Method comparison for the detection of hepatitis E virus in lettuce and water samples

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@FoodViruses

Hepatitis E workshop

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Methods for virus detection

Shellfish

Berries

Leafy greens

Bottled water

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Enteric viruses (Norovirus and HAV) in food (CEN/TC 275– WG 6– TAG4)

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

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Virus detection workflow (ISO 15216)

Elution & concentration → RNA extraction → RT-qPCR

Chop 2.0g of **digestive gland**, add equal volume of **proteinase K** solution, incubate 37°C and 60°C then clarify by centrifugation

25g sample to 40ml TGBE buffer (with pectinase for soft fruit), elute viruses by shaking and filter eluate. Precipitate using **PEG/NaCl.** For soft fruit further clarify using chloroform:butanol.

Filter onto a **positively charged membrane**, elute in TGBE buffer and concentrate using centrifugal filter device

Based on virus capsid disruption with **chaotropic reagents** and adsorption of RNA to **silica particles**

One-step RT-qPCR assay
Use of hydrolysis probes
Use of a standard curve for quantification

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 8 June 2017

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Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen

EFSA Panel on Biological Hazards (BIOHAZ),
Antonia Ricci, Ana Allende, Declan Bolton, Marianne Chemaly, Robert Davies,
Pablo Salvador Fernandez Escamez, Lieve Herman, Kostas Koutsoumanis, Roland Lindqvist,
Birgit Nørrung, Lucy Robertson, Giuseppe Ru, Moez Sanaa, Marion Simmons,
Panagiotis Skandamis, Emma Snary, Niko Speybroeck, Benno Ter Kuile, John Threlfall,
Helene Wahlström, Ilaria Di Bartolo, Reimar Johne, Nicole Pavio, Saskia Rutjes,
Wim van der Poel, Petra Vasickova, Michaela Hempen, Winy Messens, Valentina Rizzi,
Francesca Latronico and Rosina Girones

5. Recommendations

- The validation and standardisation of methods for detection and quantification of HEV from meat and meat products should be a high priority. Also, detection methods for other food matrices (e.g. shellfish, fruit and vegetables, food contact surfaces) and bottled water as described in ISO15216 should be validated in order to demonstrate their suitability for the detection of HEV.

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RT-qPCR comparison

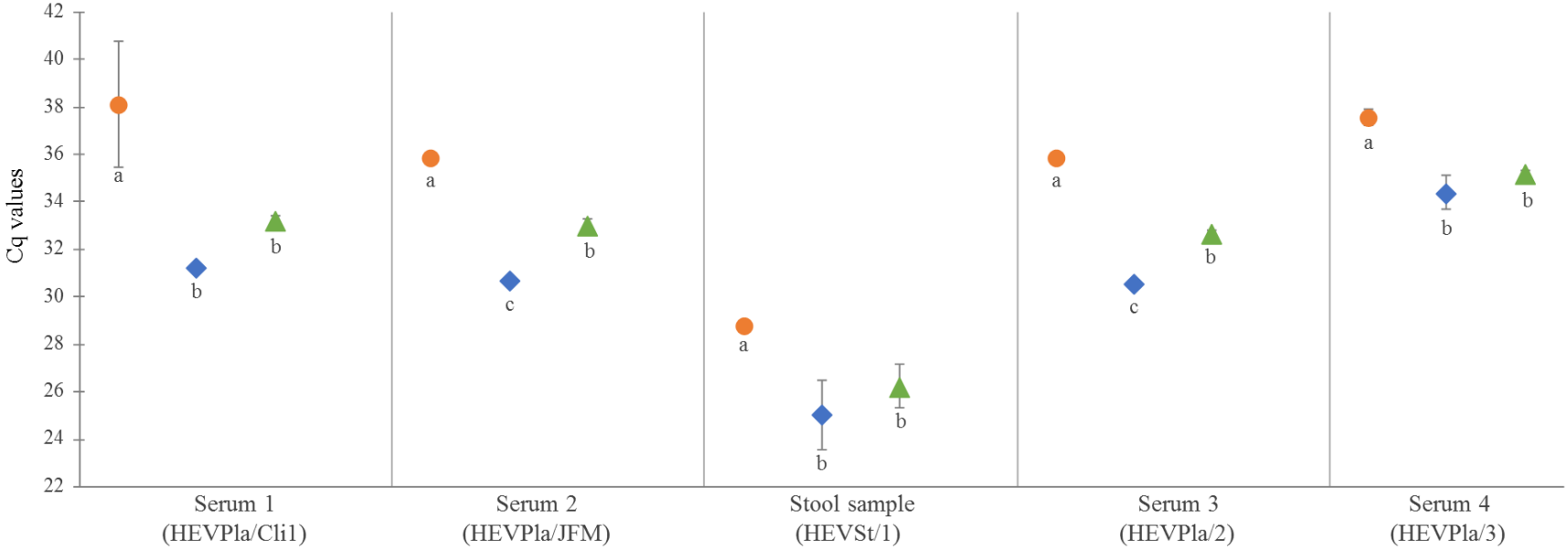
Assay	Amplification region	Primers and probe	Sequence 5'-3'	RT-qPCR conditions	Location*	Reference
A	ORF2/3	JVHEVF	GGTGGTTTCTGGGGTGAC	RT 50°C for 30 min 95°C for 15 min PCR (45x) 95°C for 10" 55°C for 20" 72°C for 15"	5304-5373 (69 nt)	**Jothikumar et al. (2006)**
		JVHEVR	AGGGGTTGGTTGGATGAA			
		JVHEVP	FAM-TGATTCTCAGCCCTTCGC-BHQ			
B	ORF2/3	NA	NA	RT 45°C for 10 min 95°C for 10 min PCR (40x) 95°C for 15" 60°C for 45"	NA	**HepatitisE@CeeramTools**
C	ORF3	HEV.Fa	GTGCCGGCGGTGGTTTC	RT 50°C for 30 min 95°C for 15 min PCR (45x) 95°C for 10" 55°C for 25" 72°C for 25"	5296-5377 (81 nt)	**Schlosser et al. (2014) with modified probe**
		HEV.Fb	GTGCCGGCGGTGGTTTCTG			
		HEV.R	GCGAAGGGGTTGGTTGGATG			
		HEV.P	FAM-TGACMGGGT/ZEN/TGATTCTCAGCC/3IABkFQ			

RT-qPCR comparison

		Jothikumar et al. (2006)		CeeramTools		Schlosser et al. (2014)	
		(+/total)	Mean Cq ± SD	(+/total)	Mean Cq ± SD	(+/total)	Mean Cq± SD
WHO HEV standard	2.5×10^5	4/4	31.14 ± 0.30^a	4/4	28.62 ± 0.15^b	4/4	28.82 ± 0.10^b
6329/10	2.5×10^4	4/4	34.33 ± 0.81^a	4/4	29.56 ± 0.46^b	4/4	32.43 ± 0.15^c
(IU/ml)	2.5×10^3	3/4	37.45 ± 0.70^a	4/4	32.65 ± 0.25^b	4/4	35.55 ± 0.30^c
	2.5×10^2	0/4		4/4	35.70 ± 0.55^a	1/4	36.98^b
Efficiency (%)			105.82		96.69		98.23
Regression coefficient			0.9502		0.9963		0.9914
Slope			-3.190		-3.404		-3.365
Intercept			40.012		35.577		38.329

Randazzo et al., 2018

RT-qPCR comparison



Randazzo et al., 2018

Elution method

Elution & concentration → RNA extraction → RT-qPCR

A **ISO 15216**
25 g lettuce
40 ml TGBE buffer/**shaker**
Precipitate using
PEG/NaCl precipitation

B **Modified ISO 15216**
25 g lettuce
90 ml TGBE buffer/shaker
PEG/NaCl precipitation

C **Sánchez et al., 2012**
10 g lettuce
90 ml BPW/Pulsifier
PEG/NaCl precipitation

Schlosser et al. (2014)

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Randazzo et al., 2018

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Elution method

Concentration method	Undiluted RNA			10-fold diluted RNA		
	*HEV samples (+/total)*	*Cq± SD*	*Mean HEV recovery (min-max) (%)*	*HEV samples (+/total)*	*Cq± SD*	*Mean HEV recovery (min-max) (%)*
A ISO 15216	3/3	29.62±0.47a	3.98 (2.56-5.20)	3/3	33.51±0.88	2.89 (1.72-4.85)
B Modified ISO 15216	3/3	31.27±0.22b	1.21 (1.10-1.37)	3/3	34.65±0.73	1.21 (0.50-1.50)
C Sánchez et al. 2012	3/3	31.43±0.69b	1.15 (0.46-1.51)	1/3	34.47*	1.25

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Randazzo et al., 2018

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Validation of eluting conditions described on the ISO15216

	Levels of inoculated HEV (IU/25 g)						Mean mengovirus recovery (min-max)
	Undiluted RNA			10-fold diluted RNA			
	1.9×10^6	1.9×10^5	1.9×10^4	1.9×10^6	1.9×10^5	1.9×10^4	(%)
	3/3 (1.29±0.81)*	2/3	2/3	3/3 (1.38±0.68)*	1/3	0/3	11.08 (5.20-18.12)
	3/3 (0.46±0.34)	3/3	2/3	2/3 (0.71±0.62)	1/3	0/3	9.64 (3.04-17.36)
	3/3 (3.95±1.12)	3/3	1/3	3/3 (1.21±0.65)	1/3	0/3	10.11 (3.61-21.07)

Average detection limit of 1,47 x 10^5 IU/25 g

Randazzo et al., 2018

Screening for HEV contamination in lettuce and water samples

Nov 2016 to Dec 2016

0/36

Randazzo et al., 2018

Screening for HEV contamination in lettuce and water samples

Comparative HEV detection in sewage by three different RT-qPCR.

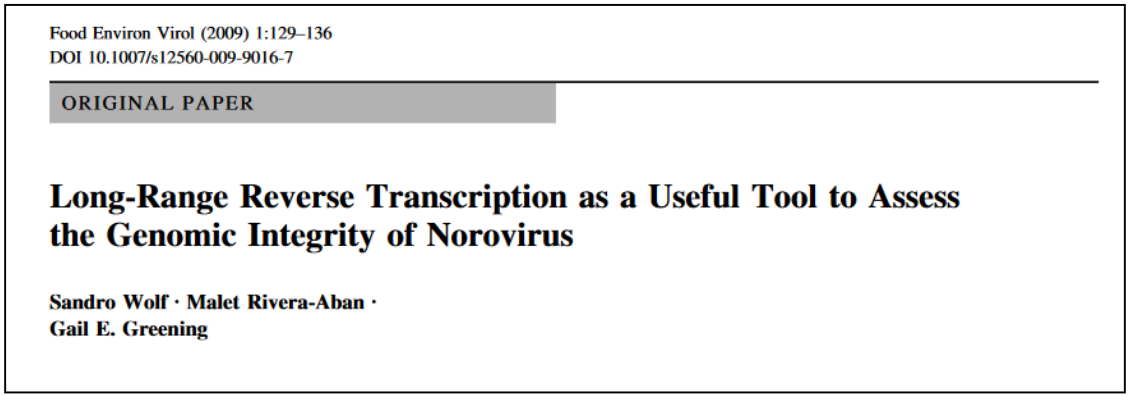
#	Sample	Assay A Jothikumar et al., 2006	Assay B HepatitisE@CeeramTools	Assay C Schlosser et al. (2014)	Concentration averages* (Log IU/l)
1	March-1	**2/2**	**1/2**	**2/2**	4.07 ± 0.32
2	March-2	**2/2**	**2/2**	**1/2**	< LOQ
3	Abril-1	0/2	0/2	**2/2**	< LOQ
4	Abril-2	**2/2**	**1/2**	**2/2**	< LOQ
5	May-1	0/2	0/2	0/2	nd
6	May-1	0/2	**2/2**	**1/2**	< LOQ
7	June-1	0/2	0/2	0/2	nd
8	June-2	0/2	0/2	0/2	nd
9	July-1	0/2	**2/2**	**2/2**	4.82 ± 0.02
10	July-2	0/2	**2/2**	**1/2**	4.49
11	August-1	0/2	**1/2**	**1/2**	4.99
12	August-1	0/2	0/2	0/2	nd
13	September-1	0/2	**1/2**	**1/2**	4.86
14	September-2	**2/2**	**2/2**	**2/2**	< LOQ
Total		4/14	9/14	10/14	
Prevalence (%)		28.57	64.29	71.43	

0/36

Randazzo et al., 2018

Assessing infectivity by RT-qPCR

Genome integrity



Food Environ Virol (2009) 1:129–136
DOI 10.1007/s12560-009-9016-7

ORIGINAL PAPER

Long-Range Reverse Transcription as a Useful Tool to Assess the Genomic Integrity of Norovirus

Sandro Wolf · Malet Rivera-Aban · Gail E. Greening

Capsid integrity

- ✓ RNase/Proteinase K pre-treatment
- ✓ Ligand binding
- ✓ Viability dyes

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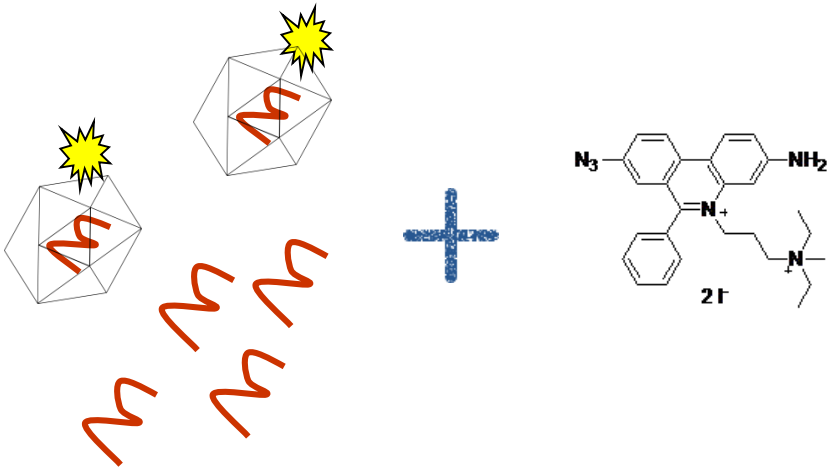
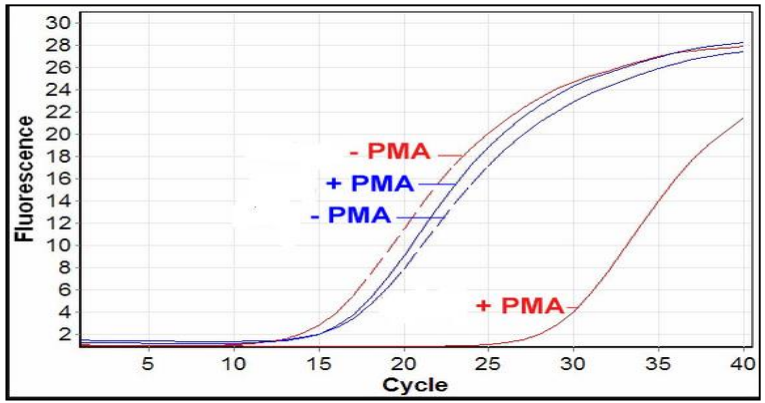
Viability RT-qPCR





Parshionikar et al., 2010

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Binding of intercalating dyes to purified HEV RNA

		81 bp		69 bp		189 bp	
Intercalating dye	Concentration (µM)	Assay A (Schlosser et al., 2014)		Assay B (Jothikumar et al., 2006)		Assay C (Mansuy et al., 2014)	
		Cq values	Reduction	Cq values	Reduction	Cq values	Reduction
PMAxx	0	23.03 ± 0.62A	-	24.78 ±1.15A	-	26.54 ± 0.27A	-
	50	25.46 ± 0.26B	2.43	26.89 ±0.30A	2.11	35.83 ±1.24B	9.29
	100	25.70 ± 0.44B	2.67	27.36 ±0.65A	2.58	37.04 ±1.19B	10.5
	250	24.67 ±0.35B	1.64	27.64 ±2.24A	2.86	34.98 ±0.57B	8.44

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	100	25.70 ± 0.44B	2.67	27.36 ±0.65A	2.58	37.04 ±1.19B	10.5
	250	24.67 ±0.35B	1.64	27.64 ±2.24A	2.86	34.98 ±0.57B	8.44
$PtCl_4$	0	22.55 ±0.10A	-	25.08 ± 0.76A	-	24.49 ±0.89	-
	50	36.92*B	14.37	35.61 ± 6.21**A	10.53	nd	-
	100	38.19*C	16.64	39.46*A	14.38	nd	-
	500	nd	-	35.03 ± 0.40**A	9.95	nd	-
	1000	nd	-	nd	-	nd	-

Fraise et al., 2018

Quantification of thermally inactivated HEV suspensions

	PtCl4 500 µM	Levels of HEV (IU/ml)			
		2×10^4	Reduction	2×10^5	Reduction
Infectious	-	4.92 ± 0.10AB		5.68 ± 0.15A	
	+	4.67 ± 0.23A	0.26	5.27 ± 0.01B	0.41
Inactivated (5 min at 99 °C)	-	5.12 ± 0.12B	- 0.20	5.73 ± 0.13A	- 0.05
	+	<LOQbC	>2.80	2.93 ± 0.19C	2.75

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Conclusions

- Assays B and C demonstrated the best results for HEV RNA detection and quantification, as 250 IU/ml of the first HEV WHO international standard could be detected.
- The ISO 15216 procedure is suitable for recovering HEV in vegetable samples.
- Considering the low HEV recovery rates in vegetables, improvements to the procedure must be undertaken.
- HEV circulates in sewage and has the potential to contaminate shellfish harvesting areas and water used for agricultural irrigation.
- The use of platinum compounds needs to be develop/optimized for different food manufacturing processes.

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