1st World Health Organization International Reference Panel for Hepatitis E Virus (HEV) Genotypes for Nucleic Acid Amplification Technique (NAT)-Based Assays

PEI code 8578/13

(Version 2.0, November 2015)

1. INTENDED USE

The current WHO International Standard (IS) material for hepatitis E virus (HEV) is genotype 3a (1). In order to reflect HEV genetic diversity and the global disease burden of acute hepatitis E, a genotype panel has been prepared which contains representative strains of all four HEV genotypes and important sub-genotypes.
The reference panel consists of 11 HEV positive samples (plasma and stool-derived) further diluted in pooled human plasma. The samples, their code numbers, as well are their HEV (sub-)genotype are listed in the appended Table. The panel has been evaluated in an international collaborative study where the samples were tested concurrently with the 1st WHO IS (6329/10).
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 24 laboratories performing a wide range of HEV NAT assays. Further details of the collaborative study are available in the report WHO/BS/2015.2264.

2. UNITAGE

No unitage has been assigned to the individual panel members. The IU for HEV RNA is defined by the current WHO International Standard, which is a genotype 3a virus. The panel is *not* intended to replace the IS for HEV RNA.
However, in the international collaborative study (based upon data returned by 17 laboratories from quantitative assays), the ***mean titres*** of panel members were determined and are provided below, for information only.
The values were obtained by comparison to the genotype 3a International Standard using current assays represented in the collaborative study. Further details of the collaborative study are available in the report WHO/BS/15.2264.

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 HBV DNA, HCV RNA, HEV RNA, HIV-1/2 RNA, HBsAg, anti-HEV, anti-HCV, and anti-HIV-1/2.
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-20ºC. 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. If not all the material is used immediately, laboratories may aliquot the remaining material into suitable volumes which should be stored at or below -70ºC.

6. STABILITY

As the stability studies with accelerated conditions indicate high stability of the lyophilized reference material under the recommended storage conditions (at or below-20ºC), there is no expire date assigned to the international reference panel. This approach complies with the recommendations for the preparation, characterization and establishment of international and other biological reference standards (3). The reference material is held at the Paul-Ehrlich-Institut (PEI) within assured, temperature-controlled storage facilities. During its life cycle the stability is monitored at regular intervals.
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

(1) Baylis SA, Blümel J, Mizusawa S, Matsubayashi K, Sakata H, Okada Y, Nübling CM, Hanschmann KM; HEV Collaborative Study Group. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. Emerg Infect Dis. 2013;19:729-35.
(2) Baylis SA, Terao E, Hanschmann KM. Collaborative Study to Establish the 1st World Health Organization International Reference Panel for Hepatitis E Virus RNA Genotypes for Nucleic Acid Amplification Technology (NAT)-Based Assays. WHO Report 2015, WHO/BS/2015.2264.
(3) Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Expert Committee on Biological Standardization. Fifty-fifth report, 2004. (WHO Technical Report Series, No. 932).

8. ACKNOWLEDGEMENTS

We are grateful to all study participants and the providers of the materials used in the preparation of the panel (2).

9. FURTHER INFORMATION

Further information for this material can be obtained as follows: whoccivd@pei.de or
WHO Biological Reference Preparations:
http://www.who.int/biologicals/en/

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Paul-Ehrlich-Str. 51-59
63225 Langen, Germany

Email: whoccivd@pei.de
Web: http://www.pei.de

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 correct name of the preparation, the PEI code number, the name and the 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		
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.

Results from the Collaborative Study to Evaluate a Hepatitis E Virus genotype Panel

Panel Member	Genotype	Source	Overall mean (log_{10} IU/ml)	95% Confidence Intervals (log_{10} IU/ml)		Standard Deviation	Min	Max	Range
8567/13	1a	Plasma	2.64	2.22	3.06	0.60	1.44	4.10	2.66
8568/13s*	1a	Stool	4.25	3.87	4.63	0.43	3.55	5.46	1.91
8569/13	1e	Plasma	3.25	2.91	3.59	0.51	2.40	4.81	2.41
8570/13	3b	Plasma	4.20	4.12	4.28	0.18	3.80	4.57	0.78
8571/13	3c	Plasma	3.40	3.27	3.53	0.22	2.80	3.88	1.08
8572/13	3e	Plasma	3.50	3.36	3.64	0.22	2.99	4.02	1.04
8573/13	3f	Plasma	3.84	3.60	4.07	0.41	2.40	5.14	2.74
8574/13s*	3 (rabbit-like)	Stool	4.98	4.73	5.24	0.38	4.35	6.88	2.52
8575/13	4c	Plasma	4.07	3.82	4.31	0.38	3.03	5.37	2.34
8576/13	4g	Plasma	3.77	3.49	4.04	0.38	3.13	5.05	1.92
8577/13s*	2a	Stool	5.42	5.10	5.74	0.49	3.27	6.10	2.83

Overall mean estimates (log_{10} IU/ml) relative to the concurrently tested 1st International Standard for HEV RNA (6329/10); the data are based upon results obtained from 17 laboratories performing a range of quantitative assays. Further details of the collaborative study are available in the report WHO/BS/15.2264

*These panel members are derived from HEV-positive human stool specimens, diluted in pooled human plasma containing trehalose and magnesium chloride at final concentrations of 5% and 150 mM, respectively.

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