1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques -Based Assays
PEI code 5086/08
(Version 2, 28th Nov 2011)

1. INTENDED USE

The current WHO International Standard for HBV DNA (97/750) was generated from HBV genotype A2/HBsAg subtype *adw2*. This HBV genotype is mainly prevalent in Western Europe and in North America and represent only 1% of the worldwide HBV-infected population. The majority of the HBV-infected people living in or coming from the Mediterranean area, Africa and Asia have the genotypes A1, B, C, D, and E, whereas F and H originate from the Americas. The origin of genotype G is not yet known. There was a need to develop a reference panel of well characterized samples representing the different HBV genotypes to investigate the commutability of the current standard material (97/750) in relation to the other HBV genotypes (1, 2).

The 1st WHO International Reference Panel for HBV Genotypes for nucleic acid amplification technique (NAT)-based assays (PEI code number 5086/08) was established by the WHO Expert Committee on Biological Standardization in October 2009. The reference panel consists of 15 lyophilized HBV positive plasma samples and covers the most prevalent HBV genotypes: Samples 1-3 (genotype A), Samples 4-6 (genotype B), Samples 7-9 (genotype C), Samples 10-12 (genotype D), Sample 13 (genotype E), Sample 14 (genotype F), and Sample 15 (genotype G). The panel has been evaluated in an international collaborative study with concurrent testing of the 2nd WHO International Standard for HBV DNA (97/750). The reference panel is intended for the control and/or for the validation of HBV NAT assays.

2. UNITAGE

No unitage is assigned to individual panel members.

3. CONTENTS

Each vial contains 0.5 ml of lyophilized plasma containing infectious HBV. Each of the high titre HBV positive plasma stocks have been diluted in a plasma pool negative tested for the following markers: HIV-1 RNA, HCV RNA, HBV DNA, HBsAg, anti-HBs, anti-HBc (IgG and IgM), anti-HIV-1/2, and anti-HCV. The Table (see page 3) provides the statistical data for each panel member from the outcome of the collaborative study (overall arithmetic mean estimates of $\log_{10}$ IU/ml values, confidence intervals, standard deviation, minimum and maximum values, and ranges). The values were obtained by comparison testing with the 2nd International Standard for HBV DNA (97/750). The results of the collaborative study based on 16 data sets for quantitative assays and 2 data sets for qualitative assays. Further details of the collaborative study are available in the report WHO/BS/09.2121 (3).

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

The preparation contains material of human origin, and contains infectious HBV. The reference materials has been diluted in human plasma negative for HIV-1 RNA, HCV RNA, HBV DNA, HBsAg, anti-HBs, anti-HBc (IgG and IgM), anti-HIV-1/2, and anti-HCV.

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 panel member should be **reconstituted in 0.5 ml of sterile nuclease-free water**. If all the material is not used immediately, laboratories may aliquot the remaining material into suitable volumes which should be stored at or below -70ºC.

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

(1) WHO Consultation on Global Measurement Standards and their use in the *in vitro* Biological Diagnostic Field. Geneva, Switzerland (June 2004), http://www.who.int/bloodproducts/publications/en/Minutes-220804.pdf

(2) Baylis SA, Heath AB, Chudy M, Pisani G, Klotz A, Kerby S, W. Gerlich W. An international collaborative study to establish the 2nd World Health Organization International Standard for hepatitis B virus DNA nucleic acid amplification technology-based assays. *Vox Sang* 2008, **94**:358-362

(3) Chudy M, Hanschmann KM, Kreß J, Gerlich W, Nübling CM. Collaborative Study to Establish a World Health Organization International Genotype Panel for HBV Nucleic Acid Amplification Technology (NAT) – Based Assays. WHO Report 2009, WHO/BS/09.2121

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

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

8. ACKNOWLEDGEMENTS

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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 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 B VIRUS (HBV)	
Handling:	See caution, section 4	
Toxicological properties		
Effects of inhalation:	Avoid – *contains infectious HBV*	
Effects of ingestion:	Avoid – *contains infectious HBV*	
Effects of skin absorption:	Avoid – *contains infectious HBV*	
Suggested First Aid		
Inhalation	Seek medical advice - *contains infectious HBV*	
Ingestion	Seek medical advice - *contains infectious HBV*	
Contact with eyes	Wash thoroughly with water. Seek medical advice – *contains infectious HBV*	
Contact with skin	Wash thoroughly with water. Seek medical advice – *contains infectious HBV*	
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.

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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.

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

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

Table Results from the Collaborative Study to Evaluate a Hepatitis B Virus Genotype Panel

Sample	N	Overall Mean (log_{10} IU/ml)	95%-Confidence Intervals (log_{10} IU/ml)		Standard Deviation	Min	Max	Range
1/A	**18**	**6.10**	**6.02**	**6.18**	**0.16**	**5.74**	**6.38**	**0.64**
2/A	**18**	**5.86**	**5.77**	**5.95**	**0.18**	**5.42**	**6.11**	**0.69**
3/A	**18**	**5.80**	**5.73**	**5.87**	**0.14**	**5.39**	**6.04**	**0.65**
4/B	**18**	**5.92**	**5.74**	**6.09**	**0.35**	**5.18**	**6.33**	**1.15**
5/B	**18**	**5.78**	**5.61**	**5.96**	**0.35**	**4.94**	**6.49**	**1.55**
	17[1]	*5.74*	*5.58*	*5.90*	*0.31*	*4.94*	*6.08*	*1.14*
6/B	**18**	**3.91**	**3.69**	**4.12**	**0.43**	**2.49**	**4.30**	**1.81**
	17[1]	*3.99*	*3.86*	*4.12*	*0.26*	*3.40*	*4.30*	*0.90*
7/C	**18**	**5.96**	**5.84**	**6.08**	**0.24**	**5.25**	**6.27**	**1.02**
8/C	**18**	**6.09**	**5.96**	**6.21**	**0.25**	**5.16**	**6.37**	**1.21**
	15[1]	*6.14*	*6.09*	*6.20*	*0.11*	*5.98*	*6.37*	*0.39*
9/C	**18**	**5.94**	**5.82**	**6.06**	**0.23**	**5.27**	**6.27**	**1.00**
10/D	**18**	**5.99**	**5.86**	**6.12**	**0.25**	**5.59**	**6.64**	**1.05**
	17[1]	*5.95*	*5.85*	*6.05*	*0.20*	*5.59*	*6.30*	*0.71*
11/D	**18**	**6.01**	**5.91**	**6.10**	**0.19**	**5.60**	**6.37**	**0.77**
	17[1]	*5.99*	*5.90*	*6.08*	*0.18*	*5.60*	*6.37*	*0.77*
12/D	**18**	**6.03**	**5.95**	**6.10**	**0.15**	**5.80**	**6.34**	**0.54**
	17[1]	*6.01*	*5.94*	*6.08*	*0.14*	*5.80*	*6.34*	*0.54*
13/E	**18**	**5.86**	**5.75**	**5.97**	**0.22**	**5.30**	**6.27**	**0.97**
14/F	**16**	**4.76**	**4.52**	**5.00**	**0.45**	**3.24**	**5.13**	**1.89**
	15[1]	*4.86*	*4.75*	*4.98*	*0.20*	*4.55*	*5.13*	*0.58*
15/G	**18**	**3.78**	**3.53**	**4.02**	**0.50**	**2.85**	**5.09**	**2.24**
	15[2]	*3.81*	*3.66*	*3.95*	*0.26*	*3.26*	*4.09*	*0.83*

Overall mean estimates (log_{10} IU/ml) relative to concurrently tested 2nd International Standard (97/750). Results of quantitative and qualitative assays combined. Further details of the collaborative study are available in the report WHO/BS/09.2121.

[1] – excluding data from 1 laboratory; [2] – excluding data from 3 laboratories

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