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**Report of the WHO collaborative study to establish the First
International Standard for detection of antibodies to Hepatitis B
core antigen (anti-HBc), human plasma**

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Abstract

A WHO Collaborative Study was undertaken to assess the suitability of a candidate International Standard (NIBSC 95/522) for detection of antibodies to Hepatitis B core antigen (anti-HBc) in diagnostic assays. Four different materials were evaluated: (A) the candidate anti-HBc International Standard preparation (NIBSC 95/522), (B) the current Paul-Ehrlich-Institut anti-HBc standard (PEI 82), (C) a low positive anti-HBc sample (PEI 108166) from a hepatitis B virus (HBV) infectious carrier without any other detectable HBV markers, and (D) a quality control panel (CBER Panel #11) of 10 members, prepared from individual donors.

Thirteen laboratories from 10 countries tested the materials using 20 different anti-HBc assays. The dilution range of candidate material A was within the dynamic measuring range of assays and the endpoint titers equivalent to the assay's cut-off ranged from 1:33 up to 1:622. As the PEI standard (Sample B) and unit has been used worldwide for many years, the antibody content of Sample A was expressed relative to this standard and unit (100 PEI units/ml). The overall potency of the candidate International Standard A was 50 IU/ml relative to Sample B. Sample C was detected positive by most assays but not consistently in all kits. This clinical sample may provide information about the sensitivity of anti-HBc assays. Similarly, some assays did not detect Panel D members that were specified to be positive. Kits which did either not detect or were weak positive in Sample C were the same that were weak in Panel D, and were also the kits which gave the lowest endpoint titers in the candidate material A and Sample B. Assessing the results quantitatively, low detection limits correlated significantly with positive results and high ratios for anti-HBc concentration measured in Samples C and D. One assay of the study, nevertheless, did not follow this correlation.

For Sample A, within-assay and inter-laboratory variability expressed by geometric coefficients of variation generally were $\leq 16\%$ and $\leq 33\%$ respectively. Stability studies with Sample A stored at $+4^\circ$ for over >4 years showed no loss of anti-HBc IgG activity and this indicates long-term stability when it is stored at -20°C .

The candidate International Standard (NIBSC 95/522) is proposed to be established as the 1st International Standard for the detection of human antibodies to HBcore antigen. This International Standard can be used for calibration of anti-HBc kit sensitivity, to calibrate secondary standards, and for quality control procedures. The potency is proposed to be 50 International Units per ampoule.

Sample C (PEI 108166) was found to be a suitable anti-HBc low reactive sample to show commutability of the proposed International Standard in the different assays and to provide an estimation of a minimum sensitivity of anti-HBc test kits.

Panel D (CBER panel #11) was found suitable as quality control reference panel and also supported commutability of the anti-HBc results.

1 Introduction

Hepatitis B virus (HBV) infection is a serious global health problem affecting two billion people worldwide [1]. The chain of HBV transmission is maintained partly by chronically infected HBV carriers with 350 million people worldwide [1]. Antibodies to hepatitis B virus core antigen (anti-HBc) are produced during acute HBV infection and persist lifelong so that HBV infection can be detected in chronic carriers even when negative for hepatitis B surface antigen (HBsAg) and HBV-DNA. Anti-HBc screening therefore has the potential to detect the majority of occult HBV infection. In fact, in the absence of anti-HBc testing HBV transmission occurred in blood recipients as well as after organ transplantations [2-8]. This has led some countries to improve blood safety by mandatory blood screening for anti-HBc, including Argentina, Brazil, France, Germany, Japan, Paraguay, Peru Uruguay, USA and Venezuela. In addition, anti-HBc may be the only positive serological marker in chronic HBV infections (isolated anti-HBc) which usually are low positive [9]. On the other hand, anti-HBc persists also in those who have cleared the virus so that isolated anti-HBc can be indistinguishable from serological profile of resolved HBV infection not easy to differentiate from potential false positive reactions in particular in low prevalence HBV countries [10]. Therefore high quality anti-HBc tests with high sensitivity and specificity are required.

The PEI anti-HBc standard has been used by many manufacturers worldwide and the sensitivity of many assays and the anti-HBc content of samples are expressed as PEI units/ml. This material has also been used for control of sensitivity of anti-HBc assays, for manufacturer's quality control in final product release testing and for official batch testing by national authorities. At the meeting of the Expert Committee for Biological Standardization (ECBS) at the World Health Organization (WHO) in October 2005 a project proposed by the Paul-Ehrlich-Institut (PEI) was endorsed to establish a WHO International anti-HBc Standard. In addition, PEI proposed to include an anti-HBc low positive material from an HBV infectious blood donor in the study (PEI 108166) in order to see whether the results with the proposed International Standard (NIBSC 95/522) are commutable with the results for a clinical sample and whether kits are sensitive enough to detect low positive anti-HBc levels. The National Institute for Biological Standards and Control (NIBSC) in UK expressed their willingness to support the project by providing the material A and stability samples and to participate in the study.

The various materials were tested in a feasibility study with 14 different anti-HBc assays conducted at PEI in 2006. The outcomes demonstrated that both, the PEI standard 82 and the NIBSC sample 95/522, were comparable and appeared to be adequate for anti-HBc calibration. Because of the limited number of vials available of the PEI 82 material, NIBSC 95/522 material which is available in sufficient volume has been proposed as a candidate to establish the anti-HBc WHO International Standard. The study also revealed that PEI 108166 was a challenging sample to assess whether kits are sensitive enough.

In a meeting of the WHO Collaborating Centres for biological standardization in January 2007 [11] the group agreed on the conclusions from the feasibility study. The group further recommended to include anti-HBc reference panel (#11) composed of 10 panel members with defined anti-HBc specifications from the Center for Biologics Evaluation and Research (CBER/FDA, USA) kindly provided by Dr. R. Biswas.

The aims of the collaborative study are to assess suitability of the candidate International Standard for use in anti-HBc diagnostic assays from manufacturers around the world and to compare the results with the other anti-HBc materials in order to support commutability.

For the time being, an international conventional reference measurement procedure for anti-HBc does not exist and this measurand is not traceable to International System of Units (SI) of quantity.

2 Materials

2.1 Sample A: Candidate Material NIBSC 95/522

This is a freeze dried pool of anti-HBc positive plasma collected from UK blood donors in the early 1990s coded NIBSC 95/522. 1 ml aliquots were freeze dried at NIBSC in 1995 following documented procedures. No stabilizers or preservatives were added. 2700 freeze dried ampoules are available. The material is high positive for anti-HBs (>1000 IU/ml). Other HBV markers including HBsAg, HBeAg, anti-HBc IgM, and anti-HBe are negative. It has residual moisture of 0.31% and a fill CV of 0.49%. 95/522 had been tested and found negative for anti-HIV 1/2 and anti-HCV. The freeze-dried preparation was tested and found negative for HCV RNA. Participants were requested to reconstitute this material in 1ml distilled water.

2.2 Sample B: PEI anti-HBc Standard PEI 82

This serum has been collected by the Paul-Ehrlich-Institut, Langen/Germany in 1982 from human blood donors and stored as liquid at -70°C in glass ampoules. Each ampoule contains 0.5 ml and has an assigned anti-HBc unitage of 100 PEI units per ml. The material is positive for HBsAg, has no detectable HBV-DNA (<12 IU/ml) and is negative for all other serological HBV markers including anti-HBc IgM, anti-HBs, HBeAg and anti-HBe. The material was found negative for anti-HIV 1/2 and anti-HCV.

2.3 Sample C: PEI 108166

This material is a pool of four consecutive plasma donations from one donor who caused transmission of HBV via labile blood components. The four single donations were tested with 14 different anti-HBc assays and compared with the results of the pool. The anti-HBc result in the pool corresponded to the expected mean value of the four single donations. 600 ml are available in 0.5 ml aliquots. The serological profile was anti-HBc low positive without other serological HBV marker (isolated anti-HBc), HBsAg negative and HBV-DNA initially negative and low positive (<12 IU/ml HBV-DNA) after retesting. The material is liquid and stored frozen at -70°C without preservative added. The material is negative for anti-HIV 1/2, anti-HCV and HCV-RNA. Corrigendum: In contrast to the study plan sent out to the participants, the material does not contain sodium azide.

2.4 Sample D: CBER Panel #11

Is a reference panel from the Center for Biological Evaluation and Research (CBER, FDA, Rockville, MD, USA) for lot release testing of licensed anti-HBc test kits. The panel was manufactured from human plasma units and consists of 8 reactive and 2 non reactive members. All reactive members are dilutions from individual reactive donors. For an assay kit to be considered acceptable by the FDA, Panel members 1, 2, 7 and 8 are required to be positive by all anti-HBc tests, panel member 5 and 9 contain anti-HBc and may be detected positive, panel member 4 and 10 contain anti-HBc but are assigned not detectable by current technology, panel members 3 and 6 must be negative. The positive and weak positive panel members are negative for anti-HBc-IgM (tested at PEI). The panel is stored at 2-8 °C and thiomersal has been added as a preservative.

3 Design of study

Participants received the samples and an accompanying study plan. They were asked to report the specifics of the assays performed, and to submit the raw data along with the corresponding cut-off value and the values for the dilution matrix. The study plan included the following:

- From each of the samples A and B seven 2-fold dilutions should be prepared and tested in the range as shown in the result's sheets, i.e. for sample A from 1:10 to 1:640 and for sample B from 1:25 to 1:1600 which were the dilution ranges found adequate by the former feasibility study. Samples A and B were requested to be tested in each anti-HBc assay in triplicate independently

- on 3 three different days by using for each day a fresh ampoule.
- The dilution matrix normally in use in the participant's laboratory should be used. Normal human serum (NHS) negative for anti-HBc is appropriate. If NHS is not available, phosphate buffered saline (PBS) with bovine serum albumin (BSA) may be used. The dilution matrix should be tested in triplicate as a control.
- Samples C and D should be tested neat without any dilution in triplicates on one day only.
- Sample C should be centrifuged before testing: 3000 g 15 minutes.
- All samples (A-D) should be tested concurrently in one test run.

3.1 Participants

Seventeen laboratories were contacted to participate in the Collaborative Study. Fifteen laboratories answered and notified to participate. Two laboratories received material but did not respond. Thirteen laboratories returned results back. The participants were from 10 countries including Australia (1), Brazil (1), China (1), France (2), Germany (1), Japan (1), Korea (1), Netherlands (1), UK (1) and USA (3). Participating laboratories are listed in Appendix 1 and were assigned a laboratory code number (1-13) not necessarily in the order listed.

3.2 Assay methods

Twenty test kits were used in the laboratories of the participants. The assays used are listed in **Table 1** together with the specific characteristics of the assays and given code 1-20. The range of anti-HBc assays used include, (i) competitive, indirect, sandwich test formats, (ii) with or without reductant pre-treatment, (iii) conducted manually, automated or as rapid assay. Assay 20 is a modification of a current assay which is still under development.

All assay's anti-HBc values were considered positive at ≥ 1 and negative at < 1 . For this, sample to cut-off values (s/co) of the competitive anti-HBc assays were transformed to the reciprocal cut-off to sample (co/s) values and for one assay the index value at 0.5 was transformed to s/co 1 by multiplication. One visual read assay (code 11) could not be included in numerical calculations.

3.3 Statistical methods

Statistical analysis was performed at PEI based on the raw data sent by the participants. The data were read from the results sheets as recorded by each participant.

The detection limits with the diluted materials Sample A (NIBSC 95/522) and Sample B (PEI 82) were calculated by linear interpolation at the intercept of the dilutions series with the assay's cut-off. Also transformation of assay's signals to U/ml was done by linear interpolation. Detection limits were also calculated by linear regression of the dilution curve but because of non linear dose response in some anti-HBc assays, it was not applicable to all assays and therefore not used. Geometric mean values (GMV) including their 95% confidence intervals (CI) were used to describe each assay. The geometric coefficient of variation (GCV) was used to describe the intra- and inter-laboratory variation [12]. Only values > 0 were considered in the evaluations.

In order to estimate the potency of Sample A relative to Sample B two approaches were used:

1. For each assay (within the different laboratories) the potency was estimated by the ratio of the GMV for this assay.
2. The potency was also estimated by means of a parallel line model [13], using either ln-transformed response data or a four parametric logistic function. The assumption of parallelism held for 84% of tests, linearity could be shown for 66% of tests, parallelism and linearity was found in 58% of tests.

As the PEI standard and unit has been in use for many years, the antibody content of Sample A was expressed relative to this standard. Potencies were expressed in International Units (IU) relative to the Sample B (PEI 82) which has an assigned unitage of 100 PEI U/ml.

Spearman's rank correlation coefficient was used to assess the correlation between analytical sensitivity with Samples A and B and anti-HBc detection in Samples C and D. Analyses were performed using SAS version 9.1 [14], R version 2.6.1 [15] and CombiStats version 4.0 [16].

4 Results

4.1 Data received

The majority of participants followed the study plan attached with the samples, with the following exceptions:

- Laboratory 5 with assay 9 tested 2 replicates only for each of the 3 days.
- Laboratories 6 and 7 tested sample A and B only for one day without days 2 and 3.
- Laboratory 9 tested one assay with sample A at days 2 and 3 with three further dilutions (1:1280, 1:2560 and 1:5120) and for sample B with one dilution more (1:3200).
- Laboratory 13 applied for two assays in the study but could return results for one assay only.

4.2 Results and Discussion

4.2.1 Candidate Material A (NIBSC 95/522) and Sample B (PEI 82)

The geometric means values (GMV) of endpoint titers of each assay, equivalent to the assay's cut-off of candidate material A and Sample B are displayed in **Table 2** including the GMVs of sensitivity relative to PEI 82 which has an assigned unitage of 100 PEI U/ml. With Sample A the majority of tests (n=22) reached endpoint titers at 1:70 and 1:150, 8 assays had endpoint titers of <1:70, 7 assays had endpoint titers of 1:150 - 1:310 and 2 assays had endpoint titers of 1:620. With Sample B dilution capacity was about 2-fold higher: most tests (n=23) being positive up to endpoint titers of 1:100 to 1:300, one assay had an endpoint titer of 1:40, 6 assays had an endpoint titer of 1:70 - 1:100 and 6 assays endpoint titers of 1:300 - 1:660 and 3 assays found the intercept at 1:900 - 1:1000. Overall, the dilution ranges for samples A and B were in the dynamic measuring range of assays. Within an individual assay, Sample A and B gave comparable dose responses. Distribution of the endpoint titers for Samples A and B among all assays and laboratories is shown in **Figure 1**. Potency estimates of the candidate material A were calculated relative to Sample B which has an assigned unitage of 100 PEI units per ml and expressed as the GMV ratios of the endpoint titers (**Table 3**). The overall potency was 49.8 U/ml (95%-CI 44.2 - 56.1 U/ml). Two assays differed: assay 1 had a lower potency of 22.8 U/ml (mean of 2 labs) while assay 8 had a potency of 73.3 U/ml. The overall potency of the candidate material A excluding these two assays was 51.1 U/ml (95%-CI 45.3 - 57.5 U/ml). **Figure 2** shows the distribution of the potency among all assays. The overall potency calculated by means of parallel line assay (PLA) was 46.8 U/ml (95%-CI 43.7 - 50.2 U/ml). Excluding assays 1 and 8 the overall potency was 47.9 U/ml (95%-CI: 45.7 - 50.2 U/ml) similar to the potencies based on the GMV ratio. Because some assays did not fulfill the assumptions of the parallel line assay, the GMV ratios rather than the potencies calculated by the PLA approach appears to be more appropriate. The potencies based on the GMV ratio of the candidate material A relative to Sample B (PEI 82) for each assay are also shown in a histogram (**Figure 2**). Each box represents the laboratory potency estimate relative to Sample B for an individual kit, and the boxes are labeled with the laboratory code number, and a code representing the kit used. The graph shows the outlying potency of assays 1 and 8.

4.2.2 Sample C (PEI 108166)

For each laboratory and assay the GMV for Sample C are displayed in **Table 4**. The majority of the assays were positive but two assays (code 1 and 7) were negative each in one test and 3 other assays (4, 8, and 19) were weak positive at the borderline of the cut-off (≤ 1.5). For these assays it is likely that they will not identify Sample C consistently. For example assay 4 was negative in

3/5 tests in the feasibility study before. By contrast assays 3, 6, 12, 13, 16 and 17 showed high signals of >5 and for assays 2, 5, 9, 10, 14, 15, 18, 20 signals were between >1.5 and 5. Rapid assay 11 was positive as well. The mean (GMV) anti-HBc content of Sample C in all assays was 1.70 PEI U/ml. The range for most assays (n=17) was 1.1 to 3.0 PEI units/ml, while the smallest and largest values differed more, i.e. assays 1 and 4 were 0.25 PEI U/ml and 0.66 PEI U/ml and assay 5 was 4.10 PEI U/ml. Without the two extremes (assay 1 and 5) the GMV anti-HBc content in Sample C is 1.8 PEI U/ml. Sample C is of particular interest as this kind of sample frequently causes discrepant results between different anti-HBc assays as in this study. Therefore Sample C can serve as a critical sample for estimation of the sensitivity of anti-HBc assays. Assays that will not detect Sample C may indicate insufficient clinical sensitivity.

4.2.3 Sample D (CBER Panel #11)

For each laboratory and assay, the GMV for Samples D1-10 are displayed in **Table 4**. As indicated in Section 2.4, for an assay kit to be considered acceptable by the FDA, Panel members 1, 2, 7 and 8 are required to be positive by all anti-HBc tests, panel member 5 and 9 contain anti-HBc and may be detected positive, panel member 4 and 10 contain anti-HBc but are assigned not detectable by current technology, panel members 3 and 6 must be negative. All assays correctly detected D1-D2 positive and D3/D6 negative. In case of D7 and D8 however, some assays did not meet the required positive result: in D7 assay 1 was negative in one lab and in D8 assays 1, 7, 14 and 15 were negative in at least one test. In addition, with D8 and D7 some assays were weak positive only close to the cut-off (<1.5): in D7 assay 7 and 10 and in D8 assays 2, 4, 12, 15, 17, and 18. By contrast, other assays also scored Panel members D4-D5 and D9 as reactive/positive; assay 16 was positive in D4, assays 3, 6, 11, 15 and 16 were positive in D5 and assays 3, 6, 11, and 16 were positive in D9. Sample D10 was negative in all assays. By this, sensitivity of kits could be arranged according to the positive score in the various Panel D members. Interestingly, the assays that were negative or weak positive in Panel D were also the same that were weak or negative with Sample C.

4.2.4 Correlation of Detection Limits in Samples A and B with anti-HBc measured in Samples C and D

Due to the qualitative design of the anti-HBc assays there is no quantification and absolute detection limit given for anti-HBc. The results were therefore analysed quantitatively for correlation of sensitivity in Samples C and D relative to analytical sensitivity in Samples A and B. As a matter of fact kits which were weak in detecting Sample C and Panel D, in general, were also the kits which gave the lowest analytical sensitivity in the candidate material A and Sample B or conversely assays that scored more positive in Samples C and D were also those that had the highest analytical sensitivity in Samples A and B. This is shown in **Table 4** where the assays are sorted according to the detection limit (U/ml) in Samples A and B. High analytical sensitivity in Samples A and B correlated with positive score in Sample C and Panel D. After transforming the assay's signals into U/ml relative to PEI 82 (100 PEI U/ml) low detection limits resulted in higher ratios for the anti-HBc concentration (U/ml) measured in Samples C and D (**Table 5**). Correlation was statistically significant (**Table 6**) relative to both, Sample A and B and for anti-HBc signals as well as the ratio of the anti-HBc concentration (p-value <0.001 for C, D1, D2, D4, D5, and of 0.003 for D9) though for Samples D7 statistical significance was for the signals only and for D8 there was no statistical significance. This appears to be due to a narrow measuring range in assays 5, 8, 14 and 19 such that low increase in assay's signal above the dilution range studied (>4 PEI U/ml) reached disproportional rises, in anti-HBc concentrations though the actual signal changes little. Assays with high ratios for anti-HBc measured provided more efficacy in detecting anti-HBc positive. The results of this study suggest that an assigned detection limit of at least <1.4 U/ml with Sample A (<0.7 U/ml with Sample B) appeared to be the limit for discrimination for sensitive anti-HBc detection, i.e. assays with detection limits of >1.4 U/ml in general resulted in ratios for anti-HBc

concentration of <1 U/ml (<2 U/ml with Sample B) and were more likely to be false negative. By contrast, assays with detection limits of <1.4 U/ml had ratios >1 U/ml and were more likely to be positive. One assay visually read (coded 11) was not included in the tables and graphs as there are no continuous values available but showed correlation as well between detection limit and anti-HBc detection (Table 4). Correlation fits with published reports, suggesting to increase sensitivity to 0.3 to 0.4 PEI U/ml (corresponding to about 0.6 to 0.8 U/ml with Sample A) in order to detect true low-level anti-HBc reactive samples [17] and showing correlation with some assays of this study for the detection limit (PEI U/ml) with score in positive clinical samples [18-20]. Correlation on the other hand was not fully consistent, i.e. assay 5 had higher anti-HBc ratios, and assay 4 had lower anti-HBc ratios than expected from the detection limits. Detection limit with assay 4 diluted in PBS has possibly been overestimated due to a matrix effect (see 4.2.6 below). Assay 1, in any case, differed significantly as it showed one of the highest analytical sensitivities (0.62 with Sample A and 0.15 U/ml with Sample B) but no reflection of it in the other samples C-D. This assay also had significant different potency for Sample A as discussed above in section 4.2.1. Therefore, this assay was neglected when assessing for sensitivity correlation (see below section 4.2.6). The only obvious difference of this assay from other assays is the sandwich test format, but whether this is of importance remains unclear. The HB core antigen (HBcAg) of the assays does not appear to have been significant in this study. All kits utilize recombinant HBcAg expressed mostly in *E.coli* [21-22] but also in yeast [23]. Moreover, different kits using the same rHBcAg resulted in different endpoint titers and otherwise assays from different manufacturers reacted similar.

4.2.5 Intra-laboratory and Inter-laboratory variability

Intra-laboratory (within-assay) and Inter-laboratory (between labs in the same assay) variation with Samples A and B are displayed in **Table 7**. Intra-laboratory variability at the assay's cut-off was mostly <15% GCV but in 5 of 39 tests with Sample A >15%: GCV 29.83% with assay 1, 17.65% with assay 2, 25.27% with assay 16, and 20.91%, and 19.77% with assays 18. This appears to be laboratory based since tests with the same assays in other labs revealed variation below <15%: GCV 6.99% (assay 1), 2.60 - 10.32% (assay 2), 5.37 and 10.60% (assay 16), 7.89 and 12.94% (assay 18). Inter-laboratory variability of Samples A and B was in the range of 12% to 33%. There were two cases of poor reproducibility: (i) with assay 16 between labs 5, 9, 12 there was GCV 59.22% and (ii) with assay 7 between labs 9 and 12 there was GCV 57.17%. Variation with assay 16 was due to a matrix effect which led to significant change in sensitivity (see below 4.2.6). Inter-lab GCV % between the other labs 5 and 12 without lab 9 was 4.7% only. Therefore the value of lab 9 was not used for calculation of the Inter-laboratory variation. Poor reproducibility with assay 7 was possibly, at least in part, due to optional assay methods (see below 4.2.6).

Intra-laboratory and Inter-laboratory variation with Samples C and D are displayed in **Table 8**. Intra-laboratory variability with Samples C and positive members of Panel D was generally also GCV <15% corresponding to the normal imprecision of assays. Some assays showed poor repeatability in some D Panel members (assay 3 and 8 in D2, assay 13 in D1; D2, D6, D7, D8) due to dose response outside of the assay's dynamic measuring range. It has to be mentioned that Intra-laboratory variation with Sample C and Panel D is based on 3 replicates only, thus, being of limited significance only. Inter-laboratory variability with Samples C-D mainly was also in an acceptable low range of <30% but with assays 1, 6, 7, 16 there was poor reproducibility between laboratories. As this was consistent across all Samples C-D it appears that poor inter-laboratory reproducibility was assay-based rather than sample-based. In fact the results for assays 6 and 16 seem, at least partly, due to the high signals outside the dynamic measuring range and for assay 7 due to different optional assay methods (manual or automated). For assay 1 there is no obvious explanation but it appears that reproducibility at high signals (Samples D1, D2) was good and less at low values close to the cut-off (Samples C, D7, D8).

In any case, inter-laboratory % GCVs figures have to be interpreted with caution as they are calculated mostly on the basis of only 2 to 3 tests per assays, maximum 4 and 5 tests in two assays. Nevertheless, the reproducibility results with the various materials in the anti-HBc assays studied appears to be in an expected range and do not indicate a deterioration of the quality of the Samples studied.

4.2.6 Special findings and results differing from the overall conclusion

1. Assay 1 showed a significant lower potency for candidate material A (22.8 U/ml) than the other assays (mean 51.6 U/ml). This assay also did behave different as there was no correlation between high analytical sensitivity in Samples A and B with anti-HBc detection in Samples C and D. The results of this assay 1 therefore were reviewed separately from the other assays.

2. Assay 16 in laboratory 9 resulted in significant change of sensitivity. Detection limit in laboratory 9 for Sample A was 0.16 U/ml and 0.09 U/ml in Sample B compared to the mean 0.36 U/ml and 0.19 U/ml in labs 5 and 12. This was due to a matrix effect as confirmed in a separate test at PEI (not shown). Still, the negative matrix tested as a control in lab 9 was negative and there was linear dose response in the extended dilutions. Therefore, values were not taken out of assessment but the test was not considered for calculation of inter-laboratory variation.

As a remark here matrix effects were detected later after the study for assays 4 and 6 as well (data not shown). In assay 4, endpoint titers with normal human plasma as diluent were consistently about 40% lower than with PBS/BSA. Detection limit for assay 4 estimated in normal human plasma would correlate more consistent with the results for detection of anti-HBc in Samples C and D in the present study. Similarly in assay 6 PBS/BSA led to significant higher titers compared to normal human plasma (not affected in this study).

Phosphate buffered saline (PBS) with bovine serum albumin (BSA) as diluent therefore was not always applicable. Normal (negative) human serum or plasma seems to be the more appropriate dilution matrix for all anti-HBc assays.

3. Laboratory 5 observed clot formation in Samples A and B using human serum as diluent.

4. With assay 7 there was another significant difference in sensitivity between laboratories 9 and 12. A matrix effect seems to be improbable as the matrix of the two laboratories was similar. One point, at least, could be difference in the test procedure applied by the two laboratories, i.e. manually and automated.

4.3 Stability

Ampoules of Sample A were incubated at NIBSC from 24.04.2001 to 30.05.2005 at 4°C, 20°C, 37°C, 45°C. The ampoules were tested at PEI on one assay kit (coded 2) in the same scheme as the collaborative study, i.e. 3 replicates on 3 days opening an ampoule for each day. As baseline the activity of fresh reconstituted material was taken. There was a very slight activity loss for the ampoules incubated at 4°C of 1.5 % and at 20°C of 7% based on the GMV ratio. At 37°C there was still 50% activity and only after 45°C activity was completely lost. However these samples were difficult to reconstitute and this may have been responsible for some loss of activity. In terms of relative potency by parallel line assay (PLA) the activity loss was close to that calculated by linear interpolation (GMV ratio), i.e. 3.1 % at 4°C, 7.42% at 20°C, and 52.1% at 37°C. The difference at 4°C of GMV 1.5 %, 3.1 % by PLA respectively, may be in partly in the range of the background error. The results are displayed in **Table 9**. Stability was also tested for the reconstituted material A. Reconstituted Sample A was stored 2 weeks at +2-8°C, frozen at -70°C, stored for 200 days, freeze/thawed 2 times, and tested in assay 2. The results are shown in **Table 10**. There was no loss of activity of the reconstituted material compared with the results obtained directly after reconstitution in the same assay (coded 2).

Overall, the results of the incubated ampoules indicate that after more than 4 years and at elevated temperatures up to 4°C there is almost no activity loss with anti-HBc in the candidate material A. The candidate standard is therefore likely to be highly stable when stored at the

recommended temperature of -20°C and is of adequate stability to serve as an International Standard. In addition, the candidate International Standard is stable after reconstitution as liquid at $2-8^{\circ}\text{C}$, for at least 14 days and after freezing at -70°C for at least 200 days. Ongoing stability studies will be undertaken periodically.

5 Conclusions and Proposals

Sample A, the anti-HBc candidate International Standard (NIBSC 95/522), was assessed relative to Sample B (PEI 82) which is widely used and has an assigned unitage of 100 PEI units per ml. The dilution capacity of the candidate material A covered the dynamic measuring range of the anti-HBc assays. The potency of the candidate material A was 50 U/ml. Analytical sensitivity determination with candidate material A, in general, provided efficacy for estimation of sensitivity performance, i.e. the lower the detection limit the higher the ratio for the anti-HBc concentration detected in samples C and D and the more likely the assays were positive in Samples C and D. Intra-assay and inter-laboratory variation for the candidate material A did not indicate unsuitable sample conditions. Adequate stability when stored at -20°C has been demonstrated. The candidate International Standard A therefore was found suitable for calibration of anti-HBc kit sensitivity, to calibrate secondary standards, and for quality control procedures, e.g. in batch release testing.

Thus, Sample A is proposed to be established as the 1st International Standard for detection of antibodies to Hepatitis B core antigen (anti-HBc) with an assigned unitage of 50 International Units per ampoule. The Paul Ehrlich Unit is therefore now equivalent to the IU. However the International Standard is the higher order standard.

The proposed unitage does not carry an uncertainty associated with its calibration. The only uncertainty is therefore derived from the variability of the dry fill weight of the ampoule content which had a coefficient of variation of 0.49%.

The results for Sample C (PEI 108166) showed commutability to the proposed International Standard in the different assays. It was assigned an average anti-HBc value of 1.8 IU/ml relative to the candidate International Standard (NIBSC 95/522). This clinical sample may provide information about the sensitivity of anti-HBc assays. Thus, Sample C will be used within PEI as an additional reference material to estimate a minimum level of sensitivity in anti-HBc test kits.

6 Comments from participants

All participants responded to the request for comments on the report. All comments of the participants were addressed and corrections were performed where appropriate. On the proposal and suitability of the material, six of the 13 participants did not provide a specific opinion. Six participants agreed with the proposal that NIBSC 95/522 (Sample A) should be established as the 1st IS for detection of anti-HBc with a potency of 50 IU/ampoule and that PEI 108166 (Sample C) can be an additional reference material to be used as sensitivity index.

One participant questioned the use of data generated using qualitative assays on dilutions of samples. The report has been amended to respond to this participant. Although this is the first collaborative study to assess the suitability of a candidate International Standard for anti-HBc, a standard has been available from the PEI for many years and found useful by kit manufacturers and laboratories.

Acknowledgements

We are grateful to NIBSC for the donation of the material 95/522 used for the candidate standard and also to CBER for providing Panel #11. Special thanks to Dr. M. Nübling PEI for providing the plasma donations for Sample C (PEI 108166). We are also grateful to all of the participants for taking part in the study. We also thank Dr. Ana Padilla for critical review of the report.

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Table 1: Anti-HBc Assays used in the Collaborative Study and Characteristics of the Anti-HBc Assays

| Assay Code | Product name | Manufacturer | Assay procedure | Test format | Antigen | Conjugate | Ig-class | Measuring ring | Pretreatment |
|------------|---|--------------------------------------|-----------------|-------------|---------|---------------------------|----------|----------------|--------------|
| 1. | ADVIA Centaur HBcT | Siemens Med. Solutions ¹⁾ | Centaur | Sandwich | rHBcAg | rHBcAg | IgG/IgM | ECL | No |
| 2. | Architect Anti-HBc | Abbott GmbH Co. KG | Architect | Indirect | rHBcAg | Anti-human IgG/IgM | IgG/IgM | CMIA | No |
| 3. | AxSYM Core 2.0 | Abbott GmbH Co. KG | AxSYM | Competitive | rHBcAg | MAb rHBcAg | IgG/IgM | MEIA | DTT |
| 4. | Bioelisa anti-HBc | Biokit S.A. | Manually | Competitive | rHBcAg | PAb rHBcAg | IgG/IgM | ELISA | No |
| 5. | Corzyme | Abbott Diagnostics | Manually | Competitive | rHBcAg | PAb rHBcAg | IgG/IgM | ELISA | No |
| 6. | Elecsys Anti-HBc | Roche Diagnostics GmbH | Elecsys | Competitive | rHBcAg | MAb rHBcAg | IgG/IgM | ECL | DTT |
| 7. | Enzygnost Anti-HBc monoclonal | Siemens Med. Solutions ²⁾ | BEPI/III | Competitive | rHBcAg | MAb rHBcAg | IgG/IgM | ELISA | No |
| 8. | Genedia Anti-HBc ELISA Plus | Green Cross Corp. | BF:P2000 | Competitive | rHBcAg | Human anti-HBc | IgG/IgM | ELISA | No |
| 9. | Hepanostika anti-HBc Uniform | bioMérieux SA | Manually | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | ELISA | No |
| 10. | Immulite 2000 anti-HBc | Siemens Med. Solutions ³⁾ | Immulite | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | CLFIA | No |
| 11. | Immuncomb II HBc IgG | Organics Ltd. | Rapid | Indirect | rHBcAg | Anti-human IgG | IgG | EIA | No |
| 12. | IMx Core | Abbott GmbH Co. KG | IMx | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | MEIA | DTT |
| 13. | Anti-HBc EIA | Shanghai Kehua Co. Ltd. | Manually | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | EIA | No |
| 14. | Lumipuls Presto HBc Ab-N | Fujirebio Diagnostics Inc. | Lumipuls Presto | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | CLFIA | No |
| 15. | Monolisa anti-HBc Plus | Bio-Rad Laboratories | Manually | Indirect | rHBcAg | MAB anti-human IgG+IgM | IgG/IgM | ELISA | No |
| 16. | Murex anti-HBc | Abbott/Murex Biotech Ltd. | Manually | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | ELISA | No |
| 17. | Ortho anti-HBc | Ortho Clinical Diagnostics | Manually | Indirect | rHBcAg | MAB anti-human IgG+IgM | IgG/IgM | ELISA | No |
| 18. | PRISM HBcCore | Abbott GmbH Co. KG | PRISM | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | ChLIA | Cysteine |
| 19. | ST AIA-Pack HBcAb | Tosoh Bioscience | Tosoh | Competitive | rHBcAg | MAB rHBcAg | IgG/IgM | EIA | No |
| 20. | Architect Anti-HBc II (ex-US)/Core (US) ⁴⁾ | Abbott Diagnostics | Architect | Indirect | rHBcAg | MAB anti-human IgG/IgM | IgG/IgM | CMIA | Reductant |

Footnotes:

- 1) formerly Bayer Health Care
- 2) formerly Dade-Behring Marburg
- 3) formerly DPC Biermann
- 4) reformulated Architect Anti-HBc assay in development not yet on the market as a current assay.

rHBcAg = recombinant HB core antigen; Ig = immunoglobulin; MAb = monoclonal antibody; PAb = polyclonal antibody; DTT = dithiothreitol; ECL = electrochemiluminescence; EIA = Enzyme immunoassay; MEIA = microparticle enzyme immunoassay; ELISA = enzyme linked immunosorbent assay; CMIA = chemiluminescent microparticle immunoassay; ChLIA = chemiluminescent immunoassay; CLFIA = enzyme linked immunoassay; CMIA =

Table 2: Mean Endpoint Titers and U/ml with Sample A and Sample B

| Assay code | Laboratory code | Sample A (95/522 NIBSC) | | | Sample B (PEI 82) | | |
|------------|-----------------|-------------------------|-------------------|----------------------|--------------------|-------------------|----------------------|
| | | U/ml ¹⁾ | GMV ²⁾ | | U/ml ¹⁾ | GMV ²⁾ | |
| | | | Titer | 95%-CI ³⁾ | | Titer | 95%-CI ³⁾ |
| 1 | 7 | 0.55 | 182.7 | 154.5 - 216.1 | 0.11 | 899.7 | 762.1 - 1062 |
| 1 | 12 | 0.64 | 155.7 | 127.4 - 190.3 | 0.16 | 615.8 | 488.4 - 776.5 |
| 2 | 10 | 1.36 | 73.6 | 68.3 - 79.41 | 0.72 | 139.4 | 128.1 - 151.6 |
| 2 | 12 | 1.49 | 67.3 | 66.0 - 68.68 | 0.84 | 119.4 | 115.8 - 123.2 |
| 2 | 13 | 0.91 | 109.5 | 96.6 - 124.0 | 0.48 | 209.4 | 197.9 - 221.6 |
| 3 | 1 | 0.33 | 306.6 | 291.2 - 322.9 | 0.15 | 664.3 | 644.0 - 685.3 |
| 3 | 7 | 0.58 | 172.4 | 140.2 - 211.9 | 0.25 | 407.7 | 336.1 - 494.6 |
| 3 | 12 | 0.46 | 219.5 | 208.1 - 231.6 | 0.21 | 467.5 | 444.3 - 491.9 |
| 4 | 12 | 1.15 | 87.1 | 80.8 - 93.94 | 0.55 | 182.0 | 168.1 - 197.1 |
| 5 | 1 | 2.79 | 35.9 | 33.7 - 38.28 | 1.40 | 71.4 | 62.5 - 81.65 |
| 5 | 4 | 2.37 | 42.2 | 37.7 - 47.12 | 1.37 | 72.8 | 62.3 - 84.99 |
| 6 | 7 | 0.76 | 131.6 | 127.7 - 135.6 | 0.37 | 271.7 | 228.0 - 323.9 |
| 6 | 12 | 0.89 | 112.7 | 106.8 - 119.0 | 0.48 | 207.3 | 196.4 - 218.9 |
| 7 | 9 | 2.91 | 34.4 | 30.4 - 37.97 | 1.45 | 68.8 | 63.6 - 74.38 |
| 7 | 12 | 1.55 | 64.4 | 60.8 - 68.21 | 0.77 | 130.0 | 124.0 - 136.3 |
| 8 | 7 | 3.03 | 33.0 | 31.5 - 34.51 | 2.22 | 45.0 | 34.9 - 57.95 |
| 9 | 5 | 1.19 | 84.0 | 65.4 - 107.9 | 0.55 | 181.4 | 167.9 - 196.0 |
| 10 | 12 | 2.18 | 45.8 | 43.2 - 48.50 | 1.06 | 94.6 | 90.4 - 99.05 |
| 11 | 2 | 0.16 | 619.9 | 575.8 - 667.3 | 0.10 | 1048.3 | 897.0 - 1225 |
| 12 | 6 | 0.71 | 141.7 | 134.2 - 149.5 | 0.33 | 302.3 | 295.1 - 309.6 |
| 12 | 12 | 0.87 | 115.0 | 107.8 - 122.6 | 0.43 | 234.4 | 214.5 - 256.1 |
| 13 | 10 | 1.38 | 72.3 | 69.1 - 75.65 | 0.80 | 124.4 | 107.6 - 143.8 |
| 14 | 8 | 2.91 | 34.4 | 33.0 - 35.97 | 1.41 | 71.0 | 68.5 - 73.50 |
| 15 | 2 | 1.09 | 91.8 | 88.8 - 94.96 | 0.64 | 156.7 | 150.2 - 163.5 |
| 15 | 5 | 1.10 | 91.3 | 87.3 - 95.44 | 0.49 | 202.2 | 192.5 - 212.3 |
| 15 | 7 | 0.76 | 131.6 | 104.2 - 166.3 | 0.33 | 299.7 | 289.4 - 310.3 |
| 15 | 11 | 0.68 | 146.6 | 133.7 - 160.8 | 0.36 | 279.0 | 251.7 - 309.3 |
| 15 | 12 | 0.95 | 105.0 | 100.6 - 109.6 | 0.50 | 199.4 | 190.9 - 208.3 |
| 16 | 5 | 0.37 | 271.4 | 260.7 - 282.6 | 0.18 | 564.3 | 531.7 - 599.0 |
| 16 | 9 | 0.16 | 626.3 | 526.7 - 744.7 | 0.09 | 1056.5 | 805.4 - 1386 |
| 16 | 12 | 0.35 | 289.6 | 268.0 - 312.9 | 0.20 | 506.2 | 425.6 - 602.2 |
| 17 | 4 | 0.92 | 108.3 | 103.3 - 113.6 | 0.54 | 185.6 | 167.5 - 205.6 |
| 17 | 12 | 1.13 | 88.7 | 82.8 - 95.06 | 0.66 | 150.4 | 142.2 - 159.2 |
| 18 | 1 | 0.97 | 103.5 | 94.3 - 113.7 | 0.44 | 228.4 | 217.5 - 239.9 |
| 18 | 3 | 0.92 | 108.4 | 94.4 - 124.6 | 0.35 | 283.1 | 273.3 - 293.4 |
| 18 | 4 | 0.97 | 102.7 | 84.2 - 125.4 | 0.49 | 205.5 | 142.7 - 295.9 |
| 18 | 12 | 0.80 | 125.1 | 118.0 - 132.7 | 0.40 | 251.6 | 231.4 - 273.5 |
| 19 | 8 | 2.40 | 41.6 | 38.9 - 44.56 | 1.24 | 80.5 | 73.0 - 88.89 |
| 20 | 1 | 0.88 | 113.4 | 111.2 - 115.7 | 0.42 | 235.3 | 229.8 - 240.9 |

Footnotes:

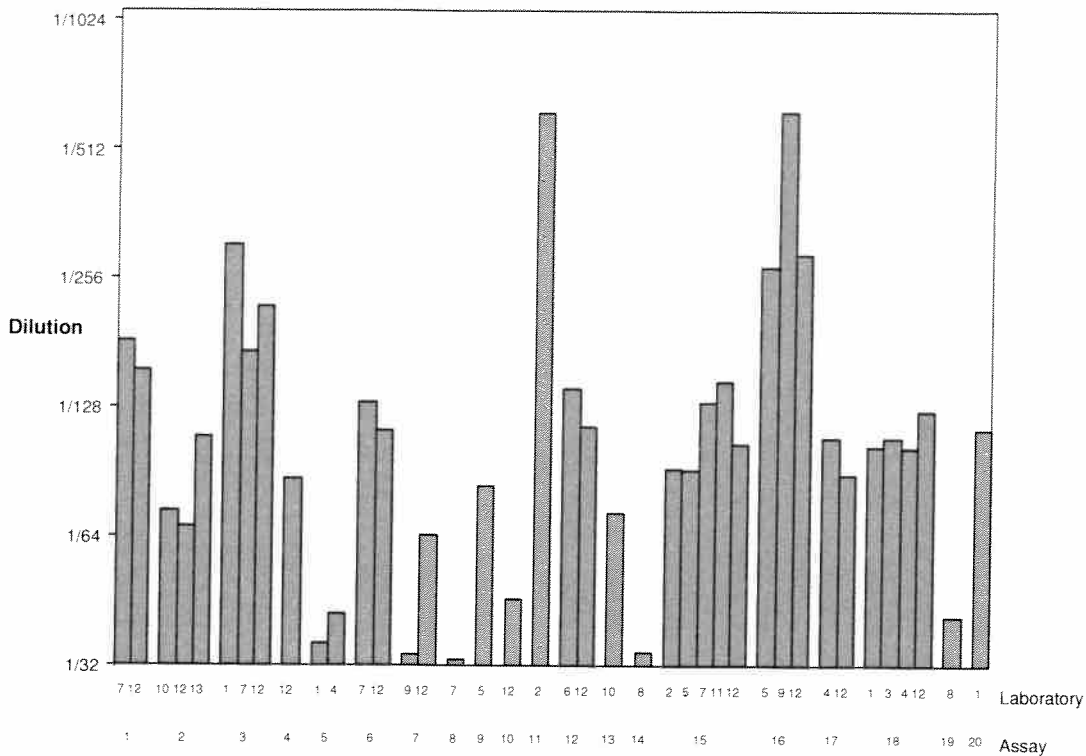
1) U/ml relative to PEI 82 (100 PEI U/ml)

2) GMV = geometric mean value

3) CI = Confidence interval

Figure 1: Mean Endpoint Titers with Sample A (NIBSC 95/522) and Sample B (PEI 82)

Dilution equivalent to cut-off - 95/522 NIBSC



Dilution equivalent to cut-off - PEI82

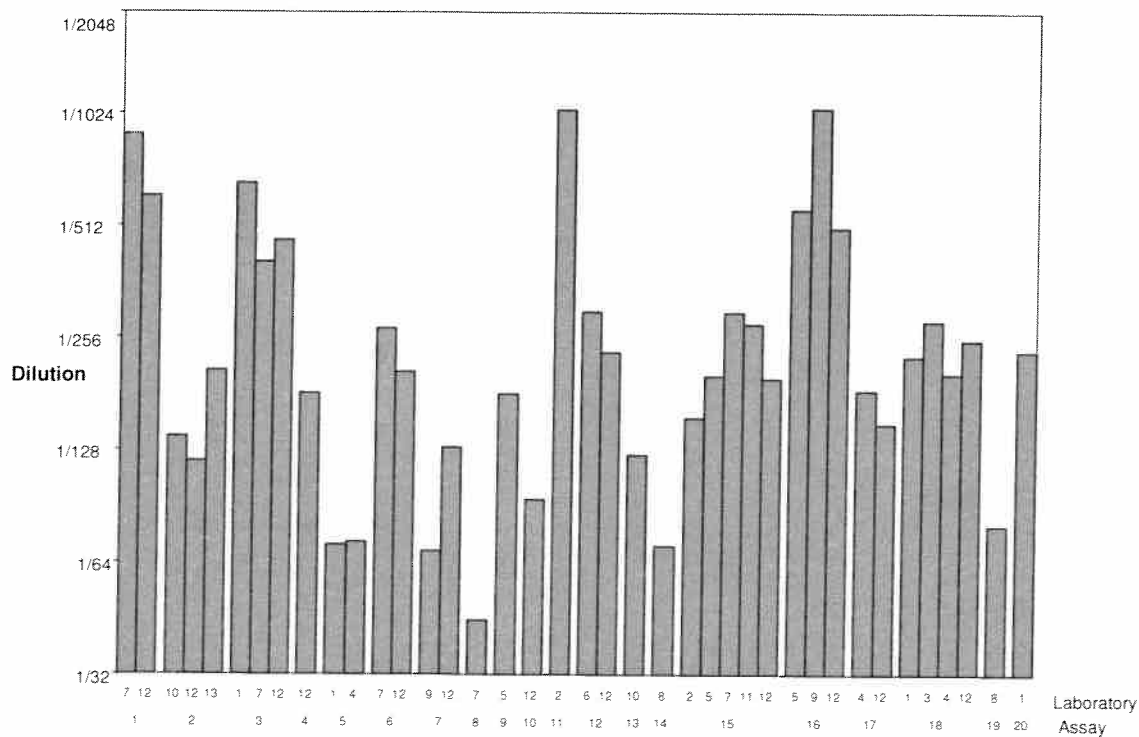


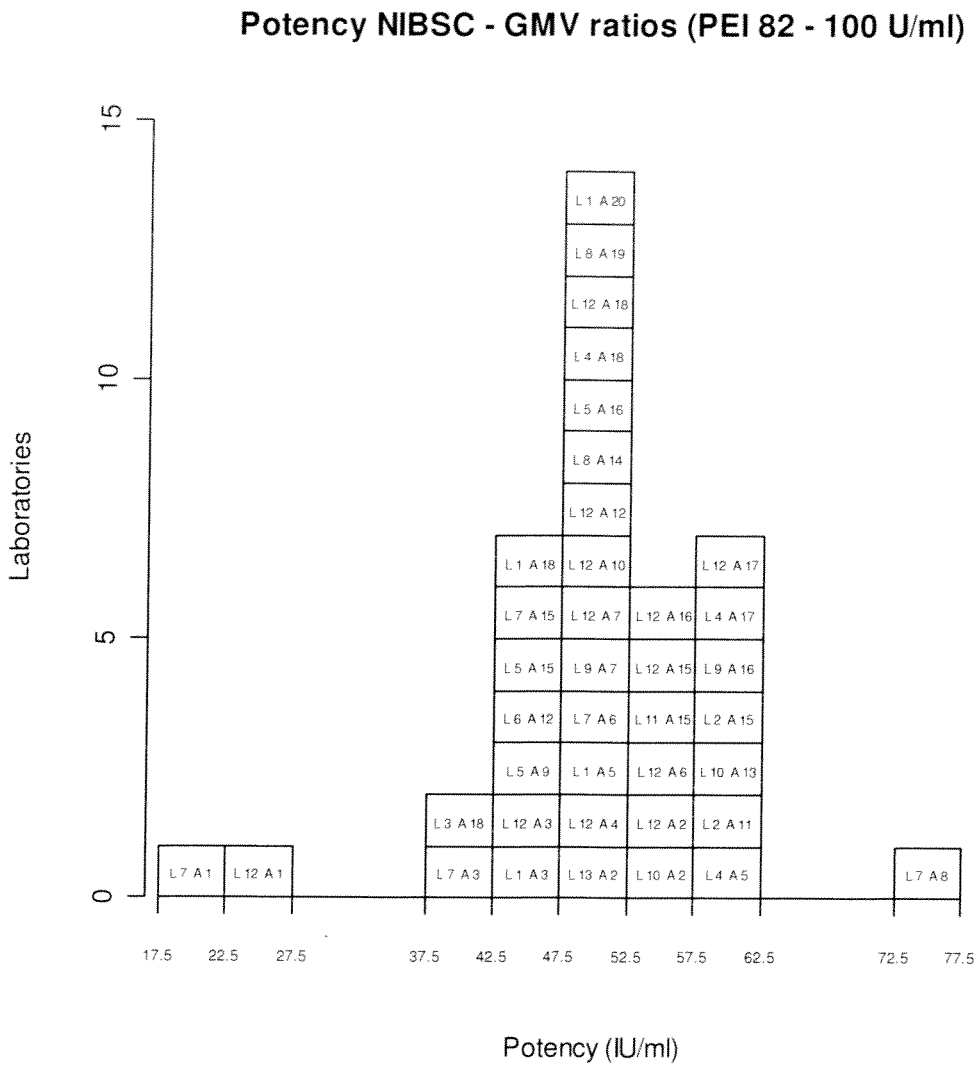
Table 3: Potency estimates of Sample A (NIBSC 95/522) relative to Sample B (PEI 82) which has an assigned unitage of 100 PEI units/ml

| Assay | Laboratory | Potency based on | | | |
|-----------------------------|------------|----------------------|-------------|---------|---------------|
| | | GMV Ratio Potency | 95%-CI | Potency | PLA 95%-CI |
| 1 | 1 | 20.3 | 17.4 - 23.7 | 23.8 | 22.6 - 25.0 |
| | 12 | 25.3 | 19.1 - 33.5 | 26.1 | 25.2 - 27.1 |
| 2 | 10 | 52.8 | 47.6 - 58.6 | 51.5 | 48.8 - 54.5 |
| | 12 | 56.4 | 54.5 - 58.3 | 57.0 | 56.0 - 58.1 |
| | 13 | 52.3 | 46.1 - 59.3 | 53.0 | 49.8 - 56.5 |
| 3 | 1 | 46.2 | 43.7 - 48.8 | 43.1 | 40.7 - 45.7 |
| | 7 | 42.3 | 35.2 - 50.7 | 35.2 | 32.8 - 37.7 |
| | 12 | 47.0 | 43.9 - 50.3 | 45.6 | 44.1 - 47.2 |
| 4 | 12 | 47.9 | 43.3 - 52.9 | 46.6 | 43.4 - 50.1 |
| 5 | 1 | 50.3 | 43.9 - 57.7 | 57.3 | 52.2 - 62.8 |
| | 4 | 58.0 | 48.6 - 69.1 | 52.7 | 40.7 - 66.7 |
| 6 | 7 | 48.4 | 43.2 - 54.3 | 43.7 | 42.1 - 45.7 |
| | 12 | 54.4 | 50.7 - 58.3 | 43.7 | 43.0 - 44.5 |
| 7 | 9 | 49.4 | 43.6 - 55.9 | 49.5 | 46.6 - 52.6 |
| | 12 | 49.5 | 46.2 - 53.0 | 49.4 | 46.5 - 52.5 |
| 8 | 7 | 73.3 | 62.1 - 86.6 | 75.4 | 66.3 - 85.7 |
| 9 | 5 | 46.3 | 39.1 - 54.8 | 48.1 | 45.2 - 51.2 |
| 10 | 12 | 48.4 | 45.2 - 51.8 | 47.9 | 45.9 - 50.0 |
| 11 | 2 | 59.1 | 50.5 - 69.3 | n.a. | n.a. n.a. |
| 12 | 6 | 46.9 | 45.1 - 48.7 | 36.3 | 30.9 - 42.4 |
| | 12 | 49.1 | 44.4 - 54.2 | 35.8 | 34.5 - 37.2 |
| 13 | 10 | 58.1 | 50.6 - 66.8 | 54.2 | 49.6 - 59.2 |
| 14 | 8 | 48.5 | 46.1 - 51.1 | 47.7 | 46.0 - 49.5 |
| | 2 | 58.6 | 55.8 - 61.6 | 53.8 | 52.4 - 55.2 |
| | 5 | 45.2 | 42.5 - 48.0 | 48.3 | 46.9 - 49.7 |
| | 7 | 43.9 | 37.7 - 51.2 | 46.0 | 42.3 - 50.0 |
| | 11 | 52.5 | 46.3 - 59.7 | 51.5 | 48.5 - 54.7 |
| 15 | 12 | 52.7 | 49.8 - 55.7 | 54.0 | 52.3 - 55.8 |
| | 5 | 48.1 | 45.0 - 51.4 | 42.9 | 40.7 - 45.3 |
| | 9 | | | 48.5 | 44.7 - 52.5 |
| 16 | 12 | 57.2 | 48.0 - 68.1 | 58.7 | 53.6 - 64.1 |
| | 4 | 58.3 | 52.6 - 64.7 | 59.9 | 56.7 - 63.4 |
| 17 | 12 | 59.0 | 54.4 - 64.0 | 58.7 | 56.6 - 60.9 |
| | 1 | 45.3 | 41.1 - 49.9 | 43.6 | 42.4 - 44.8 |
| 18 | 3 | 38.3 | 33.6 - 43.7 | 41.9 | 39.2 - 44.9 |
| | 4 | 50.0 | 34.9 - 71.7 | 43.2 | 38.3 - 48.7 |
| | 12 | 49.7 | 45.3 - 54.6 | 42.5 | 40.9 - 44.2 |
| | 8 | 51.7 | 46.3 - 57.7 | 50.7 | 47.8 - 53.7 |
| 19 | 8 | 51.7 | 46.3 - 57.7 | 50.7 | 47.8 - 53.7 |
| 20 | 1 | 48.2 | 46.9 - 49.6 | 46.2 | 45.6 - 46.8 |
| Overall | | 49.8 | 44.2 - 56.1 | 46.8 | 43.7 - 50.2 |
| Overall assays 1&8 excluded | | 51.1 | 45.3 - 57.5 | 47.9 | 45.7 - 50.2 |

Footnotes:

GMV = geometric mean value; PLA = parallel line assay; CI = confidence interval; n.a. = not applicable.

Figure 2: Geometric mean potencies of Sample A (NIBSC 95/522) relative to Sample B (PEI 82, 100 PEI U/ml)



Footnotes:

Each box represents the laboratory potency estimate relative to Sample B for an individual kit.

The boxes are labeled with the laboratory (=L) code number, and a code representing the kit used (= A)

Table 4 cont'nd

| Laboratory code | Assay code ^{b)} | S/Co ²⁾ , Co/S ²⁾ or visual interpretation ³⁾ | | | | | | | | | | |
|-----------------|--------------------------|--|------|-------|-----------------|-----------------|-------|-----------------|------|------|-------|------------------|
| | | C | D1 * | D2 * | D3 ^N | D4 [#] | D5 ** | D6 ^N | D7 * | D8 * | D9 ** | D10 [#] |
| 13 | 2 | 3.44 | 3.18 | 8.36 | 0.11 | 0.20 | 0.88 | 0.11 | 7.75 | 2.42 | 0.51 | 0.15 |
| 10 | 13 | 8.77 | 1.38 | 4.09 | 0.22 | 0.25 | 0.35 | 0.27 | 4.30 | 1.61 | 0.35 | 0.26 |
| 9 | 7 | 0.97 | 1.18 | 2.45 | 0.16 | 0.18 | 0.24 | 0.15 | 1.10 | 0.80 | 0.21 | 0.17 |
| 12 | 7 | 1.41 | 1.95 | 5.28 | 0.17 | 0.19 | 0.25 | 0.16 | 1.72 | 1.18 | 0.24 | 0.17 |
| 12 | 10 | 1.69 | 1.88 | 6.03 | 0.31 | 0.32 | 0.40 | 0.31 | 1.44 | 1.59 | 0.37 | 0.33 |
| 8 | 19 | 1.19 | 1.42 | 1.91 | 0.00 | 0.00 | 0.36 | 0.00 | 1.94 | 1.70 | 0.34 | 0.00 |
| 1 | 5 | 2.46 | 1.72 | 5.49 | 0.41 | 0.42 | 0.57 | 0.39 | 4.52 | 2.43 | 0.57 | 0.41 |
| 4 | 5 | 3.12 | 1.69 | 5.87 | 0.40 | 0.42 | 0.60 | 0.41 | 4.59 | 2.28 | 0.55 | 0.42 |
| 8 | 14 | 1.57 | 1.27 | 3.73 | 0.00 | 0.10 | 0.30 | 0.00 | 4.43 | 0.46 | 0.20 | 0.00 |
| 7 | 8 | 1.19 | 1.06 | 4.79 | 0.49 | 0.50 | 0.57 | 0.49 | 7.53 | 1.66 | 0.60 | 0.52 |
| 7 | 1 | 0.86 | 5.76 | 13.02 | 0.31 | 0.52 | 0.76 | 0.30 | 1.10 | 1.48 | 0.35 | 0.33 |
| 12 | 1 | 2.65 | 6.06 | 14.29 | 0.14 | 0.19 | 0.39 | 0.14 | 0.61 | 0.95 | 0.14 | 0.14 |

Footnotes:

- * Panel members 1, 2, 7 and 8 are required to be positive by all anti-HBc tests
- ** panel members 5 and 9 contain anti-HBc and may be detected positive
- # panel members 4 and 10 contain anti-HBc but are assigned not detectable by current technology
- N panel members 3 and 6 must be negative

1) Assays listed according to their analytical sensitivity, except assay 1

2) Values ≥ 1 are considered positive

3) Pos = positive; Neg = negative; IND = indeterminate

Shading indicates a reactive result

Table 5: Mean Analytical Sensitivities in Samples A and B correlated to Ratios of anti-HBc concentration measured in Samples C and D

| Assay code ¹⁾ | Detection Limit ²⁾ | | Ratio ⁴⁾ of anti-HBc detected in Samples C and D relative to Sample A and B | | | | | | | | | | | | | | | | | | | | | |
|--------------------------|-------------------------------|--------------------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | U/ml ³⁾ | U/ml ³⁾ | A | | B | | C | | D1 | | D2 | | D4 | | D5 | | D7 | | D8 | | D9 | | D10 | |
| | | | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio | ratio |
| 16 | 0.27 | 0.15 | 6.06 | 11.09 | 7.45 | 13.64 | 7.88 | 14.42 | 0.30 | 0.55 | 1.13 | 2.07 | 3.65 | 6.68 | 1.26 | 2.31 | 0.70 | 1.29 | 0.13 | 0.23 | | | | |
| 3 | 0.44 | 0.20 | 3.22 | 6.84 | 4.70 | 9.99 | 9.28 | 19.75 | 0.22 | 0.48 | 0.67 | 1.42 | 2.96 | 6.31 | 1.00 | 2.14 | 0.53 | 1.13 | 0.11 | 0.24 | | | | |
| 12 | 0.79 | 0.38 | 1.79 | 3.77 | 3.27 | 6.87 | 6.27 | 13.18 | 0.17 | 0.36 | 0.55 | 1.17 | 1.67 | 3.50 | 0.71 | 1.50 | 0.37 | 0.78 | 0.08 | 0.16 | | | | |
| 6 | 0.82 | 0.42 | 3.47 | 6.77 | 2.85 | 5.56 | 4.85 | 9.45 | 0.20 | 0.38 | 0.65 | 1.26 | 4.17 | 8.13 | 1.07 | 2.08 | 0.43 | 0.83 | 0.19 | 0.38 | | | | |
| 20 | 0.88 | 0.43 | 2.88 | 5.98 | 1.89 | 3.93 | 4.86 | 10.08 | 0.06 | 0.13 | 0.44 | 0.90 | 4.52 | 9.37 | 1.02 | 2.12 | 0.24 | 0.50 | 0.04 | 0.09 | | | | |
| 15 | 0.90 | 0.45 | 2.13 | 4.24 | 2.60 | 5.17 | 6.81 | 13.55 | 0.06 | 0.11 | 0.42 | 0.47 | 2.36 | 4.70 | 0.55 | 1.10 | 0.11 | 0.23 | 0.03 | 0.06 | | | | |
| 18 | 0.91 | 0.41 | 1.65 | 3.58 | 2.09 | 4.53 | 6.10 | 13.20 | 0.08 | 0.18 | 0.33 | 0.36 | 1.28 | 2.81 | 0.67 | 1.46 | 0.32 | 0.68 | 0.08 | 0.17 | | | | |
| 17 | 1.02 | 0.60 | 2.89 | 4.93 | 2.38 | 4.05 | 3.75 | 6.39 | 0.07 | 0.12 | 0.55 | 0.94 | 3.60 | 6.14 | 0.82 | 1.40 | 0.24 | 0.41 | 0.06 | 0.10 | | | | |
| 4 | 1.15 | 0.55 | 0.58 | 1.21 | 1.07 | 2.23 | 3.58 | 7.49 | 0.05 | 0.10 | 0.09 | 0.18 | 1.44 | 3.01 | 0.69 | 1.44 | 0.05 | 0.11 | 0.05 | 0.10 | | | | |
| 9 | 1.19 | 0.55 | 1.54 | 3.33 | 1.44 | 3.11 | 4.50 | 9.73 | 0.05 | 0.11 | 0.21 | 0.44 | 1.08 | 2.32 | 0.75 | 1.62 | 0.10 | 0.22 | 0.05 | 0.10 | | | | |
| 2 | 1.23 | 0.66 | 2.09 | 3.88 | 1.93 | 3.58 | 5.30 | 9.85 | 0.03 | 0.06 | 0.43 | 0.81 | 4.43 | 8.24 | 1.10 | 2.05 | 0.17 | 0.32 | 0.03 | 0.05 | | | | |
| 13 | 1.38 | 0.80 | 1.53 | 2.63 | 0.72 | 1.24 | 1.11 | 1.92 | 0.04 | 0.07 | 0.16 | 0.28 | 1.14 | 1.97 | 0.76 | 1.30 | 0.18 | 0.30 | 0.04 | 0.07 | | | | |
| 7 | 2.12 | 1.06 | 0.50 | 1.01 | 0.62 | 1.24 | 1.16 | 2.33 | 0.03 | 0.06 | 0.07 | 0.14 | 0.57 | 1.15 | 0.43 | 0.87 | 0.05 | 0.11 | 0.03 | 0.05 | | | | |
| 10 | 2.18 | 1.06 | 0.64 | 1.32 | 0.68 | 1.41 | 1.33 | 2.75 | 0.03 | 0.06 | 0.16 | 0.32 | 0.58 | 1.21 | 0.62 | 1.28 | 0.13 | 0.27 | 0.05 | 0.11 | | | | |
| 19 | 2.40 | 1.24 | 0.70 | 1.36 | 1.05 | 2.03 | 2.42 | 4.68 | 0.00 | 0.00 | 0.17 | 0.32 | 2.57 | 4.98 | 1.63 | 3.15 | 0.16 | 0.31 | 0.00 | 0.00 | | | | |
| 5 | 2.57 | 1.39 | 1.60 | 2.96 | 1.00 | 1.85 | 3.35 | 6.21 | 0.02 | 0.04 | 0.19 | 0.36 | 2.68 | 4.96 | 1.36 | 2.53 | 0.16 | 0.29 | 0.02 | 0.04 | | | | |
| 14 | 2.91 | 1.41 | 0.77 | 1.60 | 0.62 | 1.28 | 1.90 | 3.92 | 0.02 | 0.04 | 0.12 | 0.26 | 2.26 | 4.67 | 0.21 | 0.43 | 0.08 | 0.16 | 0.00 | 0.00 | | | | |
| 8 | 3.03 | 2.22 | 0.98 | 1.34 | 0.81 | 1.11 | 5.56 | 7.58 | 0.02 | 0.03 | 0.17 | 0.23 | 9.05 | 12.34 | 1.58 | 2.16 | 0.22 | 0.30 | 0.02 | 0.03 | | | | |
| 1 | 0.62 | 0.15 | 0.40 | 1.64 | 1.97 | 8.13 | 5.31 | 21.93 | 0.05 | 0.20 | 0.08 | 0.33 | 0.16 | 0.67 | 0.29 | 1.20 | 0.03 | 0.13 | 0.03 | 0.13 | | | | |

Footnotes:

- 1) Assays listed according to their analytical sensitivity, except assay 1
- 2) Geomean of all tests for each assay
- 3) Relative to PEI 82 (100 PEI U/ml)
- 4) Ratio = detection limit (U/ml) / anti-HBc concentration (U/ml) Samples C and D

**Table 6: Assessment of Correlation of Detection Limits in Samples A and B to
(1) Signals in Samples C and D and
(2) to Ratio of anti-HBc Concentration in Samples C and D**

| Sample | | Sensitivity correlation ¹⁾ relative to Sample | | | |
|--------|---------------------------------------|---|---------|---------------------|---------|
| | | A | B | A | B |
| | | Signals ²⁾ | | Ratio ³⁾ | |
| C | Correlation coefficient ⁴⁾ | -0.646 | -0.607 | -0.756 | -0.750 |
| | p-value | 0.004 | 0.008 | < 0.001 | < 0.001 |
| D1 | Correlation coefficient | -0.870 | -0.950 | -0.868 | -0.919 |
| | p-value | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| D2 | Correlation coefficient | -0.678 | -0.640 | -0.676 | -0.808 |
| | p-value | 0.002 | 0.004 | 0.002 | < 0.001 |
| D3 | Correlation coefficient | -0.472 | -0.518 | -0.662 | -0.699 |
| | p-value | 0.048 | 0.028 | 0.002 | 0.001 |
| D4 | Correlation coefficient | -0.549 | -0.594 | -0.957 | -0.950 |
| | p-value | 0.018 | 0.009 | < 0.001 | < 0.001 |
| D5 | Correlation coefficient | -0.860 | -0.820 | -0.769 | -0.795 |
| | p-value | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| D6 | Correlation coefficient | -0.470 | -0.510 | -0.868 | -0.877 |
| | p-value | 0.051 | 0.031 | < 0.001 | < 0.001 |
| D7 | Correlation coefficient | -0.335 | -0.254 | -0.211 | -0.135 |
| | p-value | 0.173 | 0.309 | 0.399 | 0.593 |
| D8 | Correlation coefficient | -0.255 | -0.158 | -0.013 | -0.138 |
| | p-value | 0.307 | 0.531 | 0.958 | 0.584 |
| D9 | Correlation coefficient | -0.481 | -0.599 | -0.664 | -0.657 |
| | p-value | 0.043 | 0.009 | 0.003 | 0.003 |
| D10 | Correlation coefficient | -0.680 | -0.519 | -0.854 | -0.853 |
| | p-value | 0.002 | 0.027 | < 0.001 | < 0.001 |

Footnotes:

¹⁾ Correlation calculated without assay coded 1

²⁾ Signals = S/Co or Co/S

³⁾ Ratio = detection limit (U/ml) / anti-HBc concentration in Samples C and D (U/ml)

⁴⁾ Spearman rank correlation coefficient and p-value for testing = 0

Table 7: Intra-laboratory and Inter-laboratory variation (GCV %) with Samples A and B

| Assay code | Laboratory code | | Samples | |
|------------|-----------------|-------------|---------|-------|
| | | | A | B |
| | | | GCV % | |
| 1 | 7 | Intra-lab.. | 6.99 | 6.91 |
| 1 | 12 | Intra-lab. | 29.83 | 35.21 |
| 1 | Interlab. | | 12.00 | 30.75 |
| 2 | 10 | Intra-lab. | 10.32 | 11.60 |
| 2 | 12 | Intra-lab. | 2.60 | 4.10 |
| 2 | 13 | Intra-lab. | 17.65 | 7.65 |
| 2 | Interlab. | | 29.52 | 33.66 |
| 3 | 1 | Intra-lab. | 6.96 | 4.12 |
| 3 | 7 | Intra-lab. | 8.67 | 8.09 |
| 3 | 12 | | 7.22 | 6.85 |
| 3 | Interlab. | | 33.53 | 28.64 |
| 4 | 12 | Intra-lab. | 10.33 | 10.91 |
| 5 | 1 | Intra-lab. | 8.57 | 19.03 |
| 5 | 4 | Intra-lab. | 15.55 | 22.41 |
| 5 | Interlab. | | 11.96 | 1.32 |
| 6 | 7 | Intra-lab. | 1.21 | 7.33 |
| 6 | 12 | Intra-lab. | 7.30 | 7.30 |
| 6 | Interlab. | | 11.58 | 21.08 |
| 7 | 9 | Intra-lab. | 14.26 | 9.80 |
| 7 | 12 | Intra-lab. | 7.78 | 6.35 |
| 7 | Interlab. | | 57.17 | 56.86 |
| 8 | 7 | Intra-lab. | 1.87 | 10.76 |
| 9 | 5 | Intra-lab. | 10.60 | 3.16 |
| 10 | 12 | Intra-lab. | 7.88 | 6.15 |
| 12 | 6 | Intra-lab. | 2.20 | 0.97 |
| 12 | 12 | Intra-lab. | 8.69 | 12.21 |
| 12 | Interlab. | | 15.91 | 19.71 |
| 13 | 10 | Intra-lab. | 6.03 | 20.77 |
| 14 | 8 | Intra-lab. | 5.81 | 4.64 |
| 15 | 2 | Intra-lab. | 4.46 | 5.64 |
| 15 | 5 | Intra-lab. | 5.96 | 6.57 |
| 15 | 7 | Intra-lab. | 9.87 | 1.41 |
| 15 | 11 | Intra-lab. | 12.75 | 14.35 |
| 15 | 12 | Intra-lab. | 5.74 | 5.84 |
| 15 | Interlab. | | 23.89 | 30.52 |
| 16 | 5 | Intra-lab. | 5.37 | 8.06 |
| 16 | 9 | Intra-lab. | 25.27 | 42.33 |
| 16 | 12 | Intra-lab. | 10.60 | 25.33 |
| 16 | Interlab. | | 59.22 | 48.75 |
| 17 | 4 | Intra-lab. | 6.39 | 14.25 |
| 17 | 12 | Intra-lab. | 9.35 | 7.62 |
| 17 | Interlab. | | 15.11 | 16.02 |
| 18 | 1 | Intra-lab. | 12.94 | 6.59 |
| 18 | 3 | Intra-lab. | 19.77 | 4.73 |
| 18 | 4 | Intra-lab. | 20.91 | 41.55 |
| 18 | 12 | Intra-lab. | 7.89 | 11.47 |
| 18 | Interlab. | | 9.59 | 14.65 |
| 19 | 8 | Intra-lab. | 9.24 | 13.70 |
| 20 | 1 | Intra-lab. | 2.64 | 3.10 |

Footnotes:

GCV = geometric coefficient of variation

Lab. = laboratory

Table 8: Intra-laboratory and Inter-laboratory variation (GCV %) with Samples C and D

| Assay code | Laboratory code | Samples | | | | | | | | | | | |
|------------|-----------------|------------|-------|-------|-------|--------|-------|-------|-------|-------|-------|-------|------|
| | | C | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | |
| | | GCV % | | | | | | | | | | | |
| 1 | 7 | Intra-lab. | 2.45 | 0.70 | 0.00 | 3.28 | 4.10 | 5.48 | 1.91 | 1.84 | 3.09 | 2.90 | 3.08 |
| 1 | 12 | Intra-lab. | 6.63 | 2.42 | 5.98 | 0.00 | 6.27 | 3.01 | 0.00 | 5.15 | 2.49 | 0.00 | 0.00 |
| 1 | Inter-lab. | 122.43 | 3.64 | 6.81 | 75.39 | 107.40 | 59.74 | 72.74 | 51.22 | 37.59 | 91.12 | 83.33 | |
| 2 | 10 | Intra-lab. | 1.26 | 2.40 | 3.37 | 6.63 | 3.01 | 2.16 | 3.80 | 0.11 | 0.41 | 2.41 | 5.41 |
| 2 | 12 | Intra-lab. | 1.42 | 1.84 | 0.91 | 3.17 | 0.00 | 1.17 | 0.00 | 0.89 | 1.54 | 2.44 | 2.86 |
| 2 | 13 | Intra-lab. | 1.56 | 2.73 | 1.75 | 0.00 | 3.01 | 0.00 | 0.00 | 3.49 | 1.46 | 2.29 | 0.00 |
| 2 | Inter-lab. | 11.02 | 13.91 | 15.54 | 32.89 | 10.06 | 11.91 | 28.90 | 23.94 | 31.79 | 10.68 | 17.28 | |
| 3 | 1 | Intra-lab. | 5.03 | 4.72 | 19.23 | 1.84 | 2.32 | 4.40 | 2.69 | 8.91 | 2.34 | 4.52 | 3.54 |
| 3 | 7 | Intra-lab. | 9.29 | 3.33 | 18.34 | 5.58 | 1.65 | 1.88 | 1.20 | 3.47 | 3.99 | 1.11 | |
| 3 | 12 | Intra-lab. | 4.73 | 8.59 | 12.47 | 6.39 | 2.19 | 4.06 | 3.99 | 11.54 | 8.18 | 6.47 | 0.99 |
| 3 | Inter-lab. | 28.03 | 22.66 | 3.73 | 12.79 | 11.15 | 33.33 | 11.38 | 29.15 | 24.55 | 22.73 | 7.42 | |
| 4 | 12 | Intra-lab. | 8.44 | 2.74 | 2.48 | 2.25 | 2.15 | 2.51 | 0.00 | 1.53 | 4.01 | 8.12 | 4.55 |
| 5 | 1 | Intra-lab. | 0.0 | 15.69 | 12.65 | 7.94 | 4.26 | 11.49 | 1.51 | 4.20 | 10.55 | 9.84 | 3.74 |
| 5 | 4 | Intra-lab. | 16.26 | 13.78 | 6.76 | 7.40 | 8.83 | 11.39 | 6.25 | 5.02 | 9.42 | 7.86 | 4.88 |
| 5 | Inter-lab. | 0.0 | 0.86 | 4.82 | 2.94 | 0.13 | 3.26 | 4.74 | 4.07 | 1.08 | 2.93 | 1.12 | |
| 6 | 7 | Intra-lab. | 4.88 | 6.77 | 11.81 | 4.91 | 4.48 | 2.46 | 2.20 | 9.31 | 4.07 | 3.94 | 1.90 |
| 6 | 12 | Intra-lab. | 4.73 | 4.73 | 7.04 | 1.24 | 2.42 | 1.95 | 2.02 | 2.19 | 1.50 | 1.99 | |
| 6 | Inter-lab. | 12.06 | 66.19 | 59.25 | 36.26 | 17.03 | 34.83 | 28.86 | 30.18 | 30.46 | 20.75 | 31.02 | |
| 7 | 9 | Intra-lab. | 8.34 | 11.98 | 8.27 | 0.00 | 3.36 | 4.93 | 3.80 | 6.30 | 6.52 | 2.86 | 3.56 |
| 7 | 12 | Intra-lab. | 8.74 | 7.38 | 3.13 | 3.56 | 3.01 | 2.29 | 0.00 | 0.34 | 3.53 | 2.49 | 3.36 |
| 7 | Inter-lab. | 30.79 | 42.39 | 71.82 | 2.90 | 6.59 | 5.95 | 3.09 | 36.63 | 31.07 | 10.06 | 2.82 | |
| 8 | 7 | Intra-lab. | 2.55 | 3.61 | 24.41 | 2.06 | 2.02 | 3.71 | 1.20 | 14.43 | 0.60 | 0.98 | 1.94 |
| 9 | 5 | Intra-lab. | 1.44 | 4.82 | 1.53 | 3.92 | 3.39 | 2.95 | 2.20 | 6.63 | 5.88 | 4.86 | 5.73 |
| 10 | 12 | Intra-lab. | 9.59 | 12.85 | 5.51 | 3.28 | 6.76 | 3.95 | 1.85 | 2.28 | 4.09 | 4.15 | 3.08 |
| 12 | 6 | Intra-lab. | 1.00 | 1.59 | 6.18 | 2.08 | 5.93 | 3.80 | 7.65 | 3.73 | 8.26 | 5.97 | 1.77 |
| 12 | 12 | Intra-lab. | 1.65 | 6.48 | 5.65 | 3.36 | 2.40 | 1.38 | 3.85 | 2.42 | 1.63 | 1.89 | 0.00 |
| 12 | Inter-lab. | 11.77 | 8.64 | 15.42 | 5.84 | 3.09 | 0.60 | 4.17 | 16.08 | 3.60 | 7.35 | 6.70 | |
| 13 | 10 | Intra-lab. | 5.76 | 18.86 | 35.30 | 11.10 | 10.90 | 14.39 | 24.34 | 61.93 | 24.00 | 5.94 | |
| 14 | 8 | Intra-lab. | 3.80 | 4.73 | 1.55 | 0.0 | 0.00 | 0.00 | 1.31 | 13.75 | 0.00 | 0.00 | |

Table 8 cont'd

| Assay code | Laboratory code | C | D1 | D2 | D3 | D4 | Samples | | | D8 | D9 | D10 |
|------------|-----------------|-------|-------|-------|-------|--------|---------|--------|-------|-------|-------|-------|
| | | | | | | | D5 | D6 | D7 | | | |
| GCV % | | | | | | | | | | | | |
| 15 | Intra-lab. | 4.12 | 4.29 | 3.08 | 13.75 | 18.42 | 10.94 | 8.01 | 5.25 | 11.74 | 3.01 | 7.04 |
| 15 | Intra-lab. | 3.23 | 3.01 | 0.75 | 0.00 | 6.76 | 7.41 | 0.00 | 4.37 | 4.34 | 3.51 | 3.36 |
| 15 | Intra-lab. | 3.03 | 5.58 | 7.16 | 15.52 | 5.15 | 2.65 | 4.37 | 4.25 | 3.43 | 1.34 | 35.55 |
| 15 | Intra-lab. | 3.74 | 1.11 | 5.44 | 8.01 | 5.66 | 1.89 | 279.92 | 5.60 | 3.85 | 0.00 | 15.48 |
| 15 | Intra-lab. | 1.90 | 3.72 | 4.04 | 0.00 | 0.00 | 4.63 | 0.00 | 2.02 | 1.01 | 2.60 | 0.00 |
| 15 | Inter-lab. | 16.12 | 13.46 | 7.14 | 46.78 | 34.90 | 28.45 | 81.67 | 11.10 | 14.57 | 37.71 | 51.28 |
| 16 | Intra-lab. | 1.55 | 5.03 | 6.39 | 4.63 | 1.82 | 4.98 | 12.40 | 0.42 | 8.53 | 0.84 | 3.11 |
| 16 | Intra-lab. | 2.39 | 7.01 | 7.65 | 1.20 | 4.86 | 6.75 | 3.07 | 1.46 | 1.78 | 7.71 | 5.12 |
| 16 | Intra-lab. | 4.04 | 4.41 | 1.25 | 4.18 | 6.07 | 2.66 | 2.26 | 1.64 | 3.46 | 16.53 | 4.05 |
| 16 | Inter-lab. | 43.47 | 40.68 | 45.10 | 23.12 | 27.06 | 36.54 | 18.39 | 28.80 | 13.97 | 19.88 | 14.29 |
| 17 | Intra-lab. | 1.94 | 5.24 | 0.00 | 15.90 | 38.14 | 8.55 | 35.84 | 0.00 | 6.84 | 18.38 | 5.15 |
| 17 | Intra-lab. | 6.68 | 1.54 | 0.88 | 13.75 | 12.99 | 2.29 | 13.40 | 1.29 | 6.38 | 36.72 | 12.99 |
| 17 | Inter-lab. | 8.40 | 19.59 | 21.05 | 15.70 | 12.31 | 1.71 | 28.07 | 15.57 | 6.43 | 21.23 | 5.85 |
| 18 | Intra-lab. | 2.89 | 0.73 | 5.16 | 1.74 | 0.80 | 1.06 | 0.86 | 0.71 | 1.53 | 0.63 | 1.46 |
| 18 | Intra-lab. | 1.45 | 6.18 | 5.16 | 1.80 | 3.93 | 3.14 | 1.98 | 3.26 | 1.01 | 6.20 | 3.33 |
| 18 | Intra-lab. | 6.87 | 2.09 | 0.00 | 1.14 | 1.07 | 3.31 | 1.12 | 0.00 | 1.73 | 0.00 | 1.12 |
| 18 | Intra-lab. | 1.32 | 3.39 | 2.64 | 1.65 | 4.04 | 1.65 | 4.91 | 0.95 | 1.86 | 1.34 | 3.03 |
| 18 | Inter-lab. | 6.11 | 3.05 | 5.36 | 7.89 | 7.86 | 5.53 | 5.98 | 8.88 | 6.01 | 7.71 | 5.66 |
| 19 | Intra-lab. | 2.24 | 2.56 | 0.52 | 0.0 | 166.51 | 29.55 | | 0.00 | 0.68 | 1.69 | 17.09 |
| 20 | Intra-lab. | 1.19 | 2.01 | 4.35 | 0.00 | 2.60 | 3.24 | 4.73 | 2.54 | 1.51 | 4.21 | 6.91 |

Footnotes:
GCV = geometric coefficient of variation
Lab. = laboratory

Table 9: Stability of Sample A (NIBSC 95/522)

| Day | Replicate | Endpoint titers (intercept at assay's cut-off) | | | | |
|-----------------------------|-----------|--|-------|-------|--------|--------|
| | | 0 | 4°C | 20°C | 37°C | 45°C |
| 1 | 1 | 74.46 | 76.52 | 73.10 | 37.14 | 0.60 |
| | 2 | 76.75 | 72.44 | 71.40 | 38.76 | 0.61 |
| | 3 | 76.10 | 77.14 | 71.93 | 38.76 | 0.61 |
| 2 | 1 | 77.89 | 72.96 | 70.08 | 41.63 | 0.60 |
| | 2 | 76.03 | 73.68 | 71.19 | 43.02 | 0.60 |
| | 3 | 76.22 | 75.25 | 71.11 | 43.43 | 0.61 |
| 3 | 1 | 75.90 | 72.92 | 67.90 | 36.36 | 0.59 |
| | 2 | 73.28 | 75.68 | 69.82 | 35.96 | 0.59 |
| | 3 | 75.17 | 74.81 | 67.44 | 37.65 | 0.60 |
| Geomean | | 75.75 | 74.58 | 70.42 | 39.10 | 0.60 |
| GCV% | | 1.78% | 2.29% | 2.66% | 7.38% | 1.31% |
| Activity loss ¹⁾ | | 0 | 1.53% | 7.03% | 48.38% | 99.21% |
| Activity loss ²⁾ | | 0 | 3.14% | 7.42% | 52.10% | N/A |

Table 10: Stability of Sample A (NIBSC 95/522) after reconstitution

| | Endpoint titers (intercept at assay's cut-off) | |
|------------------------|--|--|
| | Base line directly after reconstitution | 14 days storage at + 2-8°C after reconstitution, and frozen at -70°C |
| Geomean | 68.74 | 79.29 |
| GCV% | 1.37% | 0.87% |
| Activity ¹⁾ | 100% | 115.4% |
| Activity ²⁾ | 100% | 112.4% |

Footnotes:

¹⁾ calculated by linear interpolation²⁾ calculated by parallel line assay

GCV = geometric coefficient of variation

N/A = not applicable

Appendix 1**Participants of the WHO anti-HBc Collaborative Study**

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Appendix 2 Draft IFU



WHO International Standard
International Standard for anti-HBc
NIBSC code: 95/522
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

Antibodies to hepatitis B virus core antigen (anti-HBc) are produced during acute hepatitis B virus (HBV) infection and persist lifelong so that HBV infection can be detected in chronic carriers even when negative for hepatitis B surface antigen (HBsAg) and HBV-DNA. Anti-HBc screening therefore has the potential to detect the majority of occult HBV infection. In the absence of anti-HBc testing HBV transmission occurred in blood recipients as well as after organ transplantations. This has led some countries to improve blood safety by mandatory blood screening for anti-HBc (France, Germany, Japan, USA). In addition, anti-HBc may be the only positive serological marker in chronic HBV infections.

Anti-HBc persists also in those who have cleared the virus so that isolated anti-HBc can be indistinguishable from serological profile of resolved HBV infection not easy to differentiate from potential false positive reactions in particular in low prevalence HBV countries. Therefore high quality anti-HBc tests with high sensitivity and specificity are required.

A WHO Collaborative Study organised by the Paul Ehrlich Institute was undertaken to assess the suitability of a candidate reference material (NIBSC code 95/522) for detection of antibodies to hepatitis B core antigen (anti-HBc) in diagnostic assays. Thirteen laboratories from 10 countries tested the above described materials using 20 different anti-HBc assays.

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. Its material contains high levels of anti-HBs and is therefore non-infectious. 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 is assigned a unitage of 60IU/ampoule.

4. CONTENTS

Country of origin of biological material: United Kingdom.

This preparation contains the freeze dried residue of 1ml plasma from UK blood donors which is reactive for anti-HBc and also for anti-HBs at high levels.

5. STORAGE

Ampoules should be stored at -20°C or below.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open

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the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

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

Each ampoule should be reconstituted in 1ml distilled water.

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.

Stability studies on reconstituted material are in progress. In the meantime, users should determine the stability of reconstituted material according to their own method of preparation, storage and use. However, multiple freeze/thaw cycles should be avoided.

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

9. REFERENCES

10. ACKNOWLEDGEMENTS

11. FURTHER INFORMATION

Further information can be obtained as follows:

This material: enquiries@nibsc.ac.uk

WHO Biological Standards: <http://www.who.int/biologicals/en/>

Derivation of International Units:

<http://www.nibsc.ac.uk/products/faq.asp>

Ordering standards from NIBSC:

<http://www.nibsc.ac.uk/products/faq.asp>

NIBSC Terms & Conditions: <http://www.nibsc.ac.uk/terms.html>

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.ac.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.



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14 MATERIAL SAFETY SHEET

| Physical and Chemical properties | |
|---|--|
| Physical appearance: Freeze dried powder | Corrosive: No |
| Stable Yes | Oxidising No |
| Hygroscopic: No | Instant: No |
| Flammable: No | Handling: See caution, Section 2 |
| Other (specify): Contains material of human origin | |
| Toxicological properties | |
| Effects of inhalation: Not established, avoid inhalation | |
| Effects of ingestion: Not established, avoid ingestion | |
| Effects of skin absorption: Not established, avoid contact with skin | |
| Suggested First Aid | |
| 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 |
| Action on Spillage and Method of Disposal | |
| 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. | |

above options shall constitute an entire discharge of the Institute's liability under this Condition

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: 1.0g |
| 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



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