed by the EURL, while the registration of participants, distribution of samples and collation of results was organised by PHE.

Schemes

Two distributions were made in 2018. Scheme NHV003 was distributed on 19th February 2018, while scheme NHV004 was distributed on 1st October 2018. NRL participation in scheme NHV003 was funded by the EURL, while participation in scheme NHV004 was funded by the individual participating NRLs. In both schemes additional labs were invited to participate on a cost-recovery basis.

Samples

For each scheme two test samples were distributed. Samples comprised laboratory constructed LENTICULES[®] prepared following the method of Codd *et al* (1998) with minor modifications. Depending on the desired characteristics of each test sample, norovirus genogroup I and/or genogroup II (GI and/or GII; from human faecal samples), and/or hepatitis A virus (HAV; from tissue culture) was added to the sample mix. Details of the strains of norovirus and HAV used for the samples is shown in Table 1.

Table 1: Origin and strains/genotypes of viruses used for preparation of test samples

Description	Source	Strain ID/genotype
Hepatitis A virus	Cell culture supernatant	HM175/43c
Norovirus genogroup I	Faecal material	GI.7 (NHV003) GI.1 (NHV004) based on capsid sequence)
Norovirus genogroup II	Faecal material	GII.4 (based on capsid sequence)

To demonstrate homogeneity of each sample and to assign reference values, 20 randomly selected LENTICULE[®] discs were tested by the EURL prior to distribution. In addition, to demonstrate stability of the sample a minimum of 5 randomly selected discs were tested at both the start and end of the distribution period. Intended results including reference values are shown in Table 2.

Table 2: Reference results for proficiency testing material

Scheme	Sample	Norovirus		HAV
		GI	GII	
NHV003	NHV0005	-	+ (2.36 x 10 ² - 1.33 x 10 ⁴)	+ (1.39 x 10 ³ - 3.11 x 10 ⁴)
	NHV0006	+ (1.53 x 10 ² - 2.54 x 10 ⁴)	-	-
NHV004	NHV0007	+ (6.0 x 10 ¹ - 2.5 x 10 ³)	+ (5.8 x 10 ¹ - 3.6 x 10 ³)	-
	NHV0008	-	-	+ (1.3 x 10 ² - 4.6 x 10 ⁴)

Results expressed as copies/LENTICULE[®]. Ranges based on a 95% confidence limit determined as 2 geometric standard deviations above and below the geometric mean derived from the EURL homogeneity assessment.

NRL participation

Participation of NRLs in the two schemes is detailed in Table 3. Of the 25 eligible labs, 13 participated in scheme NHV003 (Lab 493 analysed for GI and GII only) with an additional lab receiving samples but not submitting results, while 7 participated in scheme NHV004.

Table 3: NRL participation in schemes NHV003 and NHV004

Lab ID No. ^a	121 [19]	391 [9]	413 [35]	493 [22]	583 [32]	597 [41]	601 [17]	651 [43]	653 [39]	658 [23]	701 [10]	703 [3]	715 [21]	718 [7]	720 [90]	744 [47]	983 [44]	1498 [27]	1578 [13]	1798 [147]	1859 [83]	2118 [33]	2340 [42]	2341 [102]	2374 [25]
PT scheme																									
NHV003	√	√	√	√	√	√	√		√		√	√		√					√			√	(√)		
NHV004	√	√	√								√	√							√					√	

^a NRL Lab number from PHE EQA scheme. ID number for EURL PT schemes shown in brackets. (√) indicates that the lab received samples but did not return any results.

Results

Results reported by participating NRLs are tabulated in Appendices I and II.

Presence/absence

In scheme NHV003, all results submitted by NRLs were correct.

In scheme NHV004, lab 2341[102] reported a false negative result for GII in sample NHV0007. All other results submitted by NRLs were correct.

Overall accuracy for NRLs across the two schemes was 99.2%. Thirteen out of 14 labs (92.9%) scored 100% accuracy in all the schemes in which they participated.

Quantification

All participating NRLs reported Ct values for positive samples in one or both of the schemes. Ten labs additionally reported quantities in copies/LENTICULE[®] disc in the schemes in which they participated.

Quantitative results were assessed following the median absolute deviation from the median (MAD) approach described in ISO/TS 22117 Microbiology of food and animal feeding stuffs – specific requirements and guidance for proficiency testing by interlaboratory comparison (ISO 2010).

For each sample/target virus combination where the intended result was positive, a statistically robust acceptability range was determined by calculation of the median of the absolute deviations for all participants' (including non-NRLs) results (the MAD value) from the median of all participants' results. The MAD value was then multiplied by a constant (1.4826) to obtain a robust estimate of the standard deviation (σ_{MAD} ; Table 4). For each individual result, its absolute deviation from the participants' median was compared with the calculated σ_{MAD} to determine its acceptability as follows:-

- Difference between result and participants' median $< 2 \sigma_{MAD}$ = satisfactory
- Difference between result and participants' median $> 2 \sigma_{MAD}$ and $< 3 \sigma_{MAD}$ = questionable
- Difference between result and participants' median $> 3 \sigma_{MAD}$ = unsatisfactory
- Result reported as negative = unsatisfactory

The differences between individual participants' results and the participants' median, expressed in terms of σ_{MAD} are shown in Appendix III.

In scheme NHV003, lab 391[9] reported a quantity in the questionable range for sample NHV0005, HAV. All other quantitative results reported by NRLs were in the satisfactory range.

In scheme NHV004, all quantitative results reported by NRLs were in the satisfactory range.

Nine out of ten (90.0%) NRLs carrying out quantification reported all results in the satisfactory range.

Table 4: Dataset characteristics for quantitative results

Characteristic	Sample number					
	NHV0005		NHV0006	NHV0007		NHV0008
	GII	HAV	GI	GI	GII	HAV
MEDIAN	3.25	3.82	3.30	2.58	2.66	3.38
MAD	0.29	0.23	0.37	0.27	0.30	0.43
σ MAD	0.44	0.34	0.55	0.40	0.45	0.64

Values in \log_{10} copies/LENTICULE^{*}

References

Codd AA, Richardson IR, Andrews N. 1998. Lenticules for the control of quantitative methods in food microbiology. *J Appl Microbiol.* 85(5):913–7.

Anon 2010 ISO/TS 22117 Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison.

Appendix I: Participating NRLs' results for scheme NHV003

Lab number	Sample																	
	NHV0005									NHV0006								
	GI			GII			HAV			GI			GII			HAV		
	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)
121 [19]	-			+	34.57	8.07E+02	+	31.02	1.11E+04	+	32.05	3.79E+03	-			-		
391 [9]	-			+	30.82	4.21E+03	+	31.33	1.26E+03	+	29.58	1.61E+03	-			-		
413 [35]	-			+			+			+			-			-		
493 [22]	-			+	33.14	1.69E+03	NE			+	35.05	5.66E+02	-			NE		
583 [32]	-			+	34.06	2.89E+03	+	32.08	2.18E+04	+	34.84	2.29E+03	-			-		
597 [41]	-			+	35.23	1.65E+03	+	33.97	2.21E+04	+	37.02	4.98E+02	-			-		
601 [17]	-			+	31.71, 31.63	4.33E+03	+	32.72, 32.58	7.46E+03		32.07, 31.69	5.81E+03	-			-		
653 [39]	-			+	32.98	3.60E+03	+	32.29	5.80E+03	+	32.87	3.90E+03	-			-		
701 [10]	-			+	34.63	1.53E+03	+	33.8	2.81E+03	+	34.55	1.56E+03	-			-		
703 [3]	-			+	33.79, 35.56	1.77E+03	+	32.21, 32.46	2.88E+04	+	33.40, 33.38	4.71E+03	-			-		
718 [7]	-			+	34.41	7.20E+02	+	31.3	4.60E+03	+	36.95	8.40E+02	-			-		
1798 [147]	-			+	31.5		+	28.22		+	32.03		-			-		
2118 [33]	-			+	31.65		+	29.08		+	33.53		-			-		

NE = not examined.

Appendix II: Participating NRLs' results for scheme NHV004

Lab number	Sample																	
	NHV0007									NHV0008								
	GI			GII			HAV			GI			GII			HAV		
+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	+/-	Ct value	Quantity (copies/ LENTICULE)	
121 [19]	+	37.84	3.84E+02	+	35.53	1.33E+03	-			-			-			+	33.45	1.70E+03
391 [9]	+	33.03	1.52E+02	+	32.47	2.26E+02	-			-			-			+	32.38	2.44E+02
413 [35]	+	36.308		+	37.655		-			-			-			+		
701 [10]	+	38.96, 37.80	1.18E+02	+	37.90, 38.16	1.46E+02	-			-			-			+	36.60, 36.53	4.78E+02
703 [3]	+	37.07, 37.57	1.83E+02	+	35.77, 35.18	6.36E+02	-			-			-			+	34.39, 34.13	
1798 [147]	+	34.95		+	32.88		-			-			-			+	30.61	
2341 [102]	+	38		-			-			-			-			+	34	

False negative results highlighted in yellow.

Appendix III: Differences between participants' results and the participants' median, expressed in terms of σ_{MAD}

Lab number	Sample number					
	NHV0005		NHV0006	NHV0007		NHV0008
	GII	HAV	GI	GI	GII	HAV
121 [19]	-0.78	0.67	0.51	0.00	1.04	-0.23
391 [9]	0.86	-2.13	-0.16	-1.00	-0.67	-1.55
493 [22]	-0.04	NE	-0.98	x	x	x
583 [32]	0.49	1.54	0.12	x	x	x
597 [41]	-0.07	1.56	-1.08	x	x	x
601 [17]	0.89	0.16	0.84	x	x	x
653 [39]	0.71	-0.16	0.53	x	x	x
701 [10]	-0.14	-1.09	-0.19	-1.27	-1.10	-1.10
703 [3]	0.00	1.90	0.68	-0.80	0.33	NQ
718 [7]	-0.89	-0.46	-0.67	x	x	x

Questionable results (magnitude of difference between result and participants' median $>2 \sigma_{MAD}$ and $<3 \sigma_{MAD}$) highlighted in orange. NE = sample/target virus combination not examined, NQ = sample/target virus combination not quantified, x = laboratory did not participate in the relevant scheme

About us

Cefas is a multi-disciplinary scientific research and consultancy centre providing a comprehensive range of services in fisheries management, environmental monitoring and assessment, and aquaculture to a large number of clients worldwide.

We have more than 500 staff based in 2 laboratories, our own ocean-going research vessel, and over 100 years of fisheries experience.

We have a long and successful track record in delivering high-quality services to clients in a confidential and impartial manner.
(www.cefasc.defra.gov.uk)

Cefas Technology Limited (CTL) is a wholly owned subsidiary of Cefas specialising in the application of Cefas technology to specific customer needs in a cost-effective and focussed manner.

CTL systems and services are developed by teams that are experienced in fisheries, environmental management and aquaculture, and in working closely with clients to ensure that their needs are fully met.
(www.cefastechnology.co.uk)

Head office

Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK

Tel +44 (0) 1502 56 2244
Fax +44 (0) 1502 51 3865
Web www.cefasc.defra.gov.uk

Customer focus

With our unique facilities and our breadth of expertise in environmental and fisheries management, we can rapidly put together a multi-disciplinary team of experienced specialists, fully supported by our comprehensive in-house resources.

Our existing customers are drawn from a broad spectrum with wide ranging interests. Clients include:

- international and UK government departments
- the European Commission
- the World Bank
- Food and Agriculture Organisation of the United Nations (FAO)
- oil, water, chemical, pharmaceutical, agro-chemical, aggregate and marine industries
- non-governmental and environmental organisations
- regulators and enforcement agencies
- local authorities and other public bodies

We also work successfully in partnership with other organisations, operate in international consortia and have several joint ventures commercialising our intellectual property

Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601

