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)

Table 9 Stability testing

Incubation time	Incubation temperature				
	-20°C	+4°C	+20°C	+37°C	+45°C
1 month	ND	ND	ND	ND	5.03
2 months	ND	ND	ND	4.98	4.55*
4 months	5.56	5.52	5.33	ND	ND

ND Not determined
*Material could not be completely reconstituted
Titres expressed as $\log_{10}$ candidate International Units/ml

Appendix 1 List of participants

Scientist	Affiliation
Akihiro Akaishi	Nihon Pharmaceuticals Co., Ltd. Chiba, Japan
Martijn Bouwknegt/Saskia Rutjes	National Institute for Public Health and the Environment Bilthoven, The Netherlands
Silvia Dorn	Mikrogen GmbH Neuried, Germany
Thomas Gärtner	Octapharma Frankfurt am Main, Germany
Samreen Ijaz/Renata Szypulska	Health Protection Agency London, UK
Jacques Izopet	Institut Fédératif de Biologie Purpan Toulouse, France
Shintaro Kamei/Katsuro Shimose	Chemo-Sero-Therapeutic Research Institute Kumamoto, Japan
Li Ma/Mei-ying Yu	Center for Biologics Evaluation and Research/Food and Drug Administration Bethesda, USA
Thomas Laue	Astra Diagnostics Hamburg, Germany
Keiji Matsubayashi/Hidekatsu Sakata	Japanese Red Cross Hokkaido Blood Center Sapporo, Japan
Birgit Meldal/Daniel Candotti	Cambridge University and NHS Blood and Transplant Cambridge, UK
Takao Minagi	Benesis Corporation Kyoto, Japan
Saeko Mizusawa/Yoshiaki Okada	National Institute of Infectious Diseases Tokyo, Japan
Elisa Moretti/Francesca Bonci	BioSC-Kedrion S.p.A. Bolognana-Lucca, Italy
Tonya Mixson/Saleem Kamili	Centers for Disease Control and Prevention Atlanta, USA
Andreas Nitsche/Marco Kaiser	Robert Koch-Institut Berlin, Germany
Mats Olsson/Anders Olofsson	Octapharma Stockholm, Sweden
Giulio Pisani/Francesco Marino	CRIVIB, Istituto Superiore di Sanità Rome, Italy
James Wai Kuo Shih	Xiamen University Fujian, China
Ko Suzuki	Central Blood Institute, Japanese Red Cross Society Tokyo, Japan
Isabelle Thomas	Scientific Institute of Public Health,Brussels, Belgium
Youchun Wang/Yansheng Geng	National Institutes for Food and Drug Control Beijing, China
Jürgen Wenzel/Wolfgang Jilg	University of Regensburg Regensburg, Germany

Appendix 2 Draft Instructions For Use for 6329/10

Paul-Ehrlich-Institut → → A WHO Collaborating Centre ¶
Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel → for Quality Assurance of Blood Products and ¶
¶ Federal Institute for Vaccines and Biomedicines → → in vitro Diagnostic Devices → →

¶

1ST World Health Organization International Standard for Hepatitis E Virus RNA Nucleic Acid Amplification Techniques (NAT)-Based Assays¶

¶

PEI code 6329/10¶

¶

(Version 1.0, 7th July 2011)¶

¶

1.→ INTENDED USE¶

The 1st World Health Organization International Standard for hepatitis E virus (HEV) is intended to be used in the standardization of nucleic acid amplification technique (NAT)-based assays for HEV. The need to develop a standard was demonstrated in an initial study investigating performance of HEV NAT assays (Baylis *et al.*, J. Clin. Microbiol., 2011). The standard has been prepared using a genotype 3a strain of HEV, derived from the plasma of a blood donor and further diluted in human plasma. The material has been lyophilized in 0.5 ml aliquots and stored at -20°C. The material has been evaluated in an international collaborative study involving 23 laboratories performing a wide range of HEV NAT assays. Further details of the collaborative study are available in the report WHO/BS/11.XXXX.¶

¶

2.→ UNITAGE¶

This reagent has been assigned a unitage of 250,000 International Units/ml.¶

¶

3.→ CONTENTS¶

Each vial contains 0.5 ml of lyophilized plasma containing infectious HEV.¶

¶

4.→ CAUTION¶

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.¶

The preparation contains material of human origin, and contains infectious HEV. The reference materials has been diluted in human plasma negative for HIV-1 RNA, HCV RNA, HBV DNA, HBsAg, anti-HBs, anti-HBc, anti-HIV-1/2, anti-HCV and anti-HEV (IgM and IgG).¶
As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.¶

¶

5.→ USE OF MATERIAL¶

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.¶
The material is supplied lyophilized and should be stored at or below -20°C. Each vial should be reconstituted in 0.5 ml of sterile nuclease-free water. The product should be reconstituted just prior to use, once reconstituted, freeze thawing of the product is not recommended.¶

¶

6.→ STABILITY¶

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.¶

The reference materials are held at PEI within assured, temperature-controlled storage facilities. Reference materials should be stored on receipt as indicated on the label. Once, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.¶

¶

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact PEI.¶

¶

7.→ REFERENCES¶

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.¶

¶

S. A. Baylis, K. M. Hanschmann. Collaborative Study to Establish a World Health Organization International Standard for Hepatitis E Virus RNA for Nucleic Acid Amplification Technology (NAT)–Based Assays. WHO Report 2011, WHO/BS/YY.XXXX.¶

¶

8.→ ACKNOWLEDGEMENTS¶

We are grateful to the Japanese Red Cross Hokkaido Blood Center for supplying the candidate materials, the National Institute of Infectious Diseases, Japan for their collaboration and to the study participants.¶

¶

9.→ FURTHER INFORMATION¶

This material: whoccivd@pei.de¶
WHO Biological Reference Preparations:
http://www.who.int/biologicals/en/¶

¶

10.→ CUSTOMER FEEDBACK¶

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to whoccivd@pei.de¶

¶

11.→ CITATION¶

In any circumstance where the recipient publishes a reference to PEI materials, it is important that the title of the preparation and the PEI code number, and the name and address of PEI are cited correctly.¶

¶

12.→ MATERIAL SAFETY SHEET¶

Physical properties (at room temperature)¤		
Physical appearance → →	Lyophilized powder¤	¤
Fire hazard → → →	None¤	¤
Chemical properties¤		¤
Stable → → →	Yes¤	Corrosive: No¤ ¤
Hygroscopic → →	No¤	Oxidising: No¤ ¤
Flammable → →	No¤	Irritant: No¤ ¤
Other (specify) → CONTAINS HUMAN PLASMA & INFECTIOUS HEPATITIS E VIRUS (HEV)¤		¤
Handling: → ..... See caution, section 4¤		¤
Toxicological properties¤		¤

Paul-Ehrlich-Institut → → *Tel: +49 (0)6103 77 0¶*
Paul-Ehrlich-Str. 51-59 → → *Fax: +49 (0)6103 77 123¶*
63225 Langen, Germany → → *Email: whoccivd@pei.de¶*
→ → *Web: http://www.pei.de¶*

Effects of inhalation: → → → Avoid – *contains infectious HEV*	
Effects of ingestion: → → → Avoid – *contains infectious HEV*	
Effects of skin absorption: → Avoid – *contains infectious HEV*	
Suggested First Aid	
Inhalation → ..... Seek medical advice – *contains infectious HEV*	
Ingestion → ..... Seek medical advice – *contains infectious HEV*	
Contact with eyes ..... Wash thoroughly with water. Seek medical advice – *contains infectious HEV*	
Contact with skin ..... Wash thoroughly with water. Seek medical advice – *contains infectious HEV*	
Action on Spillage and Method of Disposal	
Spillage of vial contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

13. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependent on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of agreement or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.