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REPORT

Calibration of the second International Standard for hepatitis B immunoglobulin in an international collaborative study

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Background and Objectives The International Standard for hepatitis B immunoglobulin is used in the standardization of the anti-HBs content of immunoglobulins for prophylactic and therapeutic use and also in the standardization and calibration of quantitative diagnostic anti-HBs assay kits. A collaborative study was undertaken to assess the suitability of a candidate Second International Standard (2nd IS), and to calibrate it in International Units (IU).

Materials and Methods The candidate 2nd IS was prepared from a bulk of 5% hepatitis B immunoglobulin (NIBSC code 07/164). Twenty-two participants from 12 countries assayed the first IS, the candidate 2nd IS, a freeze-dried pool of plasma containing anti-HBs and a plasma from a blood donor. These samples were assayed with 19 different assay kits.

Results Data from 102 assays were received. The mean potencies of two coded samples of the candidate 2nd IS were 100·7 and 101·4 IU/ml (combined potency 101·0 IU/ml). The geometric coefficients of variation for these samples were both 13%. The predicted long-term stability of 07/164 was assessed by assaying samples stored at elevated temperatures for a period of 6 months. 07/164 was predicted to be stable at −20°C with the estimated % loss per year of below 0·2%.

Conclusion 07/164 was established as the 2nd IS for hepatitis B immunoglobulin with an assigned potency of 100 IU/ampoule by the WHO Expert Committee on Biological Standardisation. The United States Food and Drug Administration has adopted the same standard as the new Reference for Hepatitis B Immunoglobulin, Lot 3.

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Key words: anti-HBs, assay kits, hepatitis B immunization, hepatitis B vaccine, immunoglobulin, standard.

Introduction

Hepatitis B immunoglobulins are produced in many countries from human plasma donations with high concentration of antibodies against hepatitis B surface antigen (anti-HBs), and the minimum potency requirements and potencies of individual batches are expressed in International Units (IU). The international unitage is also used in diagnostic assays to determine either sero-conversion or sero-protection resulting from natural infection or vaccination. It is generally accepted that healthy individuals who achieve anti-HBs concentrations of ≥ 10 mIU/ml in plasma after pre-exposure vaccination have nearly complete protection against both acute disease and chronic infection [1].

The first International Standard (1st IS) for hepatitis B immunoglobulin was established in 1977 [2,3]. The plasma used in the preparation of this standard was therefore

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derived from individuals who had been naturally infected with hepatitis B virus as this was prior to the development of vaccines. The standard was assessed for suitability in a collaborative study in which participants used the Abbott Ausab RIA and other in-house assays such as passive haemagglutination and counter-electrophoresis. The stocks of this standard are now depleted, and a candidate 2nd IS, NIBSC code 07/164, was prepared from a production lot of anti-HBs immunoglobulin in the United States. The suitability of this material to serve as an IS for use in determining the potency of commercial immunoglobulin products and for use in the assay of serum and plasma samples is the subject of this report. To assess this suitability and to calibrate the candidate 2nd IS in International Units, an international collaborative study was conducted. In performing this study, participants used commercial enzyme immunoassays (EIAs) from manufacturers around the world.

Materials and methods

Candidate 2nd IS, NIBSC code 07/164

The candidate 2nd IS was prepared from a bulk of 5% (w/v) protein concentration hepatitis B immunoglobulin which had been formulated to a target concentration of 100 IU/ml. Each individual plasma donation from which this standard was derived was tested and found negative for HBsAg, anti-HIV 1 + 2 and anti-HCV. In addition, minipool samples were tested for HCV RNA, HIV RNA, HBV DNA, HAV RNA and Parvovirus B19 DNA. The formulated bulk derived from about 900 donors was also tested and found negative for anti-HIV 1 + 2 and HCV RNA. One millilitre aliquots of this bulk were filled in DIN ampoules and freeze-dried under an atmosphere of nitrogen at NIBSC in September 2007 following documented procedures which are accredited to ISO9001 [4] and which comply with the WHO guidelines [5] for the preparation, characterization and establishment of international and other standards and reference reagents for biological substances. This fill was 1·0 g fill weight with a mean dry weight of 0·0715 g. The coefficient of variation (CV) was 0·3%. 10228 ampoules were filled and 5000 are available for issue as the IS, as this material will also be used as the US Standard for anti-HBs (Lot 3). Residual moisture measured on 10 samples by the Karl Fischer method gave a mean of 0·2% with a CV of 20%, and oxygen measured in the headspace of 12 ampoules gave a mean of 0·5% with a CV of 13%.

The predicted long-term stability of 07/164 was assessed by assaying samples stored at +4°C, +20°C and +37°C for a period of 6 months, along with samples stored at −20°C and −70°C. The mean potencies of the samples stored at the various temperatures, including elevated temperatures, for 6 months after freeze-drying, expressed as a percentage of the −70°C baseline sample, from three independent assays using the Behring Enzygnost Anti-HBs II assay kit were 101, 102, 97 and 100% for the samples stored at −20°, +4°C, +20°C and +37°C, respectively. The material therefore appears to be very stable, with no detectable loss of potency at higher temperatures after 6 months storage. The apparent difference between the +20°C and +37°C samples is as a result of assay variability. The usual Arrhenius model was used in an attempt to predict the long-term stability. The estimated % loss per year when stored at −20°C will be below 0·2%. 07/164 was distributed in duplicate, coded as samples B and C.

Additional study samples

1st IS for hepatitis B immunoglobulin (W1042)

This material was previously distributed by Sanquin Diagnostic Services, the Netherlands. Each ampoule contains 50 IU anti-HBs immunoglobulin and hence it has an assigned potency of 100 IU/ml when reconstituted with 0·5 ml distilled water as directed.

Coded sample A

This was a freeze-dried pool of plasma containing anti-HBs and anti-HBc which was collected from UK blood donors in the early 1990s and freeze-dried as NIBSC code 95/522. All individual donations 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.

Coded sample D

Sample D was a single-donor plasma-containing anti-HBs which was tested and found negative for HCV RNA and anti-HIV 1 + 2. This sample was distributed frozen.

Design of studies

Participants were asked to store all study samples at −20°C. They were instructed to reconstitute the 1st IS (W1042) in 0·5-ml distilled water and the freeze-dried coded samples of 07/164 in 1·0 ml distilled water on the day of reconstitution. Participants used a freshly opened and reconstituted ampoule for the preparation of dilutions and to prepare and test a series of dilutions from the 1st IS and each of the coded samples. The diluents used by the study participants were those normally used in their assays. All study samples were included in each assay so that the potency of the study samples could be expressed relative to the IS. Three independent assays were performed on different days, preferably 1 week apart. Participants were asked to test the study samples on a range of assay kits, if possible, and were asked to test the three sets of study samples with the same kits on

3 different days so that intralaboratory variation could be assessed.

Assay methods

Nineteen test kits were used. These are listed in Table 1 along with the source of antigen and the number of participants submitting data obtained with each kit. One laboratory used the Abbott Ausab RIA, which was used in the study to establish the 1st IS. Participants were asked to state the source of the HBsAg in the assay kit in case any difference in the results between assay kits could be attributed to this.

Statistical methods

Participants were asked to complete an Excel spreadsheet with the raw data from each assay and to return raw data for dose–response curves from a series of dilutions. The assays were analysed at NIBSC as parallel line assays using the raw data returned by the participants. Appropriate transformations of response and linear portions of the dose–response curves were chosen after visual inspection of the plotted data. The displacement of the dose–response curves on the log-concentration/dilution axis is used to estimate the relative concentrations of the study samples (i.e. the different dilutions of the samples required to give an identical response). Based on this analysis, potencies were expressed relative to the 1st IS, which had an assigned unitage of 100 IU/ml when reconstituted as directed in 0·5-ml distilled water. Laboratory mean potency estimates were calculated as geometric means. Overall mean potencies were calculated as the geometric means of the laboratory means, along with between-laboratory geometric coefficients of variation (% GCV).

Participants

Twenty-two participants from 12 countries participated in the study. One participant passed the dilutions prepared by them to another laboratory for assay in a locally produced kit. Both are included in the list of participants, but they are

Table 1 Assay kits used in the collaborative study and the source of antigen

Kit code	Test kit	Source of antigen	Number of laboratories using kit
K01	Enzygnost Anti-HBs II, DADE BEHRING	Inactivated HBsAg, subtypes ad and ay, from human blood	7
K02	Fijirebio Inc Lumipulse Presto HBsAb-N	Human (subtype adr)	1
K03	Bio-Rad Monolisa anti-HBs Plus – Code 72566	Human-derived HBsAg	1
K04	Bio-Rad MONOLISA™ Anti-HBs EIA, Bio-Rad, 25220	Human-derived HBsAg	1
K05	Bio-Rad, Monolisa anti-HBs 3.0, catalogue number 72400	Human-derived HBsAg, subtypes ad and ay	1
K06	BIOKIT, Bioelisa anti-HBs lot #3000-1101 modified	Inactivated HBsAg, subtypes ad and ay, from human plasma	3
K07	Diasorin anti-HBs – Kit ETI-AB-AUK-3	Human-derived HBsAg	1
K08	IMx, Abbott Laboratories	Recombinant, subtypes ad and ay	1
K09	AUSAB EIA, Abbott Laboratories	Human derived HBsAg	4
K10	AxSYM, Abbott Laboratories	*E. coli*, recombinant, subtypes ad and ay (same as ARCHITECT)	2
K11	ARCHITECT, Abbott Laboratories	*E. coli*, recombinant, subtypes ad and ay (same as AxSYM)	3
K12	Ausab RIA, Abbott Laboratories	Human-derived HBsAg	1
K13	Murex 2K95-02	Inactivated human HBsAg, subtypes ad and ay	1
K14	Roche Diagnostics Elecsys Anti-HBs	HBsAg (ad/ay), human	1
K15	Siemens Medical Solutions Diagnostics (former DPC) IMMULITE Anti-Hepatitis B Surface Ag	Purified inactivated HBsAg, subtypes ad and ay, from human plasma	1
K16	Siemens Medical Solutions Diagnostics (former Bayer) ADVIA Centaur Anti-HBs 01453163	Inactivated human HBsAg, subtypes ad and ay	1
K17	Shanghai Kehua biotech CO., LTD. Catalogue number 35.Lot NO.20071127	Mixture of HBsAg derived from human plasma and recombinant protein	1
K18	GENEDIA Anti-HBs ELISA 3.0, Green Cross Medical Science Corp., 3287B004	Inactivated human derived HBsAg	1
K19	Vitros Anti-HBs Reagent, Ortho Clinical Diagnostics, catalogue # 6801925	Human-derived HBsAg subtypes ad + ay	2

assigned only a single code number. Participating laboratories listed in Appendix 1 were randomly assigned a laboratory code number (1-21), not necessarily corresponding to the order of listing.

Results and discussion

Data received

The majority of participants included dilution series of the 1st IS and the study samples A–D in their assays, and returned raw assay data as requested. In some cases, not all of the samples were able to be included in the same assay, but separate assays, each with the 1st IS, were performed. Each sample was tested in three or four assays by each participant.

Laboratory 4 tested the 1st IS and sample A–D at only one or two dilutions, but included three dilutions of an in-house standard. The optical densities for samples A and D were outside the range covered by the in-house standard, and no reliable estimates could be obtained. The potencies of sample B and C were expressed relative to the 1st IS using a parallel line analysis including the in-house standard.

Laboratory 16 did not return raw assay data, but calculated potencies based on the kit method. These were converted to potencies relative to the IS for each of the three assays, and these values have been included in the study analysis along with the calculated potencies from the other laboratories.

Laboratory 9 returned calculated potencies based on the kit method for each dilution for each sample, rather than raw assay data. These values were taken as the assay 'response' and used to perform a parallel line analysis to obtain overall estimates of potency relative to the 1st IS.

Data analysis – potencies relative to 1st IS (W1042)

The laboratory geometric mean potencies relative to the 1st IS, which has an assigned potency of 100 IU/ml when reconstituted as directed in 0·5 ml, for samples A–D are shown in Table 2, arranged according to the different assay kits used. The laboratory means are also shown in histogram form in Fig. 1a–d for samples A, B, C and D, respectively. Each box represents the laboratory mean potency estimate relative to the 1st IS for an individual kit, and the boxes are labelled with the laboratory code number and a code representing the kit used. The kit codes used are given in Table 1.

For laboratory 21, when analysing the individual assays, it became clear that there was a consistent discrepancy in the potency estimate for sample C in assay 2, compared to the estimates from assays 1 and 3, and the estimates for the duplicate sample B. The estimate for sample C in assay 2 was around 50–70% of that in assays 1 and 3. This pattern was identical across all nine kits used. It was concluded that there had been a problem with the reconstitution of sample C or the preparation of the dilution series for this assay. The results for sample C from assay 2 were excluded from subsequent analysis.

The within-lab between-assay repeatability was assessed by calculating the GCV (%) for each sample. These are shown in Table 3. Generally, the figures represent very good repeatability, with a majority being within 10%. There are some isolated instances of poor repeatability. Laboratory 9 had higher potency estimates for samples A–D for assay 1 compared to assays 2 and 3, apparently because of

Table 2 Laboratory geometric mean potency estimates relative to International Standard (W1042)

		Sample			
Laboratory	**Kit**	**A**	**B**	**C**	**D**
01	K01	7·90	116·5	115·2	4·15
02	K09	7·64	91·2	92·0	3·14
03	K09	8·00	93·2	96·2	3·22
04	K18	–	97·2	99·6	–
05	K06	7·70	98·7	95·6	3·49
06	K01	7·80	118·6	119·6	3·78
07	K01	7·65	110·8	111·2	3·77
08	K03	8·02	97·6	99·2	1·97
09	K19	8·67	121·2	126·1	3·73
10	K01	7·24	108·9	110·4	3·68
11	K01	8·29	112·1	101·2	4·73
	K02	13·27	105·5	107·1	4·18
12	K07	9·25	124·9	128·0	4·48
13	K05	5·34	87·5	95·6	2·60
14	K01	8·03	118·8	109·4	2·99
	K09	7·88	87·6	87·1	2·95
15	K04	6·41	111·5	110·2	1·61
16	K19	7·55	103·0	102·8	3·43
17	K06	7·79	101·4	102·7	3·31
18	K11	12·31	86·2	81·8	4·22
	K17	10·26	83·3	81·3	3·56
19	K10	7·02	114·2	117·7	4·39
	K11	12·45	97·6	98·3	4·06
	K12	10·01	104·6	105·3	3·91
20	K09	5·90	75·1	74·7	2·47
21	K01	5·70	108·9	114·3	2·78
	K06	5·78	100·3	104·0	2·76
	K08	4·66	97·1	100·4	3·10
	K10	4·79	96·3	107·0	3·13
	K11	8·49	86·1	89·9	2·93
	K13	6·75	94·5	93·9	2·38
	K14	7·03	90·6	87·1	3·36
	K15	8·22	106·1	108·6	3·09
	K16	9·43	100·7	99·0	2·83

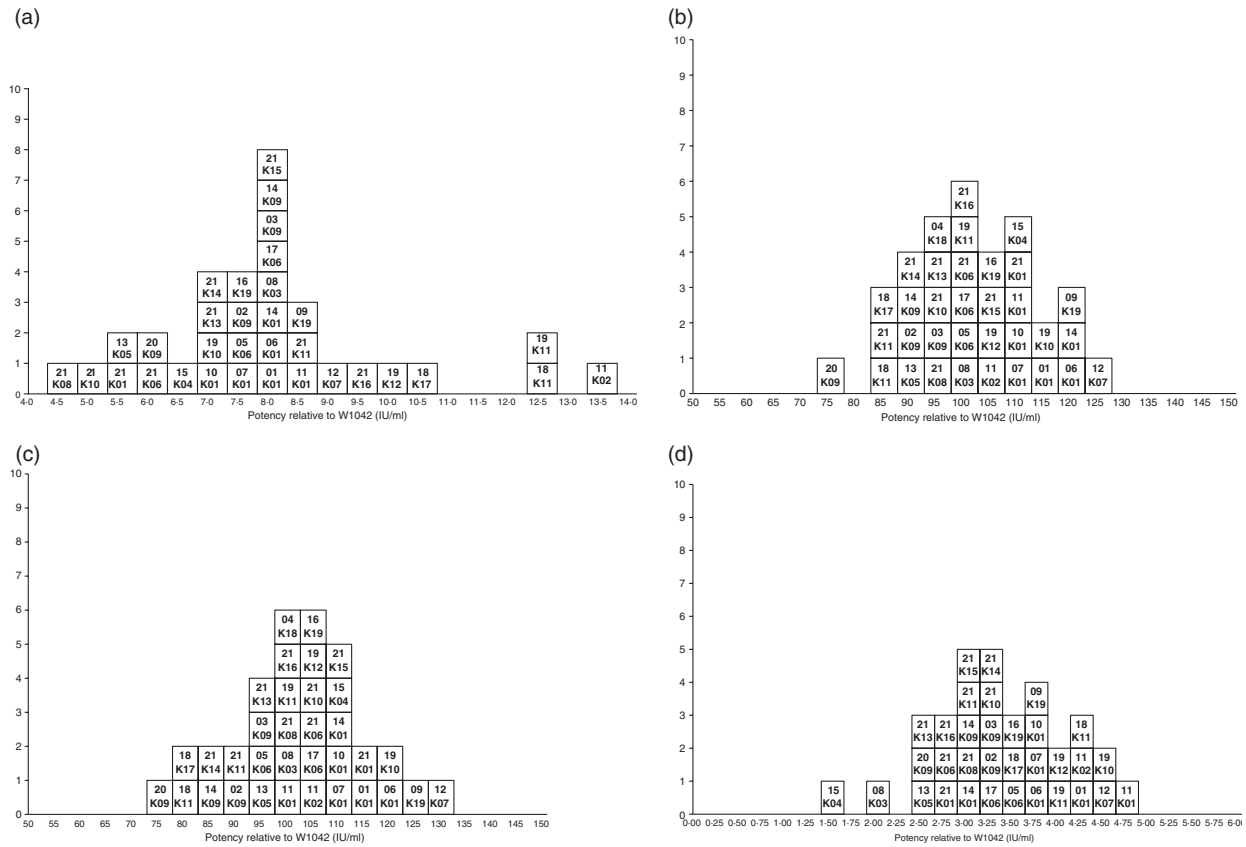

Fig. 1 (a–d) Laboratory geometric mean potencies relative to the First International Standard for samples A, B, C and D, respectively.

low responses for the 1st IS in this assay. However, the GCVs are calculated on the basis of only 3–4 assays, and are therefore sensitive to any individual assay variability. The figures should be interpreted with caution, but the overall repeatability of these assays appears to be good.

The geometric mean potency estimates for samples A–D across laboratories using the same kit, and across all laboratories, are shown in Table 4. For the candidate 2nd IS samples B and C, these were 100·7 and 101·4 IU/ml, respectively, which are very close.

The histograms indicate a reasonably symmetric distribution of potency estimates across laboratories with no obvious outlying laboratories or kits. Results are slightly more variable between laboratories for the lower potency samples A and D compared to the 2nd IS, samples B and C (GCVs between labs of 28% and 27% compared to 13% for B and C). For samples B and C, the majority of all laboratory means fall within 80–125% of the overall mean.

The potency estimates for samples B and C relative to the 1st IS appear slightly higher with kit K01 (Enzygnost Anti-HBs II) than most other kits. However, with so few of the other kits being used by more than one laboratory, it is hard to distinguish between variation between laboratories and any consistent kit effect. A comparison of laboratory mean potency estimates obtained with kit K01 to all other kits shows a significant ($P < 0{\cdot}01$) difference in the overall means with the results for kit K01 being higher for samples B and C.

The agreement in results between the duplicate samples B and C is good in all laboratories and with all kits (Tables 2 and 4). The overall geometric mean estimates (mean of 34 data sets from all laboratories) for B and C are 100·7 and 101·4 IU/ml, respectively, or 101·0 IU/ml combined. A 95% confidence interval (calculated assuming that the 34 data sets represent independent values from a normal distribution, and ignoring any consistent differences between kits) for the potency of sample B relative to the 1st IS would be 96·6–105·0 IU/ml, and for sample C, 97·1–105·8 IU/ml. The assumptions required for these calculations are not strictly met, with a lack of independence between data sets from the same laboratory with different kits, and possible consistent kit effects. However, they do give a reasonable indication of the precision, or uncertainty, of the calibration of the candidate 2nd IS, samples B and C, against the 1st IS (W1042).

Table 3 Within-Laboratory GCV (%) of potency estimates relative to International Standard (W1042)

		Sample			
Laboratory	Kit	A	B	C	D
01	K01	6	8	7	16
02	K09	8	5	6	18
03	K09	3	2	4	6
04	K18	–	15	19	–
05	K06	13	8	7	9
06	K01	2	6	7	2
07	K01	22	8	15	20
08	K03	10	17	18	9
09	K19	25	26	25	31
10	K01	6	4	4	6
11	K01	11	2	6	8
	K02	3	7	5	7
12	K07	4	8	6	5
13	K05	4	9	4	3
14	K01	9	7	20	28
	K09	6	7	8	8
15	K04	5	12	11	12
16	K19	3	4	4	4
17	K06	6	8	12	8
18	K11	6	8	13	5
	K17	12	3	6	9
19	K10	13	18	16	18
	K11	9	7	9	10
	K12	4	11	11	10
20	K09	9	9	12	12
21	K01	2	8	2	9
	K06	1	10	14	8
	K08	1	8	6	4
	K10	7	14	6	14
	K11	8	10	7	5
	K13	4	10	12	8
	K14	10	19	18	12
	K15	4	9	5	12
	K16	3	17	10	19

When the potencies of samples A and D were computed relative to that of the candidate 2nd IS 07/164, the overall values in IU/ml were nearly the same as those based on the 1st IS (Table 5). This indicates that the candidate 2nd IS, 07/164, is suitable for use in the assay of plasma pools and individual plasma samples.

The 1st IS, W1042, was established in 1977 and therefore contained antibodies from naturally infected individuals. The candidate 2nd IS, 07/164, was prepared from a production lot of anti-HBs immunoglobulin in the United States and could therefore contain antibodies from individuals who had been naturally infected or recipients of recombinant vaccines. As some assay kits now utilize recombinant antigen, participants were asked to record the source of the

Table 4 Geometric mean potency estimates for samples A–D in IU/ml

Kit	No of laboratories	Geometric mean potency	GCV (%)	Minimum potency	Maximum potency
Sample A					
K01	7	7·47	13	5·70	8·29
K02	1	13·27			
K03	1	8·02			
K04	1	6·41			
K05	1	5·34			
K06	3	7·02	19	5·78	7·79
K07	1	9·25			
K08	1	4·66			
K09	4	7·30	15	5·90	8·00
K10	2	5·80	31	4·79	7·02
K11	3	10·92	24	8·49	12·45
K12	1	10·01			
K13	1	6·75			
K14	1	7·03			
K15	1	8·22			
K16	1	9·43			
K17	1	10·26			
K19	2	8·09	10	7·55	8·67
Overall	**33**	**7·74**	**28**	**4·66**	**13·27**
Sample B					
K01	7	113·4	4	108·9	118·8
K02	1	105·5			
K03	1	97·6			
K04	1	111·5			
K05	1	87·5			
K06	3	100·1	1	98·7	101·4
K07	1	124·9			
K08	1	97·1			
K09	4	86·5	10	75·1	93·2
K10	2	104·8	13	96·3	114·2
K11	3	89·8	7	86·1	97·6
K12	1	104·6			
K13	1	94·5			
K14	1	90·6			
K15	1	106·1			
K16	1	100·7			
K17	1	83·3			
K18	1	97·2			
K19	2	111·7	12	103·0	121·2
Overall	**34**	**100·7**	**13**	**75·1**	**124·9**
Sample C					
K01	7	111·5	5	109·4	119·6
K02	1	107·1			
K03	1	99·2			
K04	1	110·2			
K05	1	95·6			
K06	3	100·7	5	95·6	104·0
K07	1	128·0			
K08	1	100·4			
K09	4	87·1	12	74·7	96·2

Table 4 Continued

Kit	No of laboratories	Geometric mean potency	GCV (%)	Minimum potency	Maximum potency
K10	2	112·2	7	107	118
K11	3	89·8	10	81·8	98·3
K12	1	105·3			
K13	1	93·9			
K14	1	87·1			
K15	1	108·6			
K16	1	99·0			
K17	1	81·3			
K18	1	99·6			
K19	2	113·9	15	102·8	126·1
Overall	**34**	**101·4**	**13**	**74·7**	**128·0**
Sample D					
K01	7	3·64	20	2·78	4·73
K02	1	4·18			
K03	1	1·97			
K04	1	1·61			
K05	1	2·60			
K06	3	3·17	13	2·76	3·49
K07	1	4·48			
K08	1	3·10			
K09	4	2·93	13	2·47	3·22
K10	2	3·70	27	3·13	4·39
K11	3	3·69	22	2·93	4·22
K12	1	3·91			
K13	1	2·38			
K14	1	3·36			
K15	1	3·09			
K16	1	2·83			
K17	1	3·56			
K19	2	3·57	6	3·43	3·73
Overall	**33**	**3·26**	**27**	**1·61**	**4·73**

antigen in the assays kits used in case this resulted in any differences in potencies obtained. These are included in Table 1. However, the antigen source does not appear to have made any difference to the overall mean potency of the candidate 2nd IS.

Conclusions

The geometric mean potency of the candidate 2nd IS in IU/ampoule is consistent for a wide range of assay kits. The geometric mean potency of the duplicate samples included in the study is 101·0 IU/ampoule and thus 101 IU/ml when reconstituted as directed in 1-ml distilled water. As this is very close to the target concentration of 100 IU/ampoule and the variation in assays is unlikely to detect the difference, 07/164 was established by the WHO as the Second International Standard for hepatitis B immunoglobulin

Table 5 Geometric mean potency estimates for Samples A and D in IU/ml calculated relative to the 1st and the candidate 2nd IS

		Sample A		Sample D	
Kit	No of Labs	1st IS	2nd IS	1st IS	2nd IS
K01	7	7·47	6·38	3·64	3·11
K02	1	13·27	12·58	4·18	3·96
K03	1	8·02	8·22	1·97	2·02
K04	1	6·41	5·75	1·61	1·45
K05	1	5·34	6·09	2·60	2·97
K06	3	7·02	7·02	3·17	3·17
K07	1	9·25	7·40	4·48	3·59
K08	1	4·66	4·80	3·10	3·20
K09	4	7·30	8·44	2·93	3·38
K10	2	5·80	5·53	3·70	3·53
K11	3	10·92	12·16	3·69	4·11
K12	1	10·01	9·57	3·91	3·74
K13	1	6·75	7·15	2·38	2·52
K14	1	7·03	7·75	3·36	3·71
K15	1	8·22	7·75	3·09	2·91
K16	1	9·43	9·36	2·83	2·81
K17	1	10·26	12·32	3·56	4·28
K19	2	8·09	7·24	3·57	3·20
Overall	**33**	**7·74**	**7·63**	**3·26**	**3·21**

with an assigned potency of 100 IU/ampoule. This material is suitable for use in the assay of prophylactic and therapeutic immunoglobulins, the calibration of quantitative diagnostic anti-HBs assay kits, and in the determination of the immune status of vaccinees and naturally infected individuals.

The 2nd IS for hepatitis B immunoglobulin, NIBSC code 07/164, is available from NIBSC (http://www.nibsc.ac.uk). Stocks of this material have been shared with the Center for Biologics Evaluation and Research (CBER)/Food and Drug Administration (FDA). It is distributed as a new CBER/FDA Reference for Hepatitis B Immunoglobulin, Lot 3, to replace the CBER/FDA Reference, Lot 2, from which the 1st IS, W1042, was derived.

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Disclaimer

The findings and conclusions in this article have not been formally disseminated by the Food and Drug

Administration and should not be construed to represent any agency determination or policy.

References

1 CDC: A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP). Part II: immunization of adults. *MMWR* 2006; **55**:1–33
2 World Health Organization: Anti-hepatitis B immunoglobulin. *WHO Tech Rep Ser* 1978; **626**:18
3 Barker LF, Lorenz D, Rastogi SC, Finlayson JS, Seligmann EB: Study of a proposed international reference preparation for anti-hepatitis B immunoglobulin. WHO Expert Committee on Biological Standardisation 29th Report BS 77.1164. Geneva, Switzerland, World Health Organization, 1977
4 Matejtschuk P, Andersen M, Phillips P: Freeze drying of biological standards. *Drugs Pharmaceut Sci* 2004; **137**:385–424
5 World Health Organization: Guidelines for the Preparation, Characterization and Establishment of International and Other Standards and Reference Reagents for Biological Substances. *WHO Tech Rep Ser* 2006; **932**:73–131

Appendix 1

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