Conclusions

In this study, a wide range of quantitative and qualitative assays were used to determine the suitability and evaluate the HEV RNA content of the candidate standards. Although the methods used by the study participants were all developed in-house, the majority of assays were able to detect the two HEV strains consistently. Based upon the data from the qualitative and the quantitative assays, the candidate WHO standard was estimated to have a potency of 5.39 $\log_{10}$ units/ml. Since the unitage assigned to the 1st WHO standard of a preparation is essentially arbitrary, for practical purposes, the candidate International Standard has been assigned a unitage of 250,000 International Units/ml. Since there was only a negligible difference in the overall means for the candidate Japanese National Standard compared to the WHO preparation, the two materials have therefore been assigned the same value i.e. 250,000 International Units/ml. In the case of the quantitative assays, laboratories reported values in HEV RNA copies/ml. The participants used plasmid DNA containing HEV sequences, synthetic oligonucleotides and *in vitro* transcribed HEV RNA to control for copy number. In some cases laboratories used HEV-containing plasma which had been calibrated against *in vitro* transcribed HEV RNA. Another laboratory prepared standard using stool-derived virus, the titre of which was determined by end-point dilution and analysis by Poisson distribution. No standard method or common quantitation standard material was used, and this is reflected in the variation observed for the quantitative results, with a variation in the order of 2 $\log_{10}$, which were improved by expressing the results against Sample 1 as a common standard. In the case of the qualitative assays, the variation in NAT-detectable units was at least 3 $\log_{10}$, and again expressing potencies relative to Sample 1 improved the agreement between the different laboratories and methods.

The collaborative study materials have been dispatched at ambient temperature, replicating the intended shipping conditions. Initial accelerated thermal degradation analysis indicates a reduction in the levels of HEV RNA at higher incubation temperatures. On-going studies on the real-time stability under normal storage conditions as well as studies concerning thermal degradation are in progress.

The standard will be of value for comparison of results between laboratories, determination of assay sensitivities and for validation. It is anticipated that the standard will find application in clinical laboratories, particularly hepatitis reference laboratories performing diagnosis and monitoring HEV viral loads in chronically infected transplant patients, research laboratories, blood and plasma centres which implement HEV NAT screening, regulatory agencies and organizations developing HEV vaccines as well as manufacturers of diagnostic kits.

Each vial of the HEV RNA standard contains the lyophilized residue of 0.5 ml of HEV RNA positive plasma. Predictions of stability indicate that the standard is stable and suitable for long-term use when stored as directed in the accompanying proposed "Instructions For Use" data sheets for the panel (Appendix 2).

Recommendations

Based upon the results of the collaborative study, it is proposed that the genotype 3a HEV strain (Samples 1 and 2, in this study) should be established as the 1st International Standard for hepatitis E virus RNA and be assigned a unitage of 250,000 International Units/ml. The standard has been given the code number 6329/10; 3800 vials are available to the WHO and custodian laboratory is the Paul-Ehrlich-Institut.

Comments from participants

After circulation of the draft report for comment, replies were received from all participants. The majority of the comments were editorial in nature and the report has been amended accordingly. All participants were in agreement with the conclusions of the report.

One participant commented on the possible incorrect estimation of the viral load by the participants who used DNA standards (synthetic oligonucleotides or plasmid DNA) due to lack of control for reverse transcription of virus RNA into cDNA. This might be better controlled using *in vitro* transcribed RNA or a virion-based preparation.

Another participant remarked that many laboratories have used the same method, showing quite different sensitivities, possibly due to differences in extraction and amplification/detection reagents and instrumentation and its set up.

Acknowledgements

The viraemic HEV donations used to prepare the candidate standards were generously provided by Keiji Matsubayashi of the Japanese Red Cross Hokkaido Blood Center. We thank all the laboratories who took part in the study and Roswitha Kleiber and Christine Hanker-Dusel for assistance.

References

Adlhoch, C., M. Kaiser, G. Pauli, J. Koch, and H. Meisel. 2009. Indigenous hepatitis E virus infection of a plasma donor in Germany. Vox Sang. 97:303-308.

Baylis, S. A., K. M. Hanschmann, J. Blümel, and C. M. Nübling; on behalf of the HEV Collaborative Study Group. 2011. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique (NAT)-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. J. Clin. Microbiol. 49:1234-1239.

Bendall, R., V. Ellis, S. Ijaz, R. Ali, and H. Dalton. 2010. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. J. Med. Virol. 82:799-805.

Boxall, E., A. Herborn, G. Kochethu, G. Pratt, D. Adams, S. Ijaz, and C. G. Teo. 2006. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. Transfus. Med. 16:79-83.

Colson, P., C.Coze, P. Gallian, M. Henry, P De Micco, and C. Tamalet. 2007. Transfusion-associated hepatitis E, France. Emerg. Infect. Dis. 13:648-649.

Drobeniuc, J., J. Meng, G. Reuter, T. Greene-Montfort, N. Khudyakova, Z. Dimitrova, S. Kamili, and C. G. Teo. 2010. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. Clin. Infect. Dis. 51:e24-27.

Guo, Q. S , Q. Yan, J. H. Xiong, S. X. Ge, J. W. Shih, M. H. Ng, J. Zhang, and N. S. Xia. 2010. Prevalence of hepatitis E virus in Chinese blood donors. J. Clin. Microbiol. 48:317-318.

Gyarmati, P., N. Mohammed, H. Norder, J. Blomberg, S. Belák, and F. Widén. 2007. Universal detection of hepatitis E virus by two real-time PCR assays: TaqMan and Primer-Probe Energy Transfer. J. Virol. Methods. 146:226-235.

Haagsma, E. B., A. Riezebos-Brilman, A. P. van den Berg, R. J. Porte, and H. G. Niesters. 2010. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. Liver Transpl. 16:474-477.

Huang, S., X. Zhang, H. Jiang, Q. Yan, X. Ai, Y. Wang, J. Cai, L. Jiang, T. Wu, Z. Wang, L. Guan, J. W. Shih, M. H. Ng, F. Zhu, J. Zhang, and N. Xia. 2010. Profile of acute infectious markers in sporadic hepatitis E. PLoS One. 5(10):e13560.

Ijaz, S., A. J. Vyse, D. Morgan, R. G. Pebody, R. S. Tedder, and D. Brown. 2009. Indigenous hepatitis E virus infection in England: more common than it seems. J. Clin. Virol. 44:272-276.

Jothikumar, N., T. L. Cromeans, B. H. Robertson, X. J. Meng, and V. R. Hill. 2006. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J. Virol. Methods 131:65-71.

Kamar, N., J. Selves, J. M. Mansuy, L. Ouezzani, J. M. Péron, J. Guitard, O. Cointault, L. Esposito, F. Abravanel, M. Danjoux, D. Durand, J. P. Vinel, J. Izopet, and L. Rostaing. 2008. Hepatitis E virus and chronic hepatitis in organ-transplant recipients.
N. Engl. J. Med. 358:811-817.

Kamar, N., L. Rostaing, F. Abravanel, C. Garrouste, L. Esposito, I. Cardeau-Desangles, J. M. Mansuy, J. Selves, J. M. Peron, P. Otal, F. Muscari, and J. Izopet. 2010a. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. Clin. Infect. Dis. 50:e30-33.

Kamar, N., L. Rostaing, F. Abravanel, C. Garrouste, S. Lhomme, L Esposito, G Basse, O Cointault, D Ribes, M. B. Nogier, L. Alric, J. M. Peron, and J. Izopet. 2010b. Ribavirin therapy inhibits viral replication on patients with chronic hepatitis e virus infection. Gastroenterology 139:1612-1618.

Kamar, N., F. Abravanel, J. Selves, C. Garrouste, L. Esposito, L. Lavayssière, O. Cointault, D. Ribes, I. Cardeau, M. B. Nogier, J. M. Mansuy, F. Muscari, J. M. Peron, J. Izopet, and L. Rostaing. 2010c. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. Transplantation 89:353-360.

Lan X., B. Yang, B. Y. Li, X. P. Yin, X. R. Li, and J. X. Liu. 2009. Reverse transcription-loop-mediated isothermal amplification assay for rapid detection of hepatitis E virus. J. Clin. Microbiol. 47:2304-2306.

Legrand-Abravanel, F., N. Kamar, K. Sandres-Saune, C. Garrouste, M. Dubois, J. M. Mansuy, F. Muscari, F. Sallusto, L. Rostaing, J. Izopet. 2010. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. J. Infect. Dis. 202:835-844.

Mallet; V., E. Nicand, P. Sultanik, C. Chakvetadze, S. Tessé, E. Thervet, L. Mouthon, P. Sogni, and S. Pol. 2010. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. Ann. Intern. Med. 153:85-89.

Matsubayashi, K., Y. Nagaoka, H. Sakata, S. Sato, K. Fukai, T. Kato, K. Takahashi, S. Mishiro, M. Imai, N. Takeda, and H. Ikeda. 2004. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 44:934-940.

Matsubayashi, K., J. H. Kang, H. Sakata, K. Takahashi, M. Shindo, M. Kato, S. Sato, T. Kato, H. Nishimori, K. Tsuji, H. Maguchi, J. Yoshida, H. Maekubo, S. Mishiro, and H. Ikeda. 2008. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. Transfusion 48:1368-1375.

Meng, J., X. Dai, J. C. Chang, E. Lopareva, J. Pillot, H. A. Fields, and Y. E. Khudyakov. 2001. Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. Virology 288:203-211.

Meng, X. J. 2010. Recent advances in Hepatitis E virus. J. Viral. Hepat. 17:153-161.

Purcell, R. H., and S. U. Emerson. 2008. Hepatitis E: an emerging awareness of an old disease. J. Hepatol. 48:494-503.

Purcell, R. H., and S. U. Emerson. 2010. Hidden danger: the raw facts about hepatitis E virus. J. Infect. Dis. 202:819-821.

Sakata, H., K. Matsubayashi, H. Takeda, S. Sato, T. Kato, S. Hino, K. Tadokoro, and H. Ikeda. 2008. A nationwide survey for hepatitis E virus prevalence in Japanese blood donors with elevated alanine aminotransferase. Transfusion 48:2568-2576.

Shrestha, M. P., R. M. Scott, D. M. Joshi, M. P. Mammen, G. B. Thapa, N. Thapa, K. S. Myint, M. Fourneau, R. A. Kuschner, S. K. Shrestha, M. P. David, J. Seriwatana, D. W. Vaughn, A. Safary, T. P. Endy, and B. L. Innis. 2007. Safety and efficacy of a recombinant hepatitis E vaccine. N. Engl. J. Med. 356:895-903.

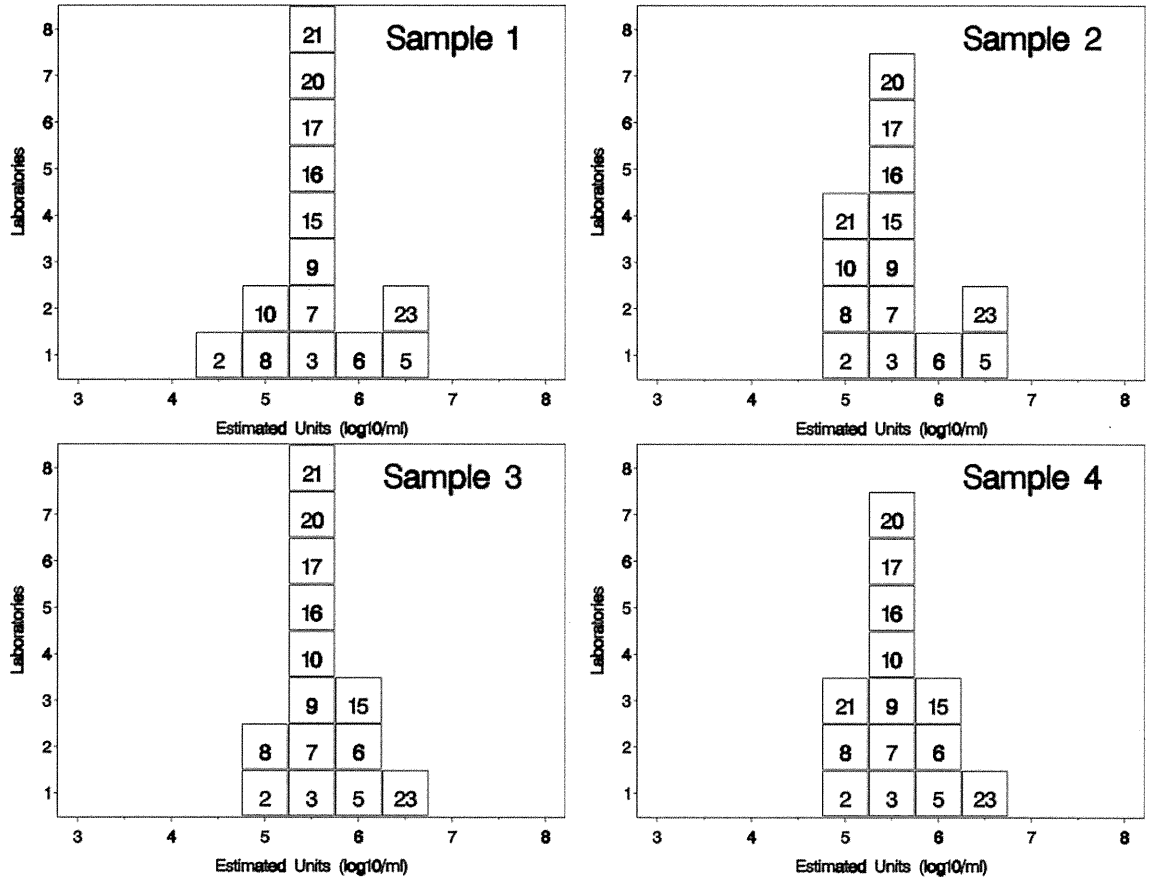
Tanaka, T., T. Masaharu, E. Kusano, and H. Okamoto. 2007. Development and evaluation of an efficient cell-culture system for Hepatitis E virus. J. Gen. Virol. 88:903-911.

Waar, K., M. M. Herremans, H. Vennema, M. P. Koopmans, and C. A. Benne. 2005. Hepatitis E is a cause of unexplained hepatitis in The Netherlands. J. Clin. Virol. 33:145-149.

Wenzel, J. J, J. Preiss, M. Schemmerer, B. Huber, A. Plentz, and W. Jilg. Detection of hepatitis E virus (HEV) from porcine livers in Southeastern Germany and high sequence homology to human HEV isolates. J. Clin. Virol. in press; DOI number 10.1016/j.jcv.2011.06.006

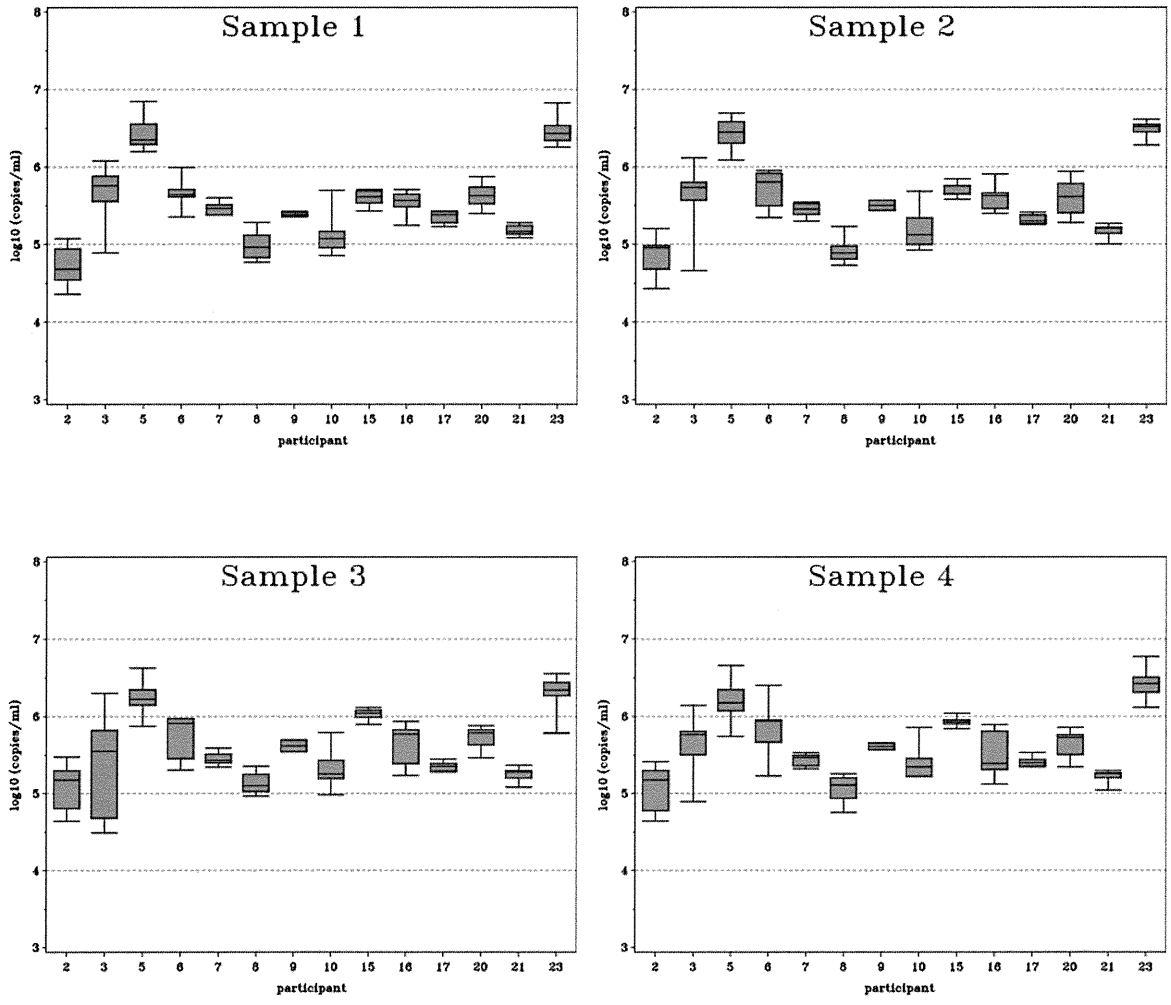
Zhu, F. C., J. Zhang, X. F. Zhang, C. Zhou, Z. Z. Wang, S. J. Huang, H. Wang, C. L. Yang, H. M. Jiang, J. P. Cai, Y. J. Wang, X. Ai, Y. M. Hu, Q. Tang, X. Yao, Q. Yan, Y. L. Xian, T. Wu, Y. M. Li, J. Miao, M. H. Ng, J. W. Shih, and N. S. Xia. 2010. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 376:895-902.

Figure 1 Estimates for quantitative assays



Histograms of the quantitative results for participating laboratories for Sample 1, Sample 2, Sample 3 and Sample 4. Estimates of $\log_{10}$ copies/ml are indicated on the x-axis. Data are shown for laboratory 16a.

Figure 2 Box and whisker plots of the quantitative data (log_{10} copies/ml)



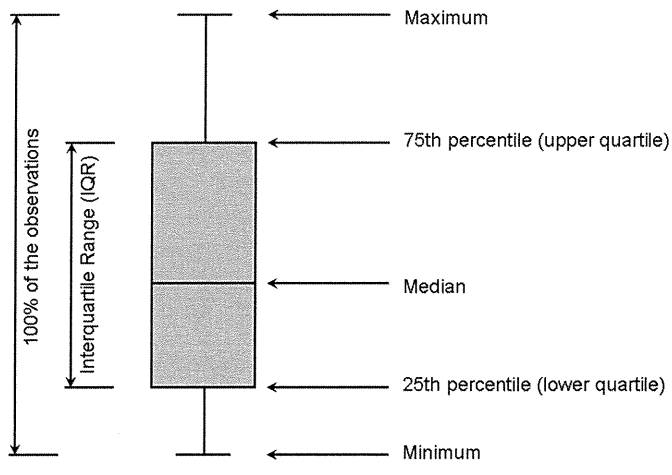
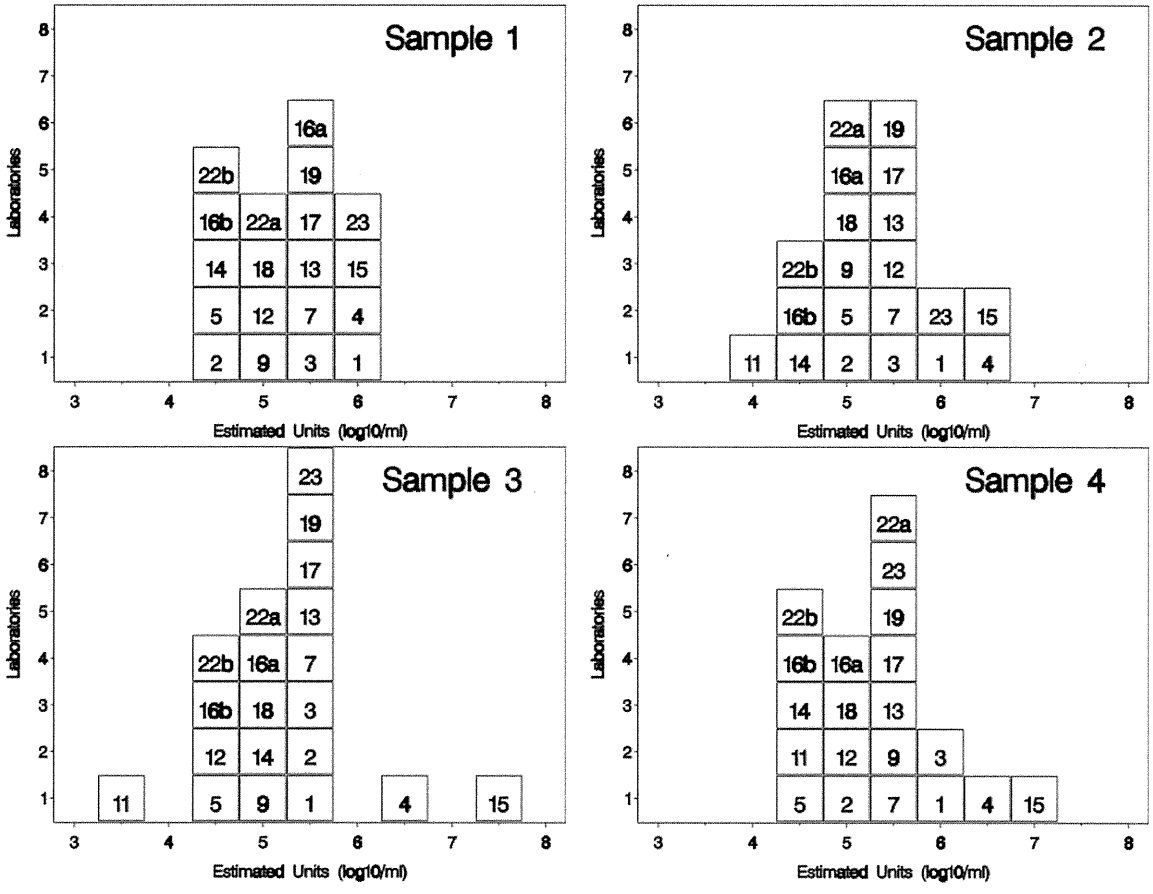
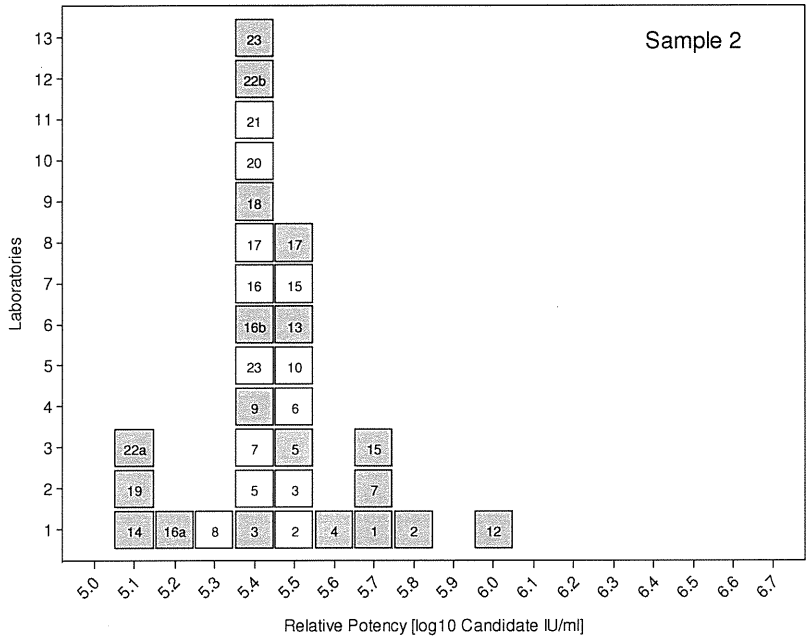


Figure 3 Estimates for qualitative assays



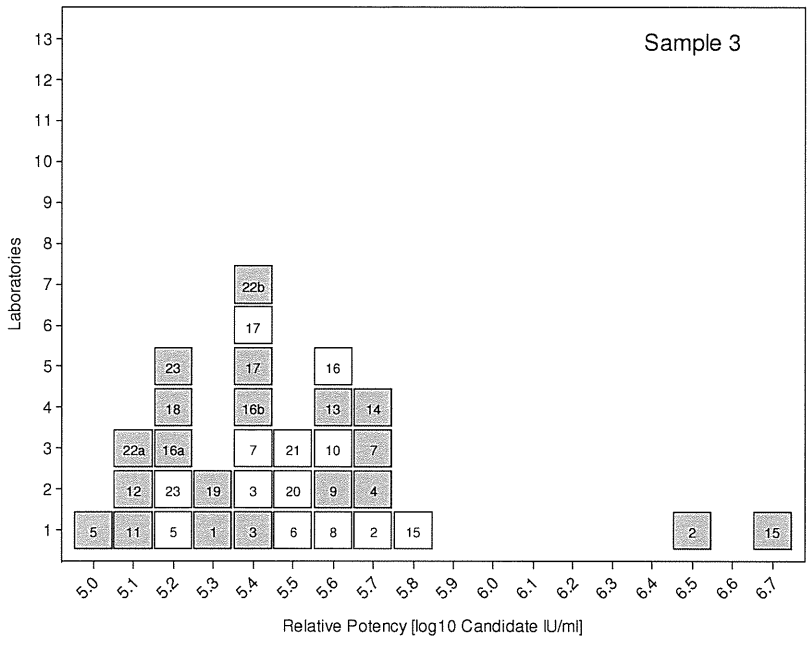
Histograms of the qualitative results for participating laboratories for Sample 1, Sample 2, Sample 3 and Sample 4. Estimates of $\log_{10}$ NAT-detectable units/ml are indicated on the x-axis. In the case of laboratory 11, data for Sample 1 have been omitted due to a 2 $\log_{10}$ higher cut-off.

Figure 4 Potency of Sample 2 relative to Sample 1



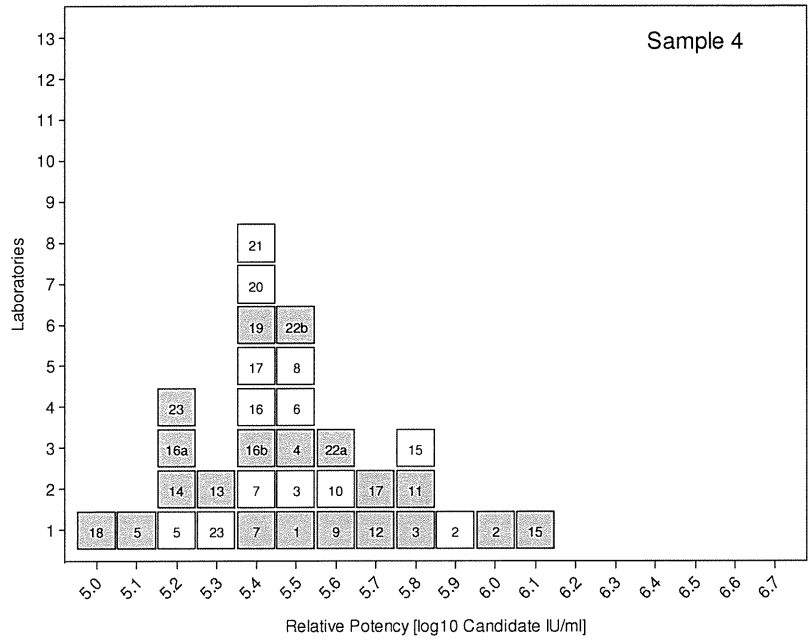
Histogram of the potency of Sample 2 relative to Sample 1 (=5.39 $\log_{10}$ units/ml); qualitative data (grey boxes) and quantitative data (white boxes). No relative potency is shown for laboratory 11 for sample 2, since no value had been determined for Sample 1 (i.e. the data were outlying and did not perform as the replicate i.e. Sample 2).

Figure 5 Potency of Sample 3 relative to Sample 1



Histogram of the potency of Sample 3 relative to Sample 1 (=5.39 $\log_{10}$ units/ml); qualitative data (grey boxes) and quantitative data (white boxes). In the case of Laboratory 11, the data have been calculated relative to Sample 2.

Figure 6 Potency of Sample 4 relative to Sample 1



Histogram of the potency of Sample 4 relative to Sample 1 (=5.39 $\log_{10}$ units/ml); qualitative data (grey boxes) and quantitative data (white boxes). In the case of Laboratory 11, the data have been calculated relative to Sample 2.

Table 1 Details of HEV strains lyophilized as candidate standards

Virus strain	HEV RNA (copies/ml)*	Genotype	Accession No.**	Anti-HEV IgM/IgG	ALT (IU/L)
HRC-HE104	1.6×10^7	3a	AB630970	-/-	36
JRC-HE3	2.5×10^7	3b	AB630971	+/-	398

*Concentrations determined by the Japanese Red Cross Hokkaido Blood Center
**Full length sequence

Table 2 Assay protocols used by participants

Laboratory code	Assay type (qualitative or quantitative)	Extraction method	NAT method	Assay target	Reference
1	Qual.	QIAamp MinElute Virus Spin kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
2	Qual./Quant.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2	Adlhoch *et al.* 2009
3	Qual./Quant.	High Pure Viral Nucleic Acid kit (Roche)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
4	Qual.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	
5	Qual./Quant.	QIAamp DNA Mini Blood kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	
6	Quant.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	
7	Qual./Quant.	QIAamp MinElute Virus Spin kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Matsubayashi *et al.* 2008
8	Quant.	SMI-TEST EX-R&D (Medical Biological Laboratories Co., Ltd.)	Real-time RT-PCR (TaqMan)	ORF2/3	Tanaka *et al.* 2007
9	Qual./Quant.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	
10	Quant.	COBAS AmpliPrep Total Nucleic Acid Isolation kit (Roche)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
11	Qual.	COBAS AmpliScreen Multiprep Specimen Preparation and Control kit (Roche)	Conventional one step RT-PCR; analysis by agarose gel electrophoresis	ORF1	
12	Qual.	QIAamp MinElute Virus Spin Kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
13	Qual.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
14	Qual.	Viral DNA/RNA Isolation kit (GenMag Biotechnology)	Nested RT-PCR; analysis by agarose gel electrophoresis	ORF2	
15	Qual./Quant.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006 (modified)
16a	Qual./Quant.	MagNA Pure LC (Roche)	Real-time PCR (SYBR Green)	ORF2/3	Jothikumar *et al.* 2006

					(modified)
16b	Qual.	MagNA Pure LC (Roche)	Nested RT-PCR; analysis by agarose gel electrophoresis	ORF2	Meng *et al.* 2001
17	Qual./Quant.	QIAamp Virus BioRobot MDx kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Matsubayashi *et al.* 2008
18	Qual.	MagNA Pure LC Total Nucleic Acid Isolation kit (Roche)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
19	Qual.	easyMag (bioMérieux)	Real-time RT-PCR (TaqMan)	ORF2	
20	Quant.	QIAamp Viral RNA Mini kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	
21	Quant.	BioRobot Universal (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
22a	Qual.	QIAamp RNA Mini kit (Qiagen)	Nested RT-PCR; analysis by agarose gel electrophoresis	ORF2	Gyarmati *et al.* 2007
22b	Qual.	QIAamp RNA Mini kit	Real-time RT-PCR (TaqMan)	ORF2/3	Jothikumar *et al.* 2006
23	Qual./Quant.	QIAamp DNA Mini Blood kit (Qiagen)	Real-time RT-PCR (TaqMan)	ORF2/3	Wenzel *et al.*, in press

Qualitative (Qual.) and quantitative (Quant.) assays

Table 3 Mean estimates from quantitative assays (log_{10} copies/ml)

Laboratory code	Sample			
	1	2	3	4
2	4.69	4.82	5.09	5.08
3	5.69	5.62	5.43	5.65
5	6.51	6.48	6.24	6.20
6	5.75	5.80	5.77	5.83
7	5.50	5.46	5.45	5.44
8	5.07	4.97	5.14	5.06
9	5.43	5.52	5.62	5.61
10	5.18	5.22	5.30	5.39
15	5.66	5.73	6.02	5.93
16a	5.59	5.62	5.64	5.51
17	5.40	5.34	5.35	5.41
20	5.70	5.65	5.74	5.65
21	5.25	5.23	5.25	5.23
23	6.54	6.53	6.31	6.41

Table 4 Mean estimates from qualitative assays (log_{10} NAT detectable units/ml)

Laboratory code	Sample			
	1	2	3	4
1	5.76	6.05	5.62	5.91
2	4.42	4.85	5.49	5.02
3	5.35	5.40	5.35	5.76
4	6.20	6.37	6.47	6.33
5	4.70	4.84	4.27	4.42
7	5.34	5.62	5.62	5.34
9	5.02	5.03	5.18	5.26
11		4.00	3.72	4.42
12	4.91	5.48	4.61	5.18
13	5.51	5.66	5.71	5.44
14	4.71	4.43	5.00	4.57
15	6.11	6.36	7.42	6.87
16a	5.32	5.17	5.17	5.17
16b	4.74	4.74	4.74	4.74
17	5.39	5.52	5.42	5.67
18	5.13	5.13	4.98	4.76
19	5.68	5.42	5.56	5.71
22a	5.21	4.92	4.91	5.44
22b	4.53	4.53	4.52	4.68
23	5.76	5.76	5.60	5.60

Laboratory 11, sample 1, omitted due to 2 log_{10} higher cut-off

Table 5a Overall mean estimates from quantitative assays (log_{10} copies/ml)

Sample	n	mean	sd	lowercl	uppercl	median	min	max	cv_geo
1	123	5.58	0.29	5.32	5.85	5.46	4.36	6.85	98%
2	125	5.60	0.28	5.33	5.87	5.46	4.43	6.69	94%
3	124	5.66	0.20	5.40	5.93	5.50	4.49	6.63	77%
4	125	5.66	0.20	5.40	5.93	5.48	4.64	6.77	76%

n – number of dilutions analysed (in linear range), sd – standard deviation, lowercl/uppercl – 95% confidence limits for the mean, cv_geo – geometric coefficient of variation [%]

Table 5b Combined mean estimates from quantitative assays (log_{10} copies/ml)

Candidate	n	mean	sd	lowercl	uppercl	median	min	max	cv_geo
WHO	248	5.59	0.30	5.33	5.86	5.46	4.36	6.85	99%
NIID	249	5.66	0.20	5.40	5.93	5.48	4.49	6.77	76%

Combined data for Samples 1 and 2, replicate samples of the candidate IS (WHO); combined data for Samples 3 and 4, replicate samples of the candidate Japanese National Standard (NIID)

Table 6a Overall means of estimates from qualitative assays (log_{10} NAT detectable units/ml)

Sample	n	mean	sd	Lower cl	Upper cl	median	min	max	cv_geo
1	19	5.25	0.51	5.01	5.50	5.32	4.42	6.20	150%
2	20	5.26	0.62	4.97	5.56	5.29	4.00	6.37	179%
3	20	5.27	0.79	4.90	5.64	5.27	3.72	7.42	226%
4	20	5.31	0.64	5.02	5.61	5.30	4.42	6.87	183%

n – number of tests, lowercl/uppercl – 95% confidence limits for the mean, cv_geo – geometric coefficient of variation [%]

Table 6b Combined means of estimates from qualitative assays (log_{10} NAT detectable units/ml)

Candidate	n	mean	sd	lowercl	uppercl	median	min	max	cv_geo
WHO	39	5.26	0.56	5.08	5.44	5.32	4.00	6.37	163%
NIID	40	5.29	0.71	5.07	5.52	5.30	3.72	7.42	202%

Combined data for Samples 1 and 2, replicate samples of the candidate IS (WHO); combined data for Samples 3 and 4, replicate samples of the candidate Japanese National Standard (NIID)

Table 7 Potency relative to Sample 1 (quantitative assays)

Sample	Laboratory code	Relative potency (log_{10} copies/ml)	95% Confidence Interval	
2	2	5.54	5.29	5.78
	3	5.45	5.15	5.74
	5	5.39	5.15	5.63
	6	5.45	5.20	5.71
	7	5.38	5.28	5.47
	8	5.31	5.17	5.45
	9			
	10	5.47	5.34	5.59
	15	5.53	5.46	5.60
	16a	5.40	5.22	5.59
	17	5.36	5.29	5.43
	20	5.36	5.26	5.46
	21	5.39	5.35	5.44
	23	5.41	5.29	5.53
3	2	5.74	5.50	5.97
	3	5.36	5.07	5.65
	5	5.21	4.97	5.46
	6	5.48	5.21	5.75
	7	5.38	5.29	5.47
	8	5.55	5.41	5.69
	9			
	10	5.55	5.43	5.68
	15	5.83	5.76	5.90
	16a	5.55	5.36	5.73
	17	5.39	5.31	5.46
	20	5.52	5.42	5.62
	21	5.46	5.41	5.50
	23	5.20	5.09	5.32
4	2	5.90	5.66	6.15
	3	5.45	5.17	5.74
	5	5.17	4.93	5.42
	6	5.54	5.29	5.80
	7	5.37	5.28	5.46
	8	5.46	5.32	5.60
	9			
	10	5.63	5.50	5.76
	15	5.75	5.68	5.83
	16a	5.35	5.17	5.53
	17	5.44	5.37	5.52
	20	5.43	5.33	5.52
	21	5.44	5.39	5.48
	23	5.27	5.16	5.39

It was not possible to estimate the relative potency for laboratory 9 since there were only two assay runs performed, each at a different dilution

Table 8 Potency relative to Sample 1 (qualitative assays)

Sample	Laboratory code	Relative potency (log_{10} NAT detectable units/ml)	95% Confidence Interval	
2	1	5.68	5.10	6.27
	2	5.82	5.26	6.38
	3	5.44	4.81	6.08
	4	5.56	4.90	6.22
	5	5.53	5.09	5.97
	7	5.68	5.16	6.23
	9	5.40	5.15	5.66
	12	5.96	5.35	6.51
	13	5.54	5.14	5.91
	14	5.11	4.71	5.50
	15	5.65	4.90	6.40
	16a	5.24	4.85	5.64
	16b	5.39	4.77	6.01
	17	5.52	4.96	6.08
	18	5.39	4.88	5.90
	19	5.13	4.71	5.56
	22a	5.10	4.57	5.63
	22b	5.39	4.79	5.99
	23	5.39	4.74	6.04
3	1	5.25	4.67	5.81
	2	6.46	5.90	7.14
	3	5.39	4.76	6.02
	4	5.66	5.00	6.32
	5	4.96	4.53	5.39
	7	5.68	5.16	6.23
	9	5.55	5.30	5.80
	11	5.11	4.52	5.69
	12	5.09	4.51	5.64
	13	5.59	5.19	5.96
	14	5.67	5.27	6.08
	15	6.67	5.90	7.44
	16a	5.24	4.85	5.64
	16b	5.39	4.77	6.01
	17	5.43	4.87	5.98
	18	5.24	4.73	5.75
	19	5.28	4.85	5.70
	22a	5.10	4.56	5.63
	22b	5.38	4.78	5.97
	23	5.24	4.59	5.89
4	1	5.54	4.96	6.12
	2	5.99	5.43	6.55
	3	5.80	5.15	6.48
	4	5.52	4.86	6.18
	5	5.11	4.70	5.51
	7	5.39	4.87	5.92
	9	5.64	5.38	5.90

	11	5.81	5.23	6.40
	12	5.65	5.07	6.20
	13	5.32	4.93	5.71
	14	5.24	4.85	5.64
	15	6.13	5.39	6.88
	16a	5.24	4.85	5.64
	16b	5.39	4.77	6.01
	17	5.68	5.12	6.23
	18	5.02	4.51	5.52
	19	5.43	5.00	5.87
	22a	5.62	5.08	6.18
	22b	5.54	4.94	6.17
	23	5.24	4.59	5.89

N.B. The relative potency for laboratory 11 was estimated relative to Sample 2 (Sample 1 had a cut-off 2 $\log_{10}$ dilutions higher)