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**Collaborative Study to Evaluate the Proposed 3<sup>rd</sup> WHO International  
Standard for Hepatitis B Virus (HBV) for Nucleic Acid Amplification  
Technology (NAT)-Based Assays**

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## **Summary**

This report describes the preparation and collaborative study evaluation of the replacement 3<sup>rd</sup> WHO International Standard for hepatitis B virus (HBV) for use in the standardization of nucleic acid amplification techniques (NAT). Two freeze-dried candidates were prepared, each comprising HBV DNA-positive plasma diluted in pooled human plasma. Sixteen laboratories from nine countries participated in a collaborative study to evaluate the fitness for purpose and potency of each candidate using their routine NAT-based assay for HBV. The freeze-dried candidates (samples 1 and 2) were evaluated alongside the 2<sup>nd</sup> WHO International Standard for HBV, NIBSC code 97/750 (sample 3). A range of HBV NAT assays were used in the evaluation, the majority of which were commercial quantitative assays based on real-time PCR technology. The overall mean potency estimates for the candidate samples 1 and 2, relative to the concurrently tested 2<sup>nd</sup> WHO International Standard for HBV (sample 3), from quantitative assays, were 5.93 and 5.98 log<sub>10</sub> IU/mL respectively. The variability in individual laboratory mean estimates for quantitative assays for samples 1-3 was approximately 0.3 log<sub>10</sub> IU/mL. The inter-laboratory variability for qualitative assays was higher. The results obtained from accelerated thermal degradation studies at 11 weeks indicate that the candidate is stable and suitable for long-term use.

The results of the study indicate the suitability of both candidates as the replacement 3<sup>rd</sup> WHO International Standard for HBV. It is proposed that candidate sample 1 (NIBSC code 10/264) is established as the 3<sup>rd</sup> WHO International Standard for HBV for NAT with an assigned potency of 850,000 IU/mL (~5.93 log<sub>10</sub> IU/mL) when reconstituted in 0.5 mL of nuclease-free water.

## **Introduction**

Stocks of the 2<sup>nd</sup> WHO International Standard for HBV are diminishing and need to be replaced. The HBV International Standard is used by IVD manufacturers, blood transfusion centres, control authorities, and clinical laboratories, to calibrate secondary reference materials and in the validation of HBV NAT assays.

The need to standardize NAT-based assays for HBV and maintain the availability of the HBV International Standard is ongoing. HBV remains a major public health problem worldwide. An estimated 350 million people worldwide have chronic HBV infection, and an estimated 600,000 people die annually from acute infections or cirrhosis and hepatocellular carcinoma caused by chronic infection<sup>1</sup>. NAT is routinely used in the management of clinical HBV infections, particularly to guide the initiation of and monitor the response to antiviral therapy in chronically-infected patients<sup>2</sup>. There remains a major risk of transfusion-transmitted infection through window-period donations, vaccine breakthrough infections and occult HBV infection<sup>3</sup>. Blood screening for HBV by NAT has been widely implemented on a voluntary basis and is mandated in some situations. A range of both commercial and laboratory-developed NAT-based assays are currently in use.

International Standards are prepared in accordance with published WHO recommendations<sup>4</sup>. The 1<sup>st</sup> and 2<sup>nd</sup> WHO International Standards for HBV were prepared by dilution of a Eurohep R1 sample<sup>5</sup> (Genotype A2, HBsAg subtype *adw2*, derived from a single donor) in HBV-negative pooled human plasma. Both materials were prepared from the same bulk, but filled and freeze-dried on two separate occasions. The standards were evaluated in parallel in a worldwide collaborative study using a range of NAT-based assays for HBV<sup>6</sup>. The first candidate (NIBSC code 97/746) was established as the 1<sup>st</sup> WHO International Standard for HBV DNA in 1999, with an assigned potency of 1,000,000 International Units (IU)/mL when reconstituted in 0.5 mL nuclease-free water. In 2006 the WHO Expert Committee on Biological Standardization (ECBS)

established the second candidate (NIBSC code 97/750) as the replacement 2<sup>nd</sup> WHO International Standard for HBV DNA following a smaller collaborative study<sup>7</sup>.

This report describes the preparation and collaborative study evaluation of two candidate materials as the replacement 3<sup>rd</sup> WHO International Standard for HBV for NAT. The candidates have been prepared from the same original HBV Eurohep R1 stock as the 1<sup>st</sup> and 2<sup>nd</sup> WHO International Standards, diluted in HBV-negative pooled human plasma. The proposal to replace the 2<sup>nd</sup> WHO International Standard for HBV for NAT was endorsed by the WHO ECBS in October 2010. The proposal and an update were also presented at the XXII Scientific Working Group on the Standardization of Genome Amplification Techniques (SoGAT) for the Safety Testing of Blood, Tissues and Organs for Blood Borne Pathogens in Rome in April 2011.

The proposed standard is intended to be used in the *in vitro* diagnostics field and it relates to ISO 17511:2003 Section 5.5.

## **Aims of study**

The aim of this collaborative study was to evaluate the suitability and potency of the candidate freeze-dried preparations in parallel with the 2<sup>nd</sup> WHO International Standard for HBV (NIBSC code 97/750) using a range of NAT-based assays.

## **Materials**

### **Candidate standards**

Two candidate materials have been prepared, comprising freeze-dried human plasma and HBV at a concentration of approximately 1,000,000 IU/mL. The HBV was sourced from a stock of the Eurohep R1 reference material stored at NIBSC and is a genotype A2, HBsAg subtype *adw2* virus. The pooled human plasma diluent was sourced from blood donations and had been tested and found negative for HIV antibody, HCV antibody, HBsAg and syphilis. It was also tested in-house and found negative for HCV RNA by NAT. The preparations were freeze-dried to ensure long-term stability.

### ***Preparation of bulk materials***

The concentration of the HBV Eurohep R1 stock was determined at NIBSC using the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, version 2.0 (Roche Diagnostics GmbH, Mannheim, Germany), alongside a dilution series of the 2<sup>nd</sup> WHO International Standard for HBV (NIBSC code 97/750).

Each bulk preparation was formulated to contain approximately 1,000,000 IU/mL of HBV in a final volume of 1.5 L of pooled human plasma, and mixed for a total of 30 minutes using a magnetic stirrer. Aliquots comprising 1 mL volumes of the liquid bulk were stored at -70 °C for evaluation against the freeze-dried product. The bulk was stored at -70 °C prior to shipping to two external facilities for filling and lyophilization into the final products, NIBSC codes 10/264 and 10/266.

### ***Filling and lyophilization of candidate standards***

The filling and lyophilization of the bulk materials was performed under contract at two external Containment Level 3 facilities, and the production summary is detailed in Table 1. Product 10/264 was filled and freeze-dried at BioReliance Ltd., Stirling, Scotland, while 10/266 was filled and freeze-dried at eQAD, UK NEQAS, Colindale, UK. On the day of the filling of each

product, the bulk was thawed in a 37 °C circulating water-bath. The bulk was removed from the water-bath when just thawed and stirred constantly during the filling process.

The bulk for product 10/264 was dispensed in 0.5 mL volumes into 3 mL crimp-cap glass vials using a dosing pump. The homogeneity of the fill was determined by performing check-weighing of approximately every fiftieth vial, with vials outside the defined specification being discarded. Filled vials were partially stoppered with 13 mm diameter igloo stoppers and lyophilized in a Virtis Genesis freeze dryer. Vials were loaded onto the shelves at 4 °C and held at this temperature for 30 mins. The freeze dryer was then cooled to -35 °C over 2 hrs and held at this temperature for a further 2 hrs. A vacuum was applied to 100 µb over 1 hr. The vacuum was maintained for 37 hrs at -35 °C and then the shelves were ramped to 25 °C over 10 hrs. The dryer was held at 25 °C and 30 µb vacuum for 16 hrs for secondary drying before releasing the vacuum and back-filling the vials with nitrogen. The vials were then stoppered in the dryer, removed and capped, before decontaminating with formaldehyde.

The bulk for product 10/266 was dispensed in 0.5 mL volumes into 3 mL screw-cap glass vials using a repeat pipettor. The homogeneity of the fill was determined by performing check-weighing of approximately every fiftieth vial, with vials outside the defined specification being discarded. Filled vials were partially stoppered with 13 mm diameter freeze drying stoppers and lyophilized in a Christ freeze dryer. Vials were loaded onto the shelves at 20 °C and the shelves were held at 4 °C for 30 mins. The freeze dryer was then cooled to -35 °C over 2 hrs and held at this temperature for a further 1.5 hrs. A vacuum was applied to 100 µb over 1 hr. The shelves were then raised to -12 °C and the vacuum maintained for 20 hrs for primary drying. The shelves were ramped to 25 °C over 5 hrs and secondary drying conditions applied. These were held at 25 °C and 30 µb vacuum for a period of 64 hrs before releasing the vacuum and back-filling the vials with nitrogen. The vials were then stoppered in the dryer, removed and capped, before decontaminating with formaldehyde.

In both cases the sealed vials were returned to NIBSC for storage at -20 °C under continuous temperature monitoring for the lifetime of the product (NIBSC to act as custodian and worldwide distributor).

### ***Post-fill testing***

Assessments of residual moisture and oxygen content, as an indicator of vial integrity after sealing, were determined for 20 vials of each freeze-dried product. Residual moisture was determined by non-invasive near-infrared (NIR) spectroscopy (MCT 600P, Process Sensors, Corby, UK). NIR results were then correlated to Karl Fischer (using calibration samples of the same excipient, measured using both NIR and Karl Fischer methods) to give % w/w moisture readings. Oxygen content was measured using a Lighthouse Infra-Red Analyzer (FMS-750, Lighthouse Instruments, Charlottesville, USA).

Samples of the liquid bulk (n=6) and freeze-dried product (n=30) were tested by HBV NAT assay as described for preparation of bulk materials, in order to determine the homogeneity of each product prior to dispatch for collaborative study.

### ***Stability of the freeze-dried candidates***

Accelerated degradation studies are underway at NIBSC in order to predict the stability of 10/264 and 10/266 when stored at the recommended temperature of -20 °C. Vials of freeze-dried product are being held at -70 °C, -20 °C, +4 °C, +20 °C, +37 °C, +45 °C. At specified time points during the life of each product, three vials will be removed from storage at each temperature and HBV DNA quantified by NAT (as described for preparation of bulk materials).

## Study samples

The freeze-dried candidates 10/264 and 10/266 were evaluated alongside the 2<sup>nd</sup> WHO International Standard for HBV (NIBSC code 97/750). Study samples were stored at -20 °C prior to shipping to participants.

Study samples shipped to participants were coded as samples 1-3 and were as follows:

- Sample 1 (S1) - Lyophilized preparation 10/264 in a 3 mL crimp cap glass vial.
- Sample 2 (S2) - Lyophilized preparation 10/266 in a 3 mL screw cap glass vial.
- Sample 3 (S3) - Lyophilized preparation 97/750 in a 3 mL crimp cap glass vial.

## Study design

The aim of this collaborative study was to evaluate the potency of the two candidate freeze-dried preparations in parallel with the 2<sup>nd</sup> WHO International Standard for HBV (NIBSC code 97/750) using a range of NAT-based assays. Three vials each of study samples 1-3 were sent to participating laboratories by courier, with specific instructions for storage and reconstitution.

## Study protocol

Participants were requested to test dilutions of each sample using their routine HBV NAT-based assay on three separate occasions, using a fresh vial of each sample in each independent assay. In accordance with the study protocol (Appendix 2), the lyophilized samples were to be reconstituted with 0.5 mL of deionized, nuclease-free molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use.

Participants were requested to dilute samples 1-3 to within the quantitative range of the assay, using the sample matrix specific to their individual assay, and to extract each dilution prior to amplification. For quantitative assays, participants were requested to test a minimum of two serial ten-fold dilutions within the linear range of the assay. For qualitative assays, participants were requested to test half-log<sub>10</sub> serial dilutions of each sample, around the assay end-point (in order to determine the actual assay end-point). For subsequent assays, participants were asked to test the dilution at the predetermined end-point, and a minimum of two half-log serial dilutions either side of the end-point (i.e., at least five dilutions in total).

Participants were requested to report the concentration of each sample in IU/mL (positive/negative for qualitative assays) for each dilution of each sample and return results, including details of methodology used, to NIBSC for analysis.

## Participants

Study samples were sent to 16 participants representing 9 countries (Appendix 1). Participants were selected for their experience in HBV NAT and geographic distribution. They represented manufacturers of *in vitro* diagnostic devices (IVDs), control and contract testing laboratories, blood transfusion centres and plasma manufacturers, and reference laboratories. All participating laboratories are referred to by a code number, allocated at random, and not representing the order of listing in Appendix 1. Where a laboratory returned data using different assay methods, the results were analyzed separately, as if from different laboratories, and are referred to as, for example, laboratory 5A, 5B etc.

## Statistical methods

Qualitative and quantitative assay results were evaluated separately. In the case of qualitative assays, for each laboratory and assay method, data from all assays were pooled to give a number

positive out of number tested at each dilution step. A single 'end-point' for each dilution series was calculated, to give an estimate of 'NAT detectable units/mL', as described previously<sup>8</sup>. It should be noted that these estimates are not necessarily directly equivalent to a genuine genome copy number/mL. In the case of quantitative assays, analysis was based on the results supplied by the participants. Results were reported as IU/mL. For each assay run, a single estimate of  $\log_{10}$  IU/mL was obtained for each sample, by taking the mean of the  $\log_{10}$  estimates of IU/mL across replicates, after correcting for any dilution factor. A single estimate for the laboratory and assay method was then calculated as the mean of the  $\log_{10}$  estimates of IU/mL across assay runs.

All analysis was based on the  $\log_{10}$  estimates of IU/mL or 'NAT detectable units/mL'. Overall mean estimates were calculated as the means of all individual laboratories. Variation between laboratories (inter-laboratory) was expressed as standard deviations (SD) of the  $\log_{10}$  estimates and % geometric coefficient of variation (%GCV)<sup>9</sup> of the actual estimates. Potencies relative to sample 3, the current International Standard (97/750), were calculated as the difference in estimated  $\log_{10}$  'units per mL' (test sample – standard) plus the value in  $\log_{10}$  IU/mL for the International Standard. Therefore for example, if in an individual assay, the test sample is 0.5  $\log_{10}$  higher than the International Standard, assigned 6.0  $\log_{10}$  IU/mL, the relative potency of the test sample is 6.5  $\log_{10}$  IU/mL.

For the quantitative assays, variation within laboratories, and between assays, (intra-laboratory) was expressed as standard deviations and %GCVs of the individual assay mean  $\log_{10}$  estimates. These estimates were pooled across all samples. The significance of the inter-laboratory variation relative to the intra-laboratory variation was assessed by an analysis of variance.

## **Results and data analysis**

### **Validation of study samples and stability assessment**

Production data for the candidate standards samples 1 (10/264) and 2 (10/266) showed that the CV of the fill mass and mean residual moistures were within acceptable limits for a WHO International Standard<sup>4</sup> (Table 1). Residual oxygen content was within the NIBSC working limit of 1.1%. Evaluation of multiple aliquots of each candidate (n=30 each for study samples 1 and 2) at NIBSC prior to dispatch indicated that the homogeneity of HBV content was similar for both study samples (2SD of 0.12  $\log_{10}$  IU/mL for each sample). Comparison of the liquid bulks versus the freeze-dried products indicated that there was a minimal loss in potency of approximately 0.03 and 0.04  $\log_{10}$  IU/mL, for 10/264 and 10/266 respectively, upon freeze-drying (data not shown).

Samples of the candidate standards 10/264 and 10/266 were stored at elevated temperatures, and assayed at NIBSC in parallel with samples stored at -20 °C and -70 °C by HBV NAT (as described for preparation of bulk materials). Three vials of each sample were evaluated after storage at each temperature for 11 weeks. The mean estimated  $\log_{10}$  IU/mL and differences ( $\log_{10}$  IU/mL) from the -70 °C baseline samples are shown in Table 2. A negative value indicates a drop in potency relative to the -70 °C baseline. The 95% confidence intervals for the differences are  $\pm 0.133 \log_{10}$  based on a pooled estimate of the standard deviation between individual vial test results, and all results fall within this range. There are no significant differences between the estimated  $\log_{10}$  IU/mL at any temperature for either of the candidate standards. As there is no observed drop in potency it is not possible to fit the usual Arrhenius model for accelerated degradation studies, or obtain any predictions for the expected loss per year with long-term storage at -20 °C. However, using the 'rule of thumb' that the decay rate will approximately double with every 10 °C increase in temperature (personal communication: Dr P K Philips), and noting that there is no detectable drop in potency after 11 weeks at +20 °C, then

there should be no detectable difference after 44 months at -20 °C. A similar argument applied to the +45 °C data would imply no detectable loss after over 15 years at -20 °C. In summary, there is no evidence of degradation at any temperature after storage for 11 weeks. It is not possible to obtain precise estimates of any degradation rates for long-term storage at -20 °C. All available data indicates adequate stability. Subsequent testing will take place at 1, 1.5, 2, 3, 4, and 5 years.

The stability of both candidates when reconstituted has not been specifically determined. Therefore, it is recommended that the reconstituted material is for single use only.

### **Data received**

Data were received from all 16 participating laboratories. Participants performed a variety of different assay methods, with some laboratories performing more than one assay method. In total, 22 data sets were received from 15 quantitative assays and 7 qualitative assays. Apart from the cases noted below, there were no exclusions of data.

#### *Qualitative Assays:*

There were no exclusions of data.

#### *Quantitative Assays:*

Laboratory 3 tested five dilutions, down to  $10^{-5}$ . The estimates from the dilutions at  $10^{-4}$  and  $10^{-5}$  were higher than those for other dilutions when corrected for dilution factor and appeared to be outside the linear range of the assay. These dilutions were excluded from further analysis.

The results from the two dilutions tested by laboratory 7 appeared to be non-linear with an approximate difference of  $0.2 \log_{10}$ , the lower estimate being at the higher concentration tested. However, the overall mean across dilutions was close to the expected value of the 2<sup>nd</sup> WHO International Standard for HBV and there was no reason to select one dilution over the other. All data were used for analysis.

The range of estimates between replicates within an assay at a single dilution (where tested) was calculated. Where this range was greater than  $0.5 \log_{10}$ , the results for the whole dilution were excluded. This occurred on five occasions for laboratory 10, and one for laboratory 11. The laboratory 11 case was noted on their results sheets as a potential mistake in loading the PCR.

### ***Summary of assay methodologies***

The majority of participants prepared dilutions of study samples 1-3 using negative human plasma, however, Roche MultiPrep Specimen Diluent (MP DIL) and nuclease-free water were also used. The range of the dilutions performed varied slightly between each laboratory. Assay methodologies for qualitative and quantitative assays are summarized in Table 3.

### ***Estimated IU/mL or 'NAT detectable units/mL'***

The laboratory mean estimates of IU/mL ( $\log_{10}$ ) from the quantitative assays and 'NAT detectable units/mL' ( $\log_{10}$ ) from the qualitative assays are shown in Tables 4 and 5 respectively. The individual laboratory mean estimates are also shown in histogram form in Figures 1a-1c. Each box represents the mean estimate from one laboratory, and the boxes are labeled with the laboratory and assay code. The results from the qualitative assays are shaded in grey.

From the Figures 1a-1c, and Tables 4 and 5, it is clear that there is good agreement between the estimates from the quantitative assays, but considerable variability between the estimates of 'NAT detectable units' from the different qualitative assays used.

It is noted that there is a significant disparity in the 'NAT detectable units/mL' reported for sample 3 (97/750) using the PROCLEIX® ULTRIO® Assay in this present study and those reported for 97/750 using the PROCLEIX® ULTRIO® Plus™ Assay in the recent collaborative study to establish the WHO International Genotype Panel for HBV NAT-based assays (4.36 and 6.31 log<sub>10</sub> 'NAT detectable units/mL' respectively)<sup>10</sup>. While the reasons for this disparity are unclear, it should be noted that the calibration of samples 1 and 2 in IU by relative potency is unaffected, as the PROCLEIX® ULTRIO® Assay results in this present study are consistent across all study samples.

Table 6 shows the overall mean estimates of log<sub>10</sub> IU/mL from the quantitative assays, and log<sub>10</sub> 'NAT-detectable units/mL' from the qualitative assays, along with the standard deviation (of log<sub>10</sub> estimates) and the %GCV (of actual estimates). For all samples, the quantitative assays have SD's around 0.08 log<sub>10</sub>, and %GCVs around 20-25%. This represents very good agreement between laboratories and assay methods. For the qualitative assays, the SD's are around 0.8 log<sub>10</sub>, with %GCVs around 450-625%.

### ***Potencies relative to the 2<sup>nd</sup> WHO International Standard for HBV (Sample 3)***

The estimated concentrations of the candidate samples 1 and 2 were expressed in IU, by direct comparison (relative potencies) to the current International Standard (sample 3), which has an assigned unitage of 10<sup>6</sup> IU/mL (6.0 log<sub>10</sub>), as described in the statistical methods section. The laboratory mean estimates are shown in Tables 7 and 8 for the quantitative and qualitative assays respectively. Units are log<sub>10</sub> IU/mL in both cases. Overall mean estimates, along with SD and %GCV, are shown in Table 9. The results are also shown in histogram form in Figures 2a-2b.

From Figures 2a and 2b, and Tables 7 and 8, it is clear that there is an improvement in the agreement between laboratories for the qualitative assays. The %GCV between laboratories has reduced from 624% and 429%, to 125% and 135%, for samples 1 and 2 respectively. There is also a small improvement for the quantitative assays, with %GCVs reducing from 24% and 21%, to 9% and 11%, for samples 1 and 2 respectively. The reduction in variability for the quantitative assays does not appear to be substantial from the figures, principally because the quantitative assays were all in good agreement initially. However, the reduction in %GCV is approximately 2-fold.

The overall mean relative potencies for samples 1 and 2 are 5.93 and 5.98 log<sub>10</sub> IU/mL, respectively, based on the quantitative assays alone, or 5.91 and 5.98 log<sub>10</sub> IU/mL based on all assays. These values compare to the direct estimates of 5.99 and 6.03 log<sub>10</sub> IU/mL from the quantitative assays which are all calibrated in IU/mL.

### ***Inter and intra-laboratory variation***

For all samples, the inter-laboratory variation was greater than the intra-laboratory variation (p<0.01). Table 10 shows the intra-laboratory standard deviations and %GCVs for each laboratory, calculated by pooling estimates for samples 1-3. There are differences between the repeatability of laboratory estimates across assays. In general, the repeatability is good for assays of this type and the average standard deviation is 0.08 log<sub>10</sub> or a %GCV of 20%. These figures represent the variability between individual assay mean estimates of IU/mL. Since each assay tested multiple replicates of samples at different dilutions, the resulting between-assay variability is lower than would be expected if only a single replicate was tested in each assay. The 'NAT detectable units' from the qualitative assays are obtained by pooling all assay data to give a



single series of number positive out of number tested at each dilution. As a result, there is no comparable analysis of intra-assay variation for the qualitative assays.

## Discussion and conclusions

In this study, a range of NAT-based assays for HBV have been used to determine the potency and evaluate the suitability of the candidate standards as the replacement 3<sup>rd</sup> WHO International Standard for HBV for NAT-based assays. The candidates were prepared from the same virus stock used for previous HBV WHO International Standards and were diluted in a similar pooled human plasma material. The candidate bulks were freeze-dried to ensure long-term stability, and the production data suggests that the batches are homogeneous. Comparison of the liquid bulks versus the freeze-dried products indicates that there was minimal loss in potency upon freeze-drying, of the order of 0.03 and 0.04 log<sub>10</sub> IU/mL for 10/264 and 10/266 respectively (data not shown). The results obtained from accelerated thermal degradation studies at 11 weeks indicate that the candidate is stable and suitable for long-term use.

In the collaborative study, the freeze-dried candidate preparations were evaluated alongside the 2<sup>nd</sup> WHO International Standard for HBV (sample 3). The overall mean estimate for the 2<sup>nd</sup> WHO International Standard for HBV determined by both quantitative and qualitative assays was 6.03 log<sub>10</sub> IU/mL. This demonstrates the long-term stability of this preparation over a period of approximately 13 years. The overall mean estimates for the candidate standard samples 1 and 2, as determined by quantitative assays, were 5.99 and 6.03 log<sub>10</sub> IU/mL respectively, based on the calibration of quantitative assay kits in IU/mL. The overall mean estimates for qualitative assays were slightly lower. The overall range in laboratory mean estimates for all study samples was 0.3 log<sub>10</sub> IU/mL for quantitative assays, and >2 log<sub>10</sub> IU/mL for qualitative assays. This difference reflects the increased variability of measurements determined by qualitative assays, and is likely, in part, to be due to the fact that the concentration was determined using 0.5 log<sub>10</sub> limited dilution steps. Inter-laboratory variability was higher than intra-laboratory variability for the quantitative assays. The agreement between laboratories for samples 1 and 2 was slightly improved when the potency was expressed relative to the 2<sup>nd</sup> WHO International Standard for HBV (sample 3). When the results were expressed in IU/mL directly relative to the concurrently tested 2<sup>nd</sup> WHO International Standard for HBV, the overall mean estimates from the quantitative assays were 5.93 (10/264) and 5.98 (10/266) log<sub>10</sub> IU/mL respectively. The qualitative assays give estimates of 'NAT detectable units/mL', which do not allow for extraction efficiency and are not calibrated in IU. Therefore, the calibration of samples 1 and 2 in IU relative to the current HBV International Standard, sample 3, is based on the IU/mL results from quantitative assays only.

The matter of commutability<sup>11</sup> of the candidate standard for HBV-positive samples has not been specifically assessed in this study. However, the material is derived from the same source as the 1<sup>st</sup> and 2<sup>nd</sup> WHO International Standards for HBV and is expected to perform in a similar manner.

In summary, this study demonstrates the stability and suitability of both candidates as the replacement 3<sup>rd</sup> WHO International Standard for HBV. Both preparations have been calibrated in IU against the 2<sup>nd</sup> WHO International Standard for HBV.

## Proposal

It is proposed that the candidate standard, NIBSC code 10/264, is established as the 3<sup>rd</sup> WHO International Standard for HBV for use in NAT-based assays, with an assigned potency of 850,000 IU/mL (~5.93 log<sub>10</sub> IU/mL) when reconstituted in 0.5 mL of nuclease-free water. The uncertainty can be derived from the variance of the fill and is 1.29%. It is noted that the second

candidate (NIBSC code 10/266) would also be a suitable replacement HBV International Standard in due course, and depending on ongoing stability assessment. The proposed standard is intended to be used by IVD manufacturers, blood transfusion centres, control authorities, and clinical laboratories, to calibrate secondary reference materials and in the validation of HBV NAT assays. Proposed Instructions for Use (IFU) for the product are included in Appendix 3.

### **Comments from participants**

Nine of sixteen participants responded to the report. There were no disagreements with the suitability of the candidate standard (NIBSC code 10/264) to serve as the 3<sup>rd</sup> WHO International Standard for HBV for use in NAT-based assays. Some comments suggested minor editorial changes and these have been implemented.

### **Acknowledgements**

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11. WHO Consultation on Global Measurement Standards and their use in the in vitro Biological Diagnostic Field, Switzerland, Geneva, Switzerland, 7-8 June 2004:  
<http://www.who.int/bloodproducts/publications/en/Minutes-220804.pdf>

**Table 1.** Production summary for the candidate standards (samples 1 - 10/264 and 2 – 10/266).

NIBSC code	10/264	10/266
Product name	Hepatitis B virus	Hepatitis B virus
Dates of processing	Filling; 7 March 2011 Lyophilisation; 7-10 March 2011 Sealing; 10 March 2011	Filling; 17 March 2011 Lyophilisation; 17-21 March 2011 Sealing; 21 March 2011
Presentation	Freeze-dried preparation in 3 mL crimp-cap glass vial	Freeze-dried preparation in 3 mL screw-cap glass vial
Appearance	Robust opaque cake	Robust opaque cake
No. of vials filled	2518	2700
Mean fill weight (g)	0.5006 (n=57)	0.506 (n=55)
CV of fill weight (%)	1.29	0.36
Mean residual moisture (%)	0.46 Karl Fischer, 0.77 NIR units (n=20)	0.29 Karl Fischer, 0.46 NIR units (n=20)
CV of residual moisture (%)	4.22	14.66
Mean oxygen content (%)	0.22 (n=20)	0.17 (n=20)
CV of oxygen content (%)	65.26	63.81
No. of vials available to WHO	2383	2533

**Table 2.** Stability of 10/264 and 10/266 at 11 weeks.

Temperature (°C)	Mean log <sub>10</sub> IU/mL		Difference in log <sub>10</sub> IU/mL from -70 °C baseline sample	
	10/264	10/266	10/264	10/266
-70	6.043	6.035	-	-
-20	6.017	6.030	-0.026	-0.005
+4	6.100	6.073	0.057	0.038
+20	6.045	6.060	0.002	0.025
+37	6.061	6.090	0.018	0.055
+45	6.054	6.126	0.011	0.091

**Table 3.** Collaborative study assay methods and codes.

<i>Quantitative Assays</i>		
Assay Code	Assay	No. of data sets
bDNA	Versant HBV DNA 3.0 Assay (bDNA)	1
CAP/CTM	COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0	4
ABT-RT	Abbott RealTime HBV	2
AFF	affigene® HBV trender	1
SMRT	SmartHBV	1
RG	RoboGene® HBV DNA Quantification Kit	1
ART-RG	<i>artus</i> ® HBV RG PCR Kit	1
CTM-HP	COBAS® TaqMan® HBV Test For Use With The High Pure System	1
IH	In-house	3
<i>Qualitative Assays</i>		
Assay Code	Assay	No. of data sets
CAS	COBAS® AmpliScreen HBV Test	2
ULT	PROCLEIX® ULTRIO® Assay	1
CTS/MPX	COBAS® TaqScreen MPX Test	1
IH	In-house	2
GFE	Confirmatory PCR Kit HBV (GFE Blut mbH)	1

**Table 4.** Laboratory mean estimates from quantitative assays ( $\log_{10}$  IU/mL).

Lab	Assay	Sample		
		S1	S2	S3
1	bDNA	5.94	5.96	5.98
2	CAP/CTM	6.10	6.11	6.23
3	ABT-RT	5.96	6.00	6.00
4	ART-RG	5.96	5.99	6.08
5A	AFF	6.02	6.12	6.11
5B	SMRT	6.15	6.19	6.17
6A	ABT-RT	5.86	5.93	5.94
6B	CAP/CTM	6.12	6.13	6.13
6C	RG	5.99	5.97	6.06
7	CTM-HP	6.00	6.01	6.01
8	CAP/CTM	6.08	6.12	6.16
10	IH	5.85	5.95	5.95
11	IH	5.88	5.93	5.91
13	CAP/CTM	5.97	6.06	6.04
16	IH	5.96	6.03	6.04

**Table 5.** Laboratory mean estimates from qualitative assays ( $\log_{10}$  'NAT detectable units/mL').

Lab	Assay	Sample		
		S1	S2	S3
8	CAS	5.76	5.91	6.15
9A	ULT	4.23	4.58	4.36
9B	CAS	6.57	6.57	6.41
9C	CTS/MPX	5.88	5.88	6.66
12	IH	6.72	6.72	6.50
14	IH	6.38	6.50	6.27
15	GFE	5.37	5.60	5.59

**Table 6.** Overall mean estimates and inter-laboratory variation ( $\log_{10}$  IU/mL for quantitative or 'NAT-detectable units/mL' for qualitative assays).

Sample	Assay	No. of data sets	Mean	SD	%GCV	Min	Max
S1	Qualitative	7	5.84	0.86	624	4.22	6.72
	Quantitative	15	5.99	0.09	24	5.85	6.15
S2	Qualitative	7	5.97	0.74	449	4.58	6.72
	Quantitative	15	6.03	0.08	21	5.93	6.19
S3	Qualitative	7	5.99	0.80	526	4.36	6.66
	Quantitative	15	6.05	0.09	24	5.91	6.23

**Table 7.** Laboratory estimates of potency relative to the 2<sup>nd</sup> WHO International Standard for HBV (sample 3) from quantitative assays log<sub>10</sub> IU/mL - based on assigned unitage of the International Standard of 10<sup>6</sup> (6.0 log<sub>10</sub>) IU/mL.

Lab	Assay	Sample	
		S1	S2
1	bDNA	5.96	5.98
2	CAP/CTM	5.87	5.88
3	ABT-RT	5.96	6.00
4	ART-RG	5.89	5.91
5A	AFF	5.90	6.00
5B	SMRT	5.99	6.03
6A	ABT-RT	5.92	5.99
6B	CAP/CTM	5.99	6.00
6C	RG	5.93	5.91
7	CTM-HP	5.98	6.00
8	CAP/CTM	5.92	5.96
10	IH	5.90	6.00
11	IH	5.97	6.02
13	CAP/CTM	5.92	6.02
16	IH	5.92	5.99

**Table 8.** Laboratory estimates of potency relative to the 2<sup>nd</sup> WHO International Standard for HBV (sample 3) from qualitative assays log<sub>10</sub> IU/mL - based on assigned unitage of the International Standard of 10<sup>6</sup> (6.0 log<sub>10</sub>) IU/mL.

Lab	Assay	Sample	
		S1	S2
8	CAS	5.61	5.75
9A	ULT	5.87	6.22
9B	CAS	6.16	6.16
9C	CTS/MPX	5.23	5.23
12	IH	6.21	6.21
14	IH	6.12	6.23
15	GFE	5.78	6.00



**Table 9.** Overall mean estimates and inter-laboratory variation for potency relative to the 2<sup>nd</sup> WHO International Standard for HBV log<sub>10</sub> IU/mL - based on assigned unitage of the International Standard of 10<sup>6</sup> (6.0 log<sub>10</sub>) IU/mL.

Sample	Assay	No. of data sets	Mean	SD	%GCV	Min	Max
S1	Qualitative	7	5.85	0.35	125	5.23	6.21
	Quantitative	15	5.93	0.04	9	5.87	5.99
	<i>Combined</i>	22	5.91	0.20	57	5.23	6.21
S2	Qualitative	7	5.97	0.37	135	5.23	6.23
	Quantitative	15	5.98	0.04	11	5.88	6.03
	<i>Combined</i>	22	5.98	0.20	58	5.23	6.23

**Table 10.** Intra-laboratory standard deviation of log<sub>10</sub> IU/mL and %GCV for quantitative assays.

Lab	SD	%GCV
1	0.02	5.1
2	0.10	26.7
3	0.04	9.4
4	0.12	30.8
5A	0.05	12.1
5B	0.01	2.9
6A	0.04	8.9
6B	0.03	7.2
6C	0.16	45.9
7	0.07	17.4
8	0.04	9.0
10	0.09	22.6
11	0.15	40.0
13	0.04	8.9
16	0.05	11.9
Overall	0.08	20.4

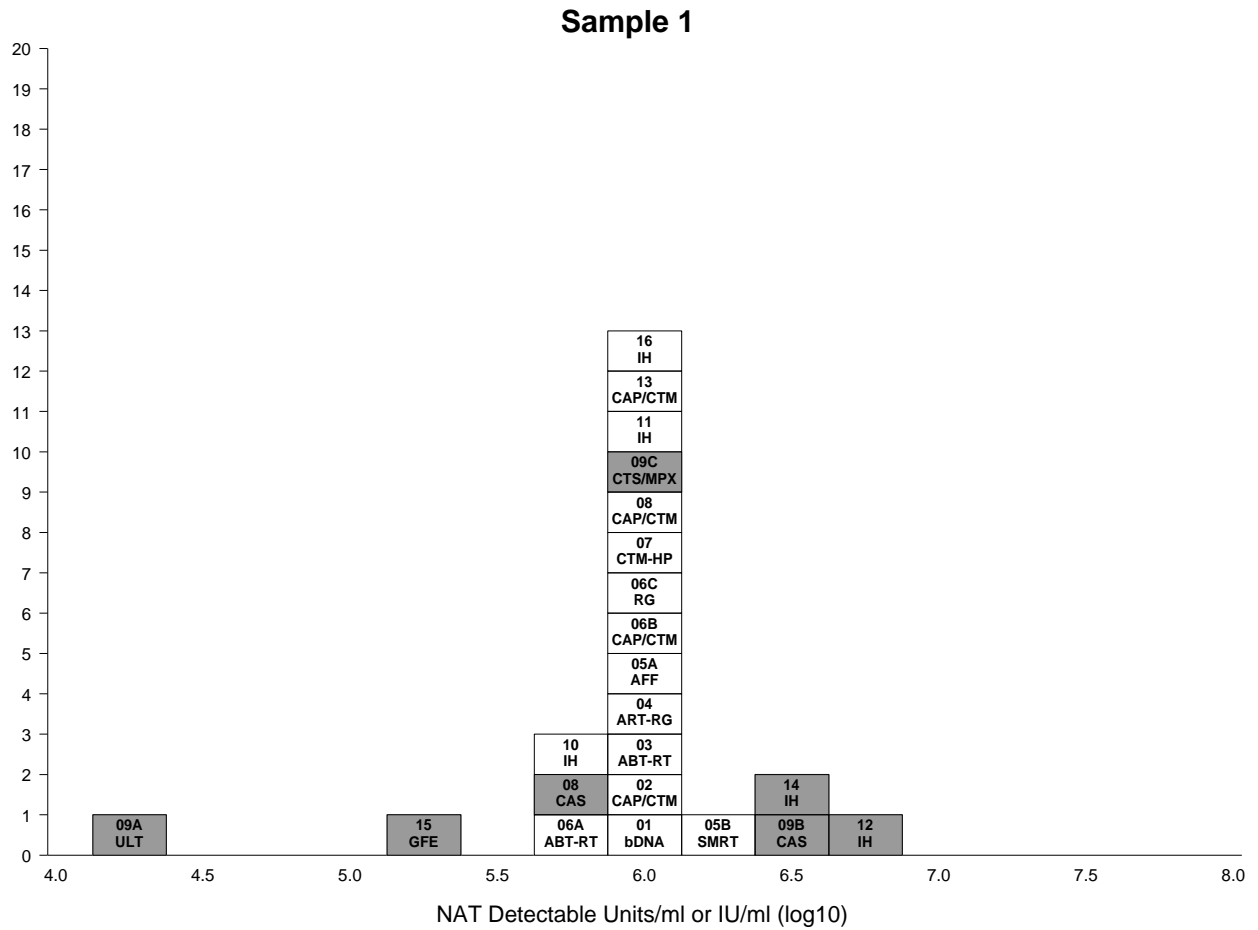
## **Figure legends**

**Figure 1.** Individual laboratory mean estimates for study samples 1-3 obtained using qualitative or quantitative NAT assays. Each box represents the mean estimate from each laboratory assay and is labeled with the laboratory and assay code. The results from the qualitative assays are shaded in grey.

**Figure 2.** Relative potencies of samples 1 and 2 against sample 3, for each quantitative or qualitative assay. Units are expressed as candidate  $\log_{10}$  IU/mL in both cases. Each box represents the relative potency for each laboratory assay and is labeled with the laboratory and assay code. The results from the qualitative assays are shaded in grey.

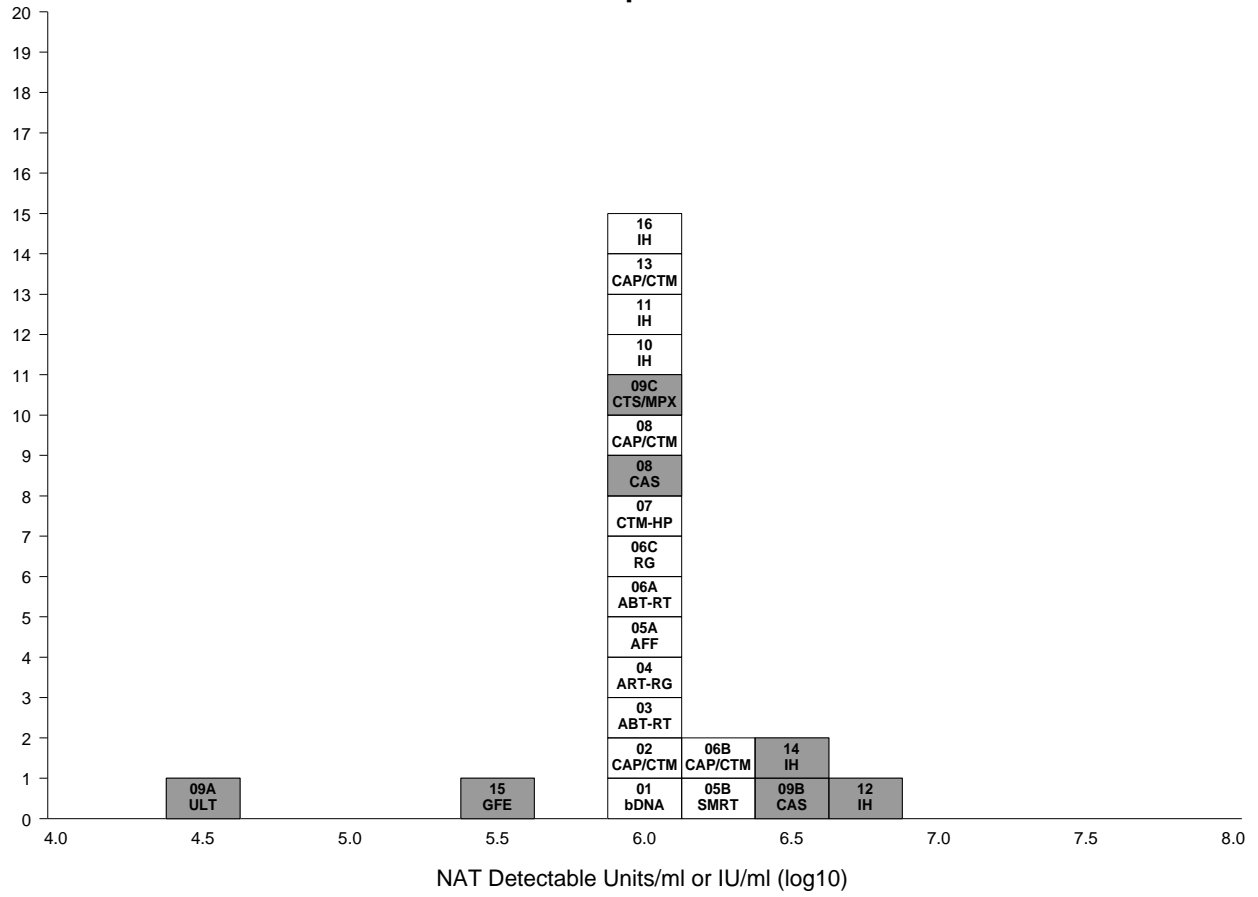
Figure 1

a



b

Sample 2



c

Sample 3

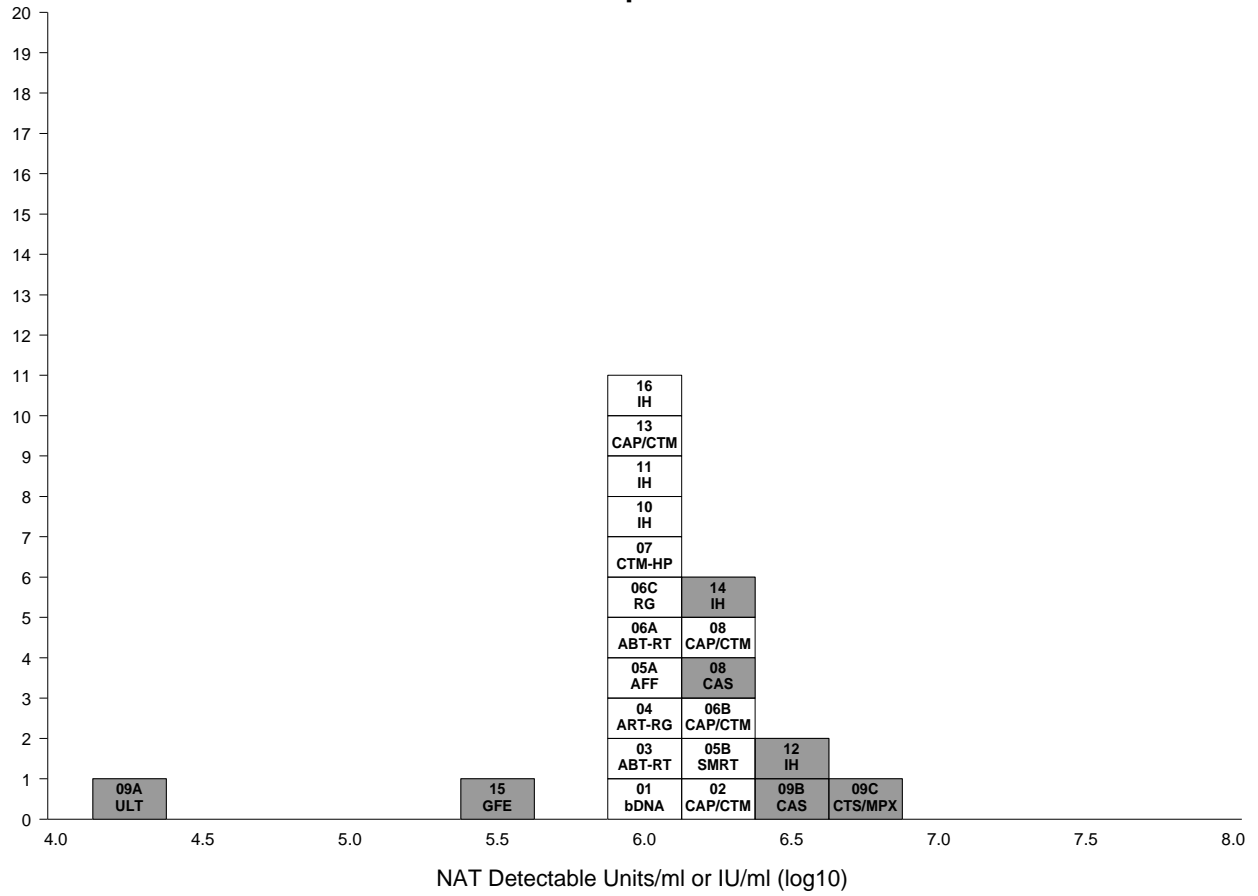
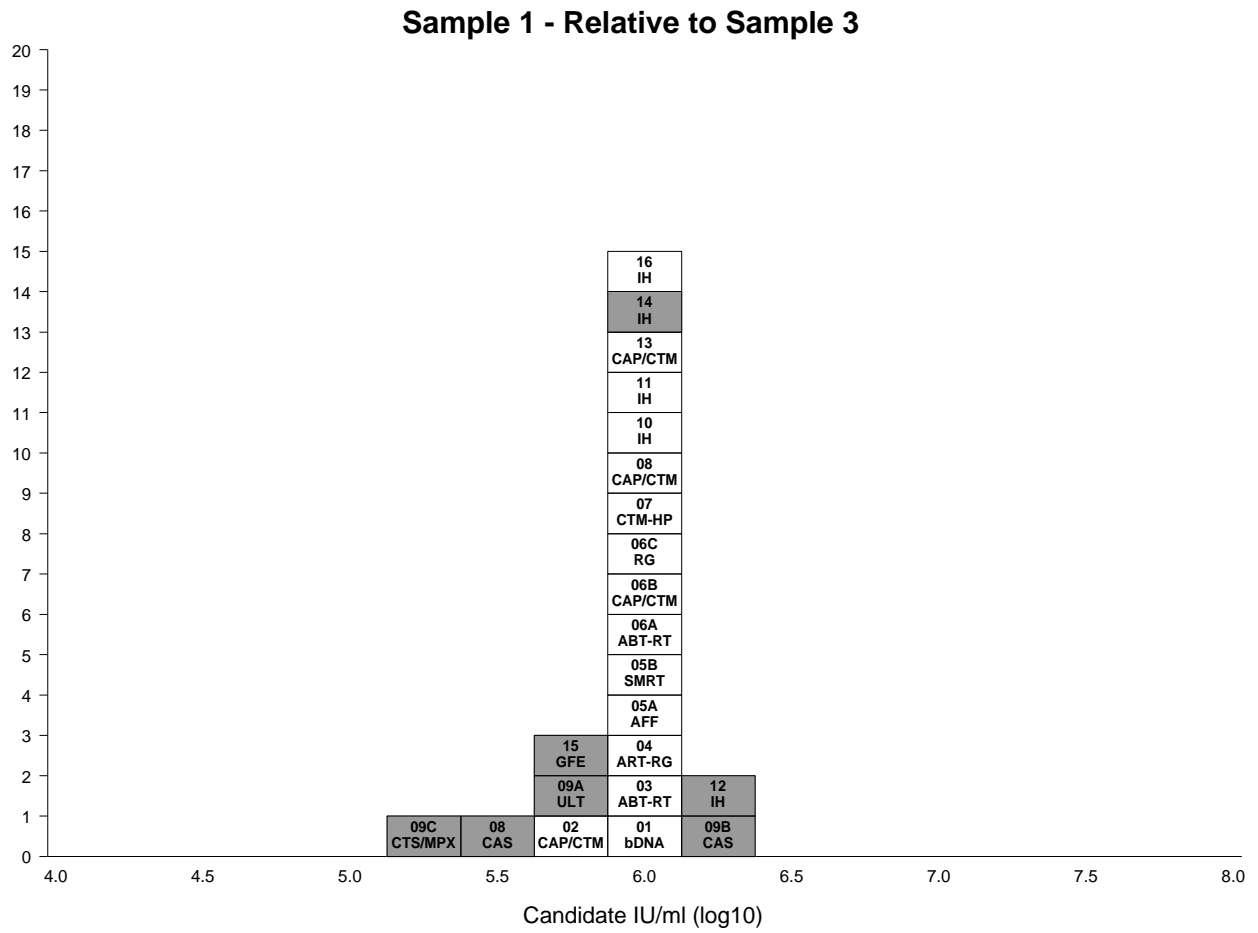


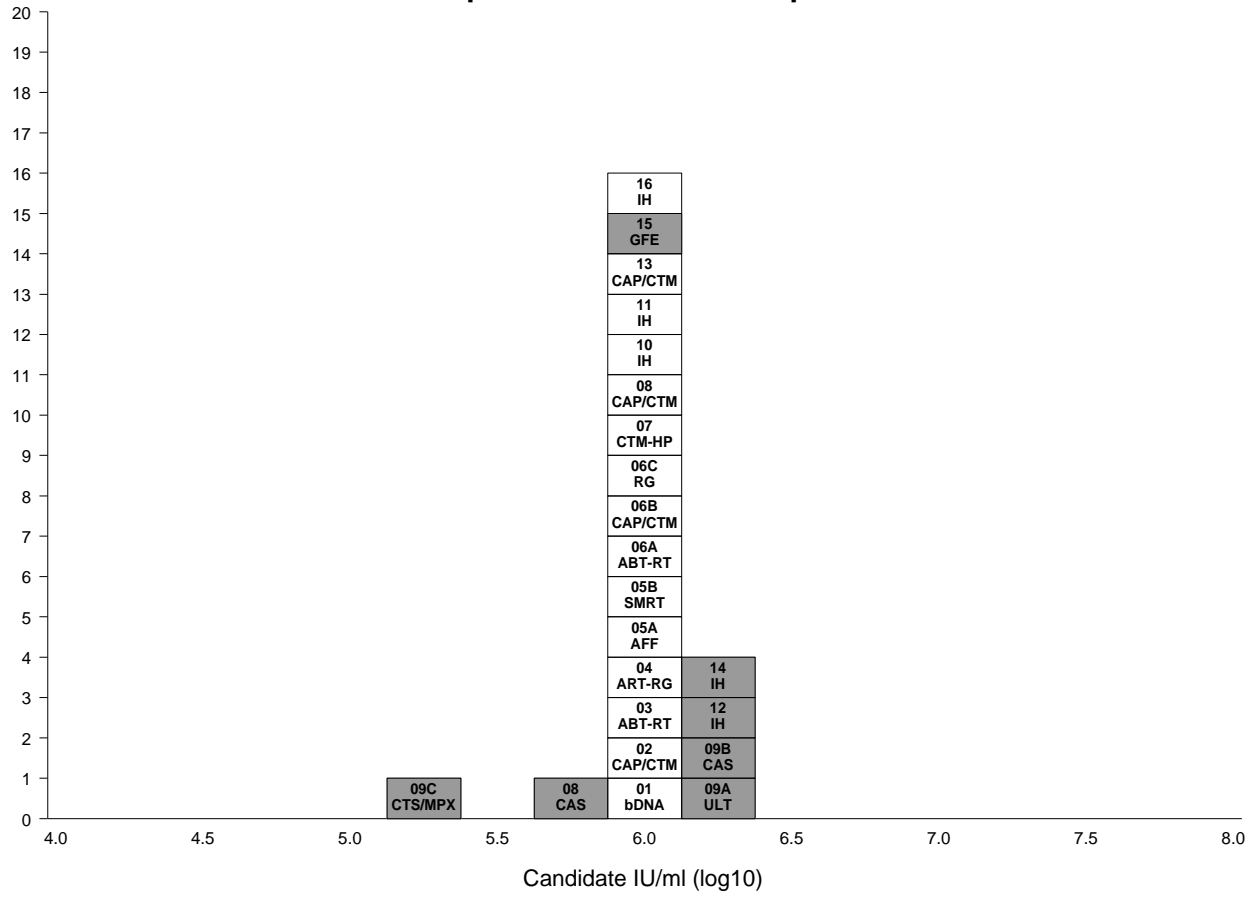
Figure 2

a



**b**

**Sample 2 - Relative to Sample 3**



## Appendix 1

## Collaborative study participants

(In alphabetical order by country)

Name	Laboratory	Country
Dr. Christina Wolf	Baxter AG, Plasma Analytics, Vienna	Austria
Prof. Cheng Zhou	2 <sup>nd</sup> Division of Viral Vaccines, National Institutes for Food and Drug Control (NIFDC), Beijing	China
Dr. Nikolaus Machuy Petra Herzog Nicole Kasdepke	QIAGEN Hamburg GmbH, Hamburg	Germany
Dr. Micha Nübling Dr. Michael Chudy Dr. Julia Kress	Paul-Ehrlich-Institut, Langen	Germany
Dr. Lutz Pichl	German Red Cross Blood Transfusion Service West GmbH, Hagen	Germany
Dr. Giulio Pisani Dr. Francesco Marino	Biologicals Unit, National Center for Immunobiologicals Research and Evaluation (CRIVIB), Istituto Superiore Di Sanita, Rome	Italy
Dr. Saeko Mizusawa <sup>1</sup> Fumihiko Ban <sup>2</sup>	<sup>1</sup> Dept. of Safety Research on Blood and Biological Products, National Institute of Infectious Diseases, Tokyo <sup>2</sup> Bio Medical Laboratories Co. Ltd., Kawagoe	Japan
Inger Bokliden Dr. Molly Vernersson	Cepheid AB, Sundbyberg	Sweden
Dr. HTM Cuypers Dr. Marco Koppelman	Sanquin Diagnostic Services, Amsterdam	The Netherlands
Dr. Simon Carne Prof. Richard Tedder	SSU/BBVU Virus Reference Department, Health Protection Agency Microbiological Services – Colindale, London	UK
Dr. Daniel Candotti Prof. Jean-Pierre Allain	National Health Service Blood and Transplant, Cambridge	UK
Dr. Jennifer Chen	Siemens Clinical Laboratory, Berkeley	USA
Dr. Zhuang Wang	Roche Molecular Systems, Branchburg	USA
Dr. Carolyn Mullen Dr. Edward Pabich	Abbott Molecular Inc., Des Plaines	USA
Dr. Stephen Kerby	Center for Biologics Evaluation and Research, FDA, Kensington	USA
Dr. Françoise Gala Dr. Jeff Albrecht	National Genetics Institute, Los Angeles	USA



## Appendix 2

### Study protocol



Page 1 of 3



#### **Collaborative study to evaluate the candidate 3rd WHO International Standard for hepatitis B virus (HBV) DNA for nucleic acid amplification techniques (NAT)**

#### **Study Protocol**

##### **Background and outline of the study**

The World Health Organisation (WHO) Expert Committee on Biological Standardisation (ECBS) has endorsed a proposal to replace the 2<sup>nd</sup> WHO International Standard for hepatitis B virus (HBV) for NAT-based assays in order to ensure continued supply of this reference material.

Two candidate materials have been prepared. These comprise freeze-dried human plasma and HBV at a concentration of ~1 E+06 IU/mL. The aim of this collaborative study is to evaluate the potency of these candidate freeze-dried preparations in parallel with the current HBV International Standard (NIBSC code 97/750) using a range of NAT-based assays. The study will allow the candidate replacements to be calibrated in terms of the HBV IU in order to maintain continuity of this unit.

Three lyophilised preparations are to be evaluated. Participants are asked to test dilutions of each sample, using their routine HBV NAT assay, on three separate occasions. Three vials of each study sample are provided. Where possible, we would encourage laboratories to use quantitative methods, however, data from qualitative assays will also be acceptable.

##### **Study samples**

Study samples comprise three lyophilised preparations in 2 mL crimp top glass vials, and are coded; sample 1, sample 2, and sample 3. Three vials of each study sample are provided for evaluation on three separate occasions. Upon receipt, all samples should be stored at -20 °C or below.

**CAUTION:** Study samples 1, 2 and 3, contain infectious HBV and should be handled only in appropriate containment facilities by fully trained and competent staff in accordance with national safety guidelines. These preparations contain human plasma, which has been tested and found negative for HIV antibody and HCV RNA by NAT. Care should be taken when opening vials to avoid cuts. See instructions for use for further details.

### Study protocol

Participants are requested to test dilutions of each study sample, using their routine HBV NAT assay, on three separate occasions.

**Prior to each assay run, samples 1-3 must be reconstituted with 0.5 mL of deionised, nuclease-free molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use.**

Dilutions should be prepared in the sample matrix normally used in the assay system (e.g. HBV DNA-negative human plasma).

Each sample must be extracted prior to amplification.

For each independent assay, study samples 1, 2, and 3 should be tested within the same assay run. Independent assays should be performed on separate days, using a fresh vial of each sample.

Below, are specific instructions for the dilution and testing of study samples, using either quantitative or qualitative assays.

#### For quantitative assays:

For each of three assays, participants are requested to test each sample, at a minimum of two serial ten-fold dilutions within the linear range of the assay (e.g.  $10^{-1}$  and  $10^{-2}$ ). If practicable, please test replicates of each dilution of each sample within the same assay run.

#### For qualitative assays:

For the first assay, participants are requested to test half- $\log_{10}$  serial dilutions of each sample, around the theoretical assay end-point, in order to determine the actual end-point (e.g.,  $10^{-2}$ ,  $10^{-2.5}$ ,  $10^{-3}$ ,  $10^{-3.5}$ ,  $10^{-4}$ ,  $10^{-4.5}$ ,  $10^{-5}$ ,  $10^{-5.5}$ ,  $10^{-6}$ ,  $10^{-6.5}$ ). It is important that the dilution series spans the limit of detection for the assay.

For the remaining assays, participants are requested to test the dilution at the assay end-point (limit of detection) determined in assay 1, and a minimum of two half- $\log_{10}$  serial dilutions either side of the pre-determined end-point (i.e., at least five dilutions in total). If practicable, please test replicates of each dilution of each sample within the same assay run.

*NB: Samples 1-3 contain approximately  $1 \times 10^6$  IU/mL HBV DNA when reconstituted in 0.5 mL nuclease-free water.*

### Reporting of results

The results of each assay (HBV concentration in IU/mL or qualitative result; positive / negative) and methodology used, should be recorded on the Result Reporting Form accompanying the samples. Where applicable, please also include the crossing point / threshold cycle for each result. Results should be returned to NIBSC **before the end of April 2011**, to allow sufficient time for statistical analysis and preparation of the final report for submission to the WHO Expert Committee for Biological Standardisation by July 2011.

The data should not be published or cited before the formal establishment of the standard by the WHO ECBS, without the expressed permission of the NIBSC study organiser.

All completed Result Reporting Forms should be returned electronically to Dr J Fryer: [Jacqueline.Fryer@nibsc.hpa.org.uk](mailto:Jacqueline.Fryer@nibsc.hpa.org.uk)

Alternatively, results may be faxed or mailed to:

Fax: +44 (0)1707 641366

Address: Dr J. Fryer, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG.

### Data analysis

All data from the study will be analysed at NIBSC. The analysis will assess the concentration of each sample, relative to each other, and the sensitivities of the different assay methods. Individual participants' data will be coded and reported "blind" to other participants during the preparation of the study report, and also in subsequent publications. Participants will receive a copy of the report of the study and proposed conclusions and recommendations for comment before it is further distributed. It is normal practice to acknowledge participants as contributors of data rather than co-authors in publications describing the establishment of the standard.

## Appendix 3

### Proposed instructions for use



**WHO International Standard  
3rd WHO International Standard for Hepatitis B Virus for Nucleic  
Acid Amplification Techniques**  
NIBSC code: 10/264  
Instructions for use  
(Version 1.00, Dated )

#### 1. INTENDED USE

The 3rd WHO International Standard for hepatitis B virus (HBV), NIBSC code 10/264, is intended to be used in the standardization of nucleic acid amplification technique (NAT)-based assays for HBV. The standard comprises a dilution of the Eurohep reference R1 (genotype A2, HBsAg subtype adw2) [1]. In HBV-negative pooled human plasma. The plasma is also negative for anti-HIV-1; anti-HCV; HCV RNA; and syphilis. The standard has been lyophilized in 0.5 mL aliquots and stored at -20°C. The material has been calibrated in International Units (IU), in parallel with the 2nd WHO International Standard for HBV, in a collaborative study involving 16 laboratories worldwide [2].

#### 2. CAUTION

**This preparation is not for administration to humans.**

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. 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 should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

#### 3. UNITAGE

This material has been assigned a unitage of 850,000 IU/mL (~5.93 log<sub>10</sub> IU/mL) when reconstituted in 0.5 mL of nuclease-free water.

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the vial content and was determined to be +/-1.29%.

#### 4. CONTENTS

Country of origin of biological material: United Kingdom.  
Each vial contains 0.5 mL of lyophilized plasma containing infectious HBV.

#### 5. STORAGE

Vials of lyophilized standard should be stored at -20 °C.

#### 6. DIRECTIONS FOR OPENING

Vials have a "flip-up" circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

#### 7. USE OF MATERIAL

**No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution**

The material should be reconstituted with 0.5 mL of deionized, nuclease-free molecular-grade water and left for a minimum of 20 minutes with

occasional agitation before use. The reconstituted material has a final concentration of 850,000 IU/mL.

The International Standard should be used to calibrate secondary reference materials, for example, by determining the equivalent concentration of secondary reference reagent being calibrated, against the International Standard, in parallel. The secondary reference reagent can then be assigned a concentration in terms of the IU. Once reconstituted, the International Standard should be diluted in the matrix appropriate to the material being calibrated, and should be extracted prior to HBV DNA measurement.

#### 8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. The stability of the material when reconstituted has not been specifically determined. Therefore, it is recommended that the standard is for single use only.

NIBSC follows the policy of WHO with respect to its reference materials.

#### 9. REFERENCES

1. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol. 1999;37:68-73.
2. Fryer JF, Heath AB, Wilkinson DE, Minor PD and the collaborative study group. Collaborative study to evaluate the proposed 3rd WHO International Standard for hepatitis B virus (HBV) for nucleic acid amplification technology (NAT)-based assays. WHO ECBS Report 2011; WHO/BS/2011.XXXX.

#### 10. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of the collaborative study participants.

#### 11. FURTHER INFORMATION

Further information can be obtained as follows:

This material:  
enquiries@nibsc.hpa.org.uk  
WHO Biological Standards:  
<http://www.who.int/biologicals/en/>  
JCTLM Higher order reference materials:  
<http://www.bipm.org/en/committees/jo/jctlm/>  
Derivation of International Units:  
[http://www.who.int/biologicals/reference\\_preparations/en/](http://www.who.int/biologicals/reference_preparations/en/)  
Ordering standards from NIBSC:  
[http://www.nibsc.ac.uk/products/ordering\\_information/frequently\\_asked\\_questions.aspx](http://www.nibsc.ac.uk/products/ordering_information/frequently_asked_questions.aspx)  
NIBSC Terms & Conditions:  
[http://www.nibsc.ac.uk/terms\\_and\\_conditions.aspx](http://www.nibsc.ac.uk/terms_and_conditions.aspx)

#### 12. 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 enquiries@nibsc.hpa.org.uk

#### 13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

#### 14. MATERIAL SAFETY SHEET

Physical and Chemical properties
----------------------------------



Physical appearance: Lyophilized powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains human plasma and infectious HBV	
<b>Toxicological properties</b>	
Effects of inhalation:	Avoid, contains infectious HBV
Effects of ingestion:	Avoid, contains infectious HBV
Effects of skin absorption:	Avoid, contains infectious HBV
<b>Suggested First Aid</b>	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
<b>Action on Spillage and Method of Disposal</b>	
Spillage of ampoule 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.	

**16. INFORMATION FOR CUSTOMS USE ONLY**

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.5 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

**15. 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 standards or reference 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 dependant 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 contract 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.

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