European Community Reference laboratory for monitoring bacteriological and contamination of bivalve molluscs

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Report on the Norovirus/Hepatitis A Ring Trial, 2006-07

CRL ring trial reference: RT19 (NoV/HAV 2006-07)

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Introduction

Regulation (EC) No 882/2004 designates the Centre for Environment, Fisheries and Aquaculture Science at Weymouth U.K. as the Community Reference Laboratory (CRL) for monitoring the viral and bacteriological contamination of bivalve molluscs. Under Article 32 the laboratory is responsible for organising comparative testing by national reference laboratories.

At the 5th annual workshop of NRLs Weymouth 2006, it was established that the Norovirus (NoV) and hepatitis A (HAV) ring trials were beneficial and should be offered in 2006/07. The workshop resolved that, "In the absence of an internationally accepted reference method for viruses, the CRL should organise a further ring trial for detection of NoV/HAV in the laboratory constructed samples only" (Resolution 4).

Preparation of samples

Samples were comprised of laboratory constructed LENTICULES (A - E). LENTICULES were constructed following the methods of Codd *et al* (1998) with minor modifications. In brief, fully characterised norovirus genogroup I (NoV GI) and genogroup II (NoV GII) as faecal material, laboratory cultured HAV, strain HM175 and phosphate buffered saline was added at a 1:5 ratio to lenticulating fluid. The lenticulating fluid was aliquoted onto parafilm in 25µl volumes and placed in a desiccating chamber at 4±2˚C for 1 week. After 1 week LENTICULES were transferred to <-15˚C for 2 days prior to analysis using the CRL standard method.

Expected results

The expected results for each LENTICULE are given in Table 1.

Table 1. Taqman™ expected results of RT19 ring trial material

Sample	Norovirus		HAV
	GI	GII	
A	-	-	-
B	+	-	-
C	-	+	-
D	+	+	-
E	-	-	+

Distribution

The NoV/HAV ring trial 2006-07 was designated as RT19. The LENTICULES were packaged according to IATA regulations, UN3373 as diagnostic specimens, division 6.2 under the packing instruction code 650. All participating laboratories received five sealed plastic bags containing LENTICULES A - E within a single thermal control unit (CL-2/4) (Air Sea Containers Ltd., Birkenhead, U.K. CH42 1LE). Relevant transport documentation, import permits, instruction sheets and result forms were included with the package. The LENTICULES were distributed at ambient temperature by City Sprint dedicated courier services on Monday 20th November 2006. On receipt, participants were requested to complete the analyses and return results by 12th January 2007.

Quality control

Prior to the distribution, 6 randomly selected LENTICULES were analysed in triplicate using the CRL standard methods. The results are given in Table 2.

Table 2. Taqman™ quality control analyses of RT19 ring trial material

Sample	Norovirus		HAV
	GI	GII	
A	-	-	-
B	+ (34.19)	-	-
C	-	+ (35.58)	-
D	+ (34.49)	+ (36.45)	-
E	-	-	+ (36.21)

Median CT values in parentheses

Participation

Invitations inviting expressions of interest in the NoV/HAV ring trial were sent to all designated MS NRLs, candidate countries laboratories, EFTA and selected third countries. Table 3 summarises the participation in the NoV/HAV ring trial distribution (RT19). Material was dispatched to twenty-eight laboratories. Addresses of participating laboratories are included as Appendix I. Twenty-five laboratories returned results for the trial (Table 4).

Table 3. Participation in the NoV/HAV ring trial distribution (RT19)

Country	Material dispatched	Results returned to CRL
Austria	No	-
Belgium & Luxembourg	Yes	Yes
Bulgaria	No	-
Czech Republic	No	-
Denmark 1[2]	Yes	Yes
Estonia	Yes	Yes
Finland	Yes	No
France 1[2]	Yes	Yes
France 2[2]	Yes	Yes
Germany 1[2]	Yes	Yes
Germany 2	Yes	Yes
Greece	Yes	Yes
Ireland[12]	Yes	Yes
Italy	Yes	Yes
Latvia	No	-
Lithuania	No	-
Netherlands[2]	Yes	Yes
Poland[2]	Yes	Yes
Portugal[2]	Yes	Yes
Romania	Yes	No
Slovakia	Yes	Yes
Slovenia[2]	Yes	Yes
Spain	Yes	Yes
Sweden	No	-
United Kingdom 1[2]	Yes	Yes
United Kingdom 2	Yes	Yes
Candidate country		
Turkey	No	-
EFTA		
Norway[2]	Yes	Yes
Third Country	Material dispatched	Results returned to CRL
Canada 1	Yes	Yes
Chile 1	Yes	Yes
Chile 2	Yes	Yes
Hong Kong	Yes	Yes
New Zealand[2]	Yes	Yes
South Korea[2]	Yes	Yes
United States[2]	Yes	Yes

[1] norovirus only; [2] reported quantitative data

Confidentiality of results

To preserve anonymity of participants a confidential identification number was used to identify each laboratory.

Reference results

Reference analyses on LENTICULES stored at 4±2˚C were undertaken using the CRL standard methods. Six randomly selected LENTICULES from each distribution set were analysed for all viral determinants on 3 separate occasions using the CRL standard method. The quantitative results of CRL reference testing are presented in Appendix II.

Participants' results

Twenty-six laboratories returned results, one of whom only used a method that did not enable discrimination of NoV GI and NoV GII. Two laboratories did not return results for for HAV. Participants' returning results with CT values are shown in Appendix III. Participants' results (shown in Table 4) were assessed as percentage relative sensitivity according to the following calculation:

$$\text{Relative sensitivity (SE)} = \frac{TP}{(TP+FN)} \times 100\%$$

percentage relative specificity:

$$\text{Relative specificity (SP)} = \frac{TN}{(TN+FP)} \times 100\%$$

and percentage relative accuracy

$$\text{Relative accuracy (AC)} = \frac{TP+TN}{N} \times 100\%$$

Where TP = true positives

FN= false negatives

FP = false positives

TN= true negatives

N = total number of tests

Note: Participants' results were expressed as percentage concordance with intended results generated by CRL. In this assessment presence/absence data was used and no consideration of quantitative measurements (C_T values) was made. Scoring was adapted to incorporate laboratories that did not fully discriminate between NoV GI and NoV GII positive results.

Table 4. Participants' results for all LENTICULES (A – E)

Lab ID number	GI			GII			HAV		
	SE	SP	AC	SE	SP	AC	SE	SP	AC
3	100	100	100	100	100	100	nt	nt	nt
7	100	100	100	100	100	100	100	100	100
9	75	50	60	75	83	80	50	100	90
10	100	100	100	100	67	80	100	100	100
11	100	100	100	100	100	100	100	100	100
14	100	100	100	100	100	100	100	100	100
15	100	100	100	100	100	100	100	100	100
17	100	100	100	100	100	100	100	75	80
19	100	100	100	100	100	100	100	100	100
21	100	100	100	100	67	80	100	100	100
22	75	100	90	100	100	100	100	100	100
24	100	100	100	100	100	100	100	100	100
25	100	100	100	100	100	100	100	100	100
27	50	100	80	100	100	100	100	100	100
29	100	100	100	0	100	60	100	100	100
31	75	100	90	75	100	90	nt	nt	nt
32	100	100	100	100	100	100	100	100	100
33	100	100	100	100	100	100	100	100	100
37	100[a]	83[a]	90[a]	100[a]	100[a]	100[a]	100	100	100
39	100	100	100	100	100	100	100	100	100
41	100	100	100	100	100	100	100	100	100
48	66[b]	100[b]	90[b]	66[b]	100[b]	90[b]	100	100	100
55	100	100	100	100	100	100	100	100	100
78	0[c]	100[c]	60[c]	50[c]	33[c]	70[c]	0	75	60
89	100	100	100	100	100	100	100	100	100
94	100	100	100	100	100	100	100	100	100

nt – not tested

[a]Lab 37 used method discriminating between NoV genogroups for replicate 1, non-discriminating method for replicate 2

[b]Lab 48 used non-discriminating NoV method, results shown are for total NoV

[c] Lab 78 recorded a positive NoV result for LENTICULE C, but was unable to discriminate between genogroups

Summary

- Laboratory constructed LENTICULES provided a stable, homogeneous reference material for the ring trial that could be transported at ambient temperature.
- 75%, 71% and 86% of laboratories returned 100% correct results for norovirus GI, GII and hepatitis A respectively
- Demonstrating that LENTICULES can be effectively and correctly analysed.
- Material was dispatched to twenty-eight laboratories. Twenty-six laboratories (93%) returned results within the stated time frame.
- Only one laboratory did not discriminate between NoV GI and NoV GII.
- Two laboratories did not test the LENTICULES for HAV**.**
- Fourteen laboratories returned quantitative real-time PCR data compared with ten in 2005-6.
- The increase in numbers of laboratories generating real-time quantitative data was encouraging and greatly enhances the ability to assess both method and laboratory performance.
- Participants used a number of different RT-PCR methods.

References

Codd A.A., Richardson I. R. and Andrews N. (1998) Lenticules for the control of quantitative methods in food microbiology J Appl Microbiol,Nov, 85(5), 913 - 7

Appendix I - Participating laboratories

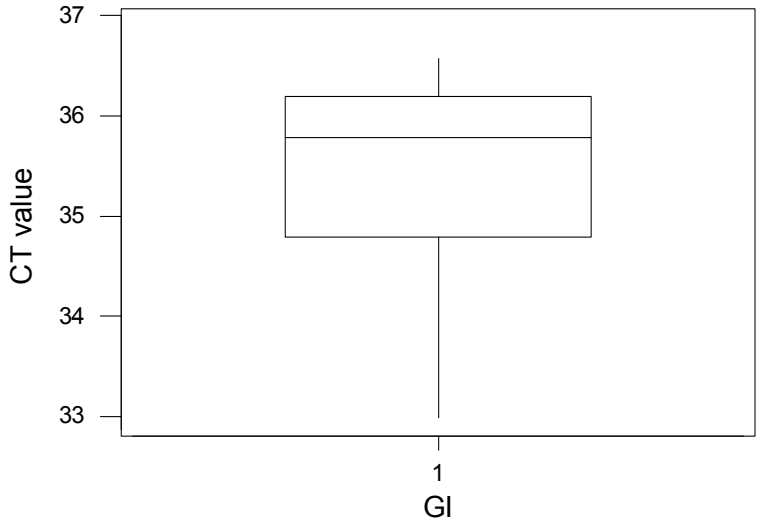
Departement des Sciences des denrees alimentaires Service de microbiologie Bd de Colonster no. 20 Bat B43Bis 4000 Liege **BELGIUM**	Health Canada Room 435 251 Fredrick Banting Driveway PL 2204A2 Ottawa Ontario K1A0K9 **CANADA**
Japan Food Safely Center Universidad de Chile Olivos 943 Santiago **CHILE**	Aquagestion S.A. Panamericana sur 581 Puerto Montt **CHILE**
State Veterinary Institute Jihlava Rantiroska 93 58605 Jihlava **CZECH REPUBLIC**	Danish Institute of Food and Veterinary Research, Department of Mikrobiological Food Safety, Mørkhøj Bygade 19, DK-2860 Søborg. **DENMARK**
Veterinary and Food Laboratory Kreutzwaldi 30 51006 Tartu **ESTONIA**	Finnish Food Safety Authority Evira Mustialankatu 3 00790 Helsinki **FINLAND**
IFREMER Lab. Microbiologie BP 21105 44311 Nantes cedex 03 **FRANCE**	CEERAM 22 Avenue de la Passagere 44800 Saint Herblain **FRANCE**
Federal Institute for Risk Assessment (BfR) Diedersdorfer Weg 1 D-12277 Berlin **GERMANY**	Veterinary Institute for Fish and Fishery Product Lower Saxony Federal State Office for Consumer Protection and Food Safety Schleusenstraβe 1 Cuxhaven **GERMANY**
Democritus university of Thrace Laboratory of Hygiene and Environmental protection, Medical school, Dragana, 68100 Alexandrouplois **GREECE**	Virology Division Centre for Health Protection 9/F Public Health Laboratory Centre 382 Nam Cheong Street Shek Kip Mei Kowloon **HONG KONG**

Marine Institute Rinville Oranmore County Galway **IRELAND**	Istituto Superiore di Sanità v.le Regina Elena, 299 00161ROME **ITALY**
National Institute for Public Health and the Environment (RIVM) Anthonie van Leeuwenhoeklaan 9 3721 MA bilthoven **THE NETHERLANDS**	Institute of Environmental Science and Research Ltd 34 Kenepuru Drive PO Box 50-348 Porirua **NEW ZEALAND**
Norweigan School of Veterinary Science Ullevålsveien 72 0033 Oslo **NORWAY**	National Veterinary Research Institute Al. Partyzantów 57 24-100 Pulawy **POLAND**
IPMAR Av. Brazilia 1449-006 Lisboa **PORTUGAL**	State Veterinary and Food Institute Janoskova 1161/58 026 01 Dolwy Kubin **SLOVAKIA**
National Veterinary Institute Veterinary Faculty University of Ljubljana Gerbiceva 60 1115 LjubljanaN **SLOVENIA**	Agencia Espanola de Seguridad Alimentaria Ctra. Majadahonda-Pozuelo, Km 2 Majadahonda 28220 Madrid, **SPAIN**
National Fisheries Research & Development Institute, #408-1, Shirang-ri, Gijang-eup, Gijang-gun, Busan, 619-902 **KOREA**	Institute for Diagnosis and Animal Health 63, Dr. Staicovici Street, sector 5, cod. 050557, Bucharest, **ROMANIA**
CEFAS - Weymouth Laboratory Barrack Road The Nothe Weymouth Dorset DT4 8UB **UNITED KINGDOM**	U.S Food and Drug Administration Gulf Coast Seafood Laboratory 1 Iberville Dr. P.O. Box 158 Dauphin Island AL 36528 **UNITED STATES**

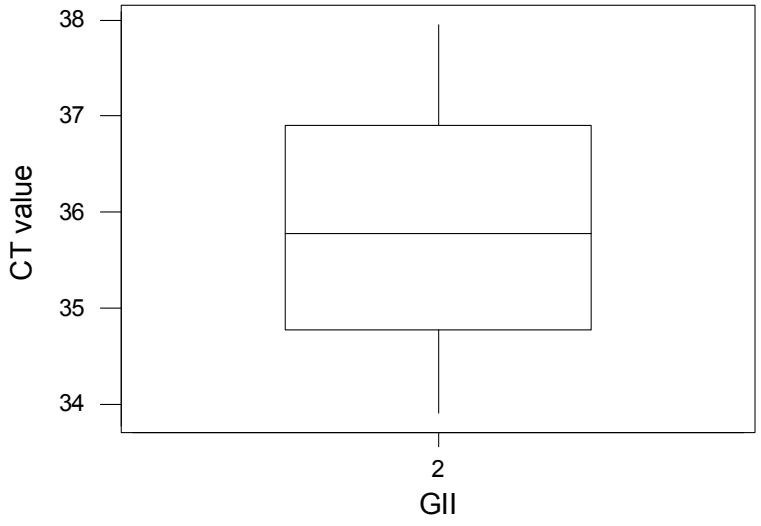
Appendix II - Reference results

CRL Reference results normalised using FCV process controlled data - Displayed as boxplots

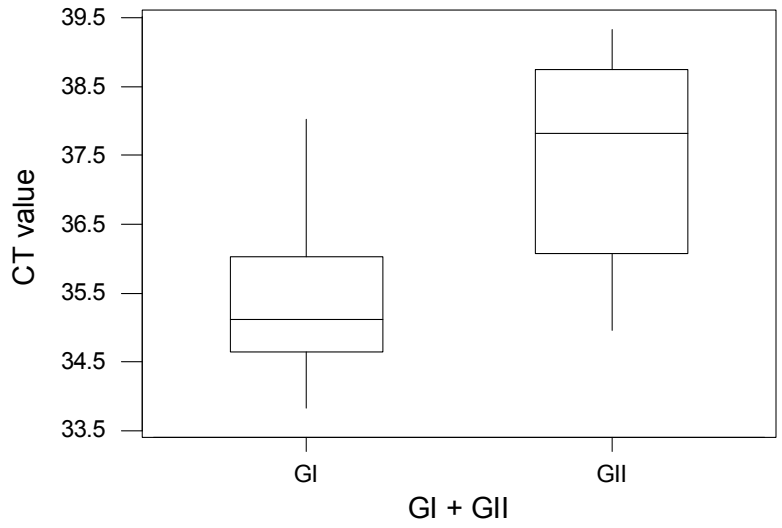
1. LENTICULE B



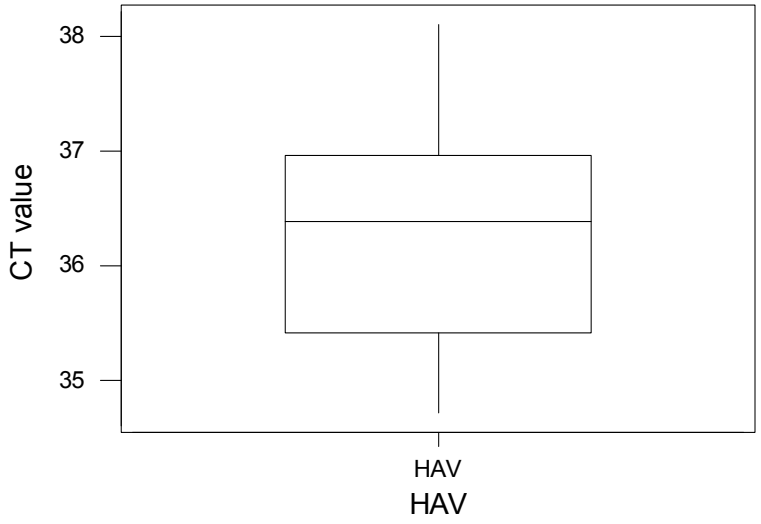
2. LENTICULE C



3. LENTICULE D



4. LENTICULE E



Appendix III - Participants' CT values for positive results

Lab ID number	LENTICULE B		LENTICULE C		LENTICULE D				LENTICULE E		Volume extracted in ul
	Norovirus GI		Norovirus GII		Norovirus GI		Norovirus GII		HAV		
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	
3*	34.39	34.19	29.24	29.06	33.17	33.43	31.06	30.99	N/A		400
9*	35.00	28.70	31.00	34.70	30.10	-	31.50	-	N/A		50
10	34.25	32.10	32.87	33.22	34.05	33.07	34.35	32.99	38.21	37.54	400
11	40.08	41.25	44.36	41.08	39.73	43.02	40.22	41.46	35.89	41.07	140
15	28.92	29.14	26.13	26.25	31.85	30.12	28.26	28.29	36.30	36.52	Not Known
19	27.30	28.24	26.26	26.08	28.55	27.64	26.13	25.99	31.58	31.70	400
21	34.40	34.40	31.30	31.10	32.90	33.50	30.50	30.40	33.00	33.10	1000
24*	36.51	36.46	29.81	28.84	35.20	35.26	29.39	30.02	N/A		200
25	34.60	34.70	37.80	38.50	33.90	33.80	37.80	38.20	34.80	34.90	1000
32	31.55	30.53	26.00	26.84	28.60	26.74	26.57	26.52	36.64	37.72	280
37**	N/A	38.70	N/A	33.84	N/A	32.70	N/A	32.70	N/A		1000
39***	-	-	33.00	28.00	-	-	32.80	36.00	29.00	34.00	250
41	29.44	29.50	25.49	25.51	29.86	30.10	25.57	25.99	26.91	27.12	1000
55	43.82	41.10	36.60	36.90	39.62	42.41	35.30	35.91	38.41	37.94	200
94	27.88	27.77	25.20	25.16	28.80	29.18	26.27	26.73	27.25	27.32	1000

* - real-time detection for HAV not carried out
** - real-time detection for norovirus only carried out on replicate 2, did not discriminate between genogroups, real-time detection for HAV not carried out
*** - GI positives only with conventional PCR