



**WORLD HEALTH ORGANIZATION**  
**ORGANISATION MONDIALE DE LA SANTE**

**EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION**  
**Geneva, 17 to 21 November 2003**

**WHO/BS/03.1987**  
**ENGLISH ONLY**

**WHO Working Group on Hepatitis and HIV Diagnostic Kits**

**Report of a collaborative study to 1) assess the suitability of a candidate replacement International Standard for HBsAg and a reference panel for HBsAg and 2) to calibrate the candidate standard in IU.**

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**Summary**

A WHO collaborative study was undertaken to characterize and assess the suitability of a candidate replacement for the first International Standard (IS) for

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**World Health Organization 2003**

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the first International Standard (IS) for HBsAg, subtype ad and a proposed reference panel for HBsAg. The candidate replacement standard is a purified heat inactivated HBsAg subtype adw2, genotype A, and the reference panel is comprised of a series of fourfold dilutions of this material. The panel also contains a negative control sample. The candidate replacement standard was assayed against the first International Standard and a range of currently available reference materials in 6 laboratories using 10 assay kits. At least 6 sets of data were available for each assay. In addition to the first IS, the reference materials assayed were: the primary Paul Ehrlich Institute standard, subtype ad, developed by Gerlich and Thomssen, which is a native, non-inactivated HBsAg preparation; the current Paul Ehrlich Standard, subtype ad, which is heat inactivated; the French standard, inactivated with methylene blue and, the Abbott Standard, subtype ad, a native HBsAg preparation which was purified by multiple sucrose and CsCl gradients. There was more variation in the assays involving the first International Standard in the different assay kits than for any other standard.

The studies demonstrated that the units used to assign values to several commonly used HBsAg reference preparations are not equivalent. Taking all assay data into consideration, the results indicated that 1 IU is equivalent to 0.58 PEI units (primary) or 0.43 PEI units (current) or 1.93 French 'ng' or 5.59 Abbott 'ng'.

The overall mean potency in IU of the candidate IS was 33IU/vial. As the panel members were manufactured as a series of fourfold dilutions, the unitages of panel members A, B, C and D, are calculated to contain 8.25, 2.06, 0.52 and 0.13 IU/vial respectively. Panel members A-D were scored positive in all assays in Phase 1. Panel member E is a negative control sample and was scored negative in all assays. The panel was also tested in seven other laboratories using 10 immunoassays and 7 rapid tests, and the use of the panel clearly demonstrated differences in the sensitivities of assay kits.

The candidate IS, NIBSC code 00/588, has a mean weight of 0.0886g with a CV of 0.486 and a residual moisture of 0.3266. 2500 vials are available. Its stability has been assessed in accelerated degradation studies and a predicted loss of <0.001% estimated per year when stored at -20°C.

The Working Group on Reference Preparations for Testing Hepatitis B, Hepatitis C and HIV Diagnostic Kits which met at WHO Headquarters, Geneva on 6 - 7 October 2003 endorsed the proposal that the candidate IS 00/588 be established as the Second International Standard for HBsAg, subtype adw2, genotype A with a unitage of 33IU/vial. As phase 2 of the collaborative study has clearly demonstrated the usefulness of a pre-diluted panel in laboratories that may not have access to characterized reference materials, they also proposed that the 5-member panel of dilutions be established as a WHO reference panel for use in the assessment of the analytical sensitivity of assay kits that detect genotype A wild type sequences.

## **Introduction**

The Working Group on Hepatitis and HIV Diagnostic Kits held at WHO in Geneva on 16 to 19 January 2000 agreed that a new WHO HBsAg Reference Standard and a series of dilutions would be of use to National Regulatory Agencies in evaluating kits. It was agreed

that the range of dilutions should approximately encompass the range of approximately 25IU to 0.1IU/ml, as this range would cover the sensitivity of the least and most sensitive kits available worldwide.

The WHO Collaborating Centres for Biological Standards proposed that the most concentrated preparation produced, NIBSC code 00/588, be designated as a candidate replacement for the First International Standard (IS) for HBsAg<sup>1,2</sup> (subtype ad; NIBSC Code 80/549). Studies to characterize the candidate panel members were divided into two phases. In phase 1, the candidate IS was assayed along the current IS and a number of reference preparations in widespread use so that the relationships of the different units of activity assigned to these preparations can be established. These are listed below in the section on study samples. The remaining panel members (panel members A-E) were included in the study but only tested undiluted.

In the second phase of the study the candidate IS and candidate reference panel members A-E were assayed using a wide range of detection kits that are in use around the world and accessible to members of the laboratories participating in the study.

### **The candidate IS and reference panel**

Plasma derived HBsAg was purified at the Central Laboratory of the Netherlands Red Cross (Sanquin Blood Supply) by PEG precipitation and ultracentrifugation to remove Dane particles<sup>3</sup>. The purified HBsAg was inactivated by heating at 101-103° for 90 seconds followed by pasteurisation at 65° for 10h. This antigen preparation was diluted in recalcified plasma which had been shown to be negative for anti-HCV, anti-HIV 1+2, HBsAg and anti-HBs as well as negative for HCV RNA, HBV DNA and HIV RNA. Fourfold dilutions of the concentrated antigen were prepared. Merthiolate was added to the recalcified plasma as preservative. This antigen has been shown to be subtype adw2, genotype A, small particles (personal communication Dr W Gerlich). Five preparations containing four-fold dilutions of the HBsAg preparation were produced along with a negative control containing the same plasma diluent. The preparations were filled as 1ml aliquots in rubber stoppered vials and were freeze-dried. Details of the each preparation are given in Table 1.

### **Study samples**

The samples distributed to participants in phase 1 of the study along with the candidate standard and panel members were:

The International Standard for HBsAg, subtype ad, 100IU/vial<sup>1,2</sup>

The original Paul Ehrlich Institute standard, developed by Gerlich and Thomssen, which is native, non-inactivated HBsAg preparation<sup>4</sup>, supplied to participants at a concentration of 50PEI units ('ng') per ml.

The current Paul Ehrlich Standard (heat inactivated), supplied to participants at a concentration of 50PEI units ('ng') per ml.

The French (AFSSAPS) standard (methylene blue inactivated and calibrated against batch No 8 of Institut Pasteur Production plasma derived hepatitis B vaccine) supplied at a concentration of 5'French ng'/ml.

The Abbott Standard which is a native HBsAg preparation purified by multiple sucrose

and CsCl gradients, supplied at a concentration of 3.8 'Abbott ng'/ml.

The unitages assigned to some of these standards are historically referred to as 'ng' although traceability to SI units has not necessarily been established for all units. In the absence of any other assigned values, 'ng' values are qualified by the source of the standard, e.g. 'French ng' or 'Abbott ng'.

### **Stability**

Accelerated degradation studies have been undertaken on the freeze-dried preparations. Samples of each preparation were stored at elevated temperatures (+4°C, +20°C, +37°C and +45°C) for periods up to 28 months. The samples stored at +45°C could not be reconstituted and were therefore not assayed. The potency of samples of the candidate IS 00/588 stored at the other elevated temperatures were expressed relative to those of samples stored at -20°C. The results of individual assays are summarized in Table 2.

The potencies of materials stored for 17 and 28 month periods show little difference. The assays with the Abbott Auszyme kit appear to have higher potencies than for the Murex kit, but this may be due to assay variation alone. As the figures in the table are ratios, there is no reason to expect an assay kit effect.

The long-term stability of the candidate IS 00/588 was predicted using the Arrhenius model for accelerated degradation studies<sup>5</sup>. The model gave a statistically good fit to the data. The predicted percentage loss per year, or loss per month, at the different temperatures of storage, are given in Table 3. The predictions are dependent on the estimated potencies at +37°C being reliable, and the apparent drop in potency not being affected by problems of reconstitution. It is not possible to obtain reliable predictions from the data for +4°C and +20°C alone, as insufficient degradation has occurred. From these data, 00/588 appears to be adequately stable to serve as an IS. The stability will continue to be monitored over a longer period of time.

Some degradation studies were also carried out on the panel members (01/400, 01/402, 01/404 and 01/406). Again, potencies were expressed relative to samples stored at -20°C (data not shown). There are insufficient data to apply the Arrhenius model to all individual panel members. However, the data obtained so far are consistent with the pattern observed for the candidate IS and suggest that these materials will be of similar stability. Additional assays are being undertaken.

The candidate panel members contain preservative because they may not be used up in a single day by users, so stability studies on reconstituted materials have also been undertaken. The reconstituted contents of samples of each preparation gave the same potency in IU after storage at 2-8° C for 6 months (data not shown).

### **Design of the study**

The collaborative study was divided into two phases. In phase 1, six laboratories assayed dilutions of the International Standard (80/549), the candidate replacement International Standard 00/588, the primary PEI standard, the French standard, and the Abbott

Diagnostics standard on three separate days, in a series of previously agreed assays. The unitage assigned to each reference preparation is given in Table 4 and the assay kits used are detailed in Table 5. Each assay was used in at least two laboratories so that at least 6 sets of data from the use of each kit were available. Diluent (recalcified plasma negative for HBsAg, anti-HCV, anti-HIV 1+2 and anti-HBs) was supplied for the preparation of dilutions by all the participating laboratories. Panel members A-E undiluted were also assayed.

In phase 2 of the study, laboratories around the world were asked to assay the candidate IS and reference panel members A-E in a range of assay kits available in their countries. They were encouraged to use locally produced kits and rapid assays

### **Participating Laboratories**

Six laboratories participated in phase 1 and 7 participants in phase 2. The laboratories are listed in the Appendix and are indicated in the figures by letter assigned randomly.

### **Statistical methods**

All assays were analyzed as parallel-line assays<sup>6</sup> using either the untransformed or the log-transformed optical density plotted against log-dilution. The optimal linear portion of the dose-response curve was selected by eye for each sample in each assay. Where necessary, responses at the extremes of the linear portion of the dose response were omitted. The between replicate variation was very small in many assays, suggesting that there may not have been adequate replication, and that the between replicate variation was not a realistic estimate of the within-assay variability. The usual tests for linearity and parallelism were therefore not applied.

The potency of each sample was calculated against each of the other preparations in each assay without taking into consideration any pre-assigned unitage as this facilitated the use of a common scale in the figures. Laboratory means were calculated as geometric means, and within assay variability was expressed by calculating a geometric coefficient of variation (%gcv)<sup>7</sup>. Overall mean potency estimates were obtained by calculating geometric means of the individual laboratory means.

Data were submitted on the use of 10 assay kits that are identified in the figures by the assay code listed in Table 5. The HBsAg content of each preparation was expressed as a percentage of each other preparation with each assay kit. The means of the HBsAg content obtained with each kit were then calculated followed by the overall mean.

### **Results**

The HBsAg content of each preparation relative to the first International Standard along with overall means and %GCV are given in Table 6 and Figure 1 and against the candidate IS in Table 7 and Figure 2. Table 7a is another way of looking at this information, where data of Table 7 have been unitized by dividing values in each column by the average value of the standard. Potency expressed relative to the International Standard in International Units is equivalent to expression as a percentage as the International Standard has an assigned unitage

of 100IU per vial. The potency of each preparation relative to each of the other study samples at the concentration supplied can be calculated from these tables. The data for each laboratory and each standard relative to the primary PEI standard are shown in Figure 3; against the current PEI standard in Figure 4; relative to the French standard in Figure 5 and to the Abbott standard in Figure 6.

The variability of the potency estimates for all assay kits is greater when measured against the current International Standard than for any other reference preparation. This is also evident from inspection of the figures; the spread of results in Figure 1 is greater than in any other figure. This observation was consistent among all laboratories as can be seen with a clustering of mean potencies of the PEI current standard against the IS determined in individual kits e.g. kit 11 (Ortho). The reason for this is not known. However, assay kits which gave the highest and lowest potencies for the primary PEI standard when assayed against the current IS and the candidate replacement IS 00/588 were the same for both of these preparations as can be seen in Tables 6 and 7. This did not hold true for potencies of the current PEI standard, the French and the Abbott standards (data not shown). The reason for the difference between primary and current PEI standards may be due to the current standard being heat inactivated or to the production of pre-dilutions of the PEI material prior to distribution of the study samples where the material was subjected to an additional freeze-thaw cycle.

Overall means for each standard relative to each other reference material at the concentration supplied are summarized in Table 8 and the %GCVs are given in Table 9.

The actual unitages of the standards were calculated from the data given in Table 8 and the relationship between all units is given in Table 10. A review of the raw data indicates that both the French and Abbott standards gave unitages in IU considerably lower than expected. A review of the raw data from some of the assays confirmed that this was the case. So approximately –

1 IU	= 0.58 PEI units (primary ng)
1 IU	= 0.43 PEI units (current)
1 IU	= 1.9 French ng
1 IU	= 5.6 Abbott ng
1 PEI unit	= 11.3 Abbott ng
1 PEI unit	= 2.9 French ng

The overall mean potency in IU of the candidate IS was 33 IU/vial. The potency of the candidate IS in terms of the other units is given in Table 10. Panel members A-D were scored positive in all assays conducted as part of phase 1 and panel member E, the negative control, was scored negative in all assays. The predicted potencies of panel members A-D, which were produced as a series of fourfold dilutions are given in Table 11.

In phase 2 of the collaborative studies, seven laboratories assayed the proposed panel in 10 immunoassays and 7 rapid tests. The results are summarized in Table 12. The use of the panel clearly demonstrates differences in the sensitivities of assay kits and shows the utility of the proposed panel as a tool for objectively judging the suitability of the numerous HBsAg tests kits worldwide.

## Traceability of the International Standard

The current WHO International Standard for HBsAg is traceable to an independent biochemical determination of its unitage. This study shows that the current WHO International HBsAg standard has the following relationship to a 'ng' of HBsAg as defined by Gerlich and Thomssen (material generated in 1974 and subsequently used as the primary reference material of the Paul Ehrlich Institute): *1.0 WHO IU of HBsAg = 0.58 ng of original PEI HBsAg*. Data from the first collaborative study of the WHO International Standard for HBsAg in 1980<sup>1</sup> showed a relationship with the primary PEI standard almost identical with that found in this study: *1.0 WHO IU of HBsAg = 0.55 ng of primary PEI HBsAg*. Thus, the immunological relationship between the two reference materials has remained constant over 20 years. PEI units for immunoassay were based on ng values found by biochemical analysis in studies performed by Drs. Gerlich and Thomssen in 1974<sup>4</sup>. These investigators extensively purified HBsAg (ad) from serum and subsequently analyzed the material by conventional biochemical technologies. The results of this extensive study have since been confirmed by at least one independent laboratory.

In recent correspondence from Dr. Gerlich in October 2003, he stated that he had performed a purification of HBsAg/ayw2/D from plasma with quantitative follow-up of the protein in the year 2000. The amounts of HBsAg associated with the protein were measured by QIE and were calibrated relative to the PEI material. Results were consistent with values he had obtained in 1974. Quoting Dr. Gerlich, "*Thus, these units are traceable even today.*" This study shows that the metrological unitage determined for the original PEI HBsAg 30 years ago is equivalent to the unitage of the same material today based on the biochemical characteristics of this material.

From these findings, it is concluded that, since the WHO HBsAg material and the PEI HBsAg material have the same immunological relationship today as they had in 1980, and since the unitage of the PEI HBsAg is traceable to biochemical values, the WHO HBsAg unitage is also traceable to these biochemical values and has not drifted since its establishment in 1985.

## Conclusions

These studies have demonstrated that the relationship between the IU and the PEI unit is the same as that found in studies undertaken in 1980 when the First IS was assessed for suitability and that this relationship holds for the candidate replacement standard for HBsAg. Traceability to the PEI ng has been shown because the latter reference standard was characterized by a biochemically reproducible method. This study, then, assigns a traceable unitage to the candidate replacement standard for HBsAg subtype adw2, genotype A, 00/588.

These studies have also demonstrated that 'ng' values assigned to other standards are not equivalent, and some of these values may have drifted over time. It is recommended that a uniform IU nomenclature and a single HBsAg preparation having the traceability characteristics presented here be used globally to avoid these discrepancies and to provide immunological equivalence.

The Phase 2 studies have demonstrated that the proposed HBsAg panel will be of use in assessing the analytical sensitivity of the many assay kits used worldwide, particularly in laboratories where it is impractical or undesirable to prepare dilutions.

**Proposals**

1. It is proposed that the candidate IS, NIBSC code 00/588, be established as the Second International standard for HBsAg, subtype adw2, genotype A with an assigned unitage of 33IU/vial.
2. It is proposed that panel members A-E be established as a WHO reference panel for HBsAg for use by national regulatory authorities in the assessment of the sensitivity of assays kits for the detection of HBsAg.

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**Appendix****Participants – phase 1**

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**Figure Legends**

Figure 1: The potency of each preparation relative to the International Standard expressed as a percentage or IU. The laboratories are by a letter assigned randomly.

Figure 2: The potency of each preparation relative to the candidate IS expressed as a percentage. The laboratories are by a letter assigned randomly.

Figure 3: The potency of each preparation relative to the primary PEI standard expressed as a percentage without taking into consideration the unitage of the materials assayed. The laboratories are by a letter assigned randomly.

Figure 4: The potency of each preparation relative the current PEI standard expressed as a percentage without taking into consideration the unitage of the materials assayed. The laboratories are by a letter assigned randomly.

Figure 5: The potency of each preparation relative to the French standard expressed as a percentage without taking into consideration the unitage of the materials assayed. The laboratories are by a letter assigned randomly.

Figure 6: The potency of each preparation relative to the Abbott standard expressed as a percentage without taking into consideration the unitage of the materials assayed. The laboratories are by a letter assigned randomly.

**Table 1** Characteristics of the candidate IS and reference panel members

Sample	NIBSC Code number	Number vials available	Residual moisture	Mean Dry Weight, g	CV
Candidate IS	00/588	2504	0.3266%	0.0886	0.486
Panel member A	01/400	2168	0.5723%	0.0852	0.467
Panel member B	01/402	2761	0.7160%	0.0888	0.526
Panel member C	01/404	2318	0.5122%	0.0875	1.123
Panel member D	01/406	2247	0.8148%	0.0902	2.942
Panel member E (Negative control)	00/616	2777	0.2290%	0.0807	0.545

**Table 2:** Potency of samples stored at elevated temperatures as proportion of that of sample stored at  $-20^{\circ}\text{C}$ 

Kit Temp	Murex GE34			Abbott Auszyme
	17 months	18 months	28 months	28 months
$+4^{\circ}\text{C}$	0.901	0.958 0.882	0.935	1.074
$+20^{\circ}\text{C}$	0.839	0.911 0.781	0.894	1.010
$+37^{\circ}\text{C}$	0.139	0.150 0.171	0.048 0.061	0.085

**Table 3** The predicted percentage loss per year or loss per month at the different temperatures of storage

Temperature of storage	% loss per month	% loss per year
$-20^{\circ}\text{C}$		<0.001
$+4^{\circ}\text{C}$		0.18
$+20^{\circ}\text{C}$	0.43	
$+37^{\circ}\text{C}$	9.84	

**Table 4 Assigned unitage of study samples included in Phase 1 at the dilution supplied**

Preparation	Assigned unitage of material supplied to participants
International Standard	100IU/ampoule.
Original Paul Ehrlich standard (50000PEI units/ml; pre-diluted 1 in 1000)	50 PEI units/ml equivalent to 50 'PEI ng'/ml
Current Paul Ehrlich Inst. standard (1000PEI units/ml; pre-diluted 1 in 20)	50 PEI Units/ml equivalent to 50 'PEI ng'/ml
AFSSAPS standard	5 'French ng'/ml
Abbott Standard	3.8 'Abbott ng'/ml
Candidate IS (00/588)	No unitage assigned.
Panel member A	1 in 4 dilution of candidate IS
Panel member B	1 in 16 dilution of candidate IS
Panel member C	1 in 64 dilution of candidate IS
Panel member D	1 in 256 dilution of candidate IS
Panel member E	Human re-calcified plasma

**Table 5 Assays kits used in Phase 1**

Test Code	Test method	Kit version, code number
1.	Abbott	Prism
2.	Abbott	AxSYM
4.	Abbott	Auszyme Cat No 1980-24
5.	Sanofi (Biorad)	Monolisa AgHBs plus 72313/72314"
6.	Abbott	Murex HBsAg version 3 GE 34/36
7.	Behring	Enzygnost
8.	Biotest/Biokit	
9.	Dia-Sorin	ETI MAK 4 ref : N0019"
10.	BioMerieux	Vidas HBs Ag ref: 30301"
11.	Ortho Clinical Diagnostics	Ab to HBsAg ELISA (test system 3 ref : 931802/00/50)

(NB Test kit 3 (Abbott Ausria) is no longer available so could not be included in the studies)

**Table 6 Potencies of Samples against the Current IS (IU or %)**

Assay	Number of laboratories each performing 3 assays	Candidate IS (00/588)	PEI Primary supplied as 50 PEI units/ml	PEI Current supplied as 50 PEI units/ml	French supplied as 5 'ng'/ml	Abbott supplied as 3.8 'ng' /ml
Abbott Prism	2	34.99	85.50	126.37	2.15	0.57
Abbott AxSym	2	26.90	55.61	109.48	1.94	0.58
Abbott Auszyme	3	32.39	82.37	129.78	2.98	0.78
Sanofi (Biorad) Monolisa	2	44.77	150.97	140.24	3.62	0.76
Abbott Murex GE 34	2	22.00	52.01	69.32	1.73	0.33
Behring Enzygnost	2	28.96	63.17	79.62	2.18	0.75
Biotest/Biokit	2	25.60	66.07	86.20	1.72	0.61
Dia-Sorin ETI MAK 4	2	26.66	76.74	112.19	2.30	0.57
BioMerieux Vidas	2	38.60	85.50	104.48	2.33	0.73
Ortho version 3.0	4	47.33	93.50	195.33	4.57	1.04
	<b>Overall</b>	<b>33.02</b>	<b>85.66</b>	<b>117.03</b>	<b>2.59</b>	<b>0.68</b>
	<b>%GCV</b>	<b>30</b>	<b>45</b>	<b>38</b>	<b>45</b>	<b>39</b>

**Table 7 Potencies of Samples against the Candidate IS (00/588)**

Assay	Number of laboratories each performing 3 assays	IS	PEI Primary supplied as 50 PEI units/ml	PEI Current supplied as 50 PEI units/ml	French supplied as 5 'ng'/ml	Abbott supplied as 3.8 'ng' /ml
Abbott Prism	2	285.76	244.34	361.10	6.16	1.64
Abbott AxSym	2	371.70	206.71	406.92	7.21	2.15
Abbott Auszyme	3	308.75	254.33	400.69	9.21	2.41
Sanofi (Biorad) Monolisa	2	223.38	337.24	313.27	8.10	1.71
Abbott Murex GE 34	2	454.51	236.40	315.05	7.88	1.48
Behring Enzygnost	2	345.30	218.14	274.94	7.53	2.60
Biotest/Biokit	2	390.56	258.03	336.65	6.73	2.37
Dia-Sorin ETI MAK 4	2	375.11	287.87	420.83	8.64	2.13
BioMerieux Vidas	2	259.05	242.23	270.66	6.03	1.88
Ortho version 3.0	4	211.30	300.02	412.75	9.65	2.20
	<b>Overall</b>	<b>302.87</b>	<b>259.43</b>	<b>354.45</b>	<b>7.85</b>	<b>2.06</b>
	<b>%GCV</b>	<b>30</b>	<b>16</b>	<b>18</b>	<b>22</b>	<b>21</b>

**Table 7a: Unitage of 5 standards with 10 commercial test kits relative to the candidate IS: the potency of each standard obtained with each kit was divided by the average potency for that standard, from Table 7.**

Assay Kit	IS (current)	PEI (primary)	PEI (current)	French	Abbott
Abbott Prism	0.95	0.94	1.02	0.79	0.80
Abbott AXSYM	1.23	0.80	1.15	0.92	1.04
Abbott Auszyme	1.02	0.98	1.13	1.17	1.17
Sanofi Monalisa	0.74	1.30	0.88	1.03	0.83
Abbott Murex	1.50	0.91	0.89	1.00	0.72
Behring Enzygnost	1.14	0.84	0.78	0.96	1.26
Biotest Biokit	1.29	1.00	0.95	0.86	1.15
Dia-Sorin ETI	1.24	1.11	1.19	1.00	1.03
Biomerieux Vidas	0.86	0.93	0.76	0.77	0.91
Ortho version 3.0	0.70	1.16	1.17	1.23	1.07
<i>Average potency (from Table 7)</i>	<i>(302.9)</i>	<i>(259.4)</i>	<i>(354.5)</i>	<i>(7.85)</i>	<i>(2.06)</i>
<i>% GCV (from Table 7)</i>	<i>(30)</i>	<i>(16)</i>	<i>(18)</i>	<i>(22)</i>	<i>(21)</i>

**Table 8** The mean concentration of each preparation at the dilution supplied, expressed as a concentration of each other as a percentage

Potency expressed as % of standard	Test Preparation with assigned unitage at dilution supplied					
	Int Std 100IU/vial	Candidate IS (00/588)	PEI original (supplied as 50PEI units/ml)	PEI current (supplied as 50PEI units/ml)	French standard 5'ng'/ml	Abbott 3.8'ng'/ml
International Standard	<b>100</b>	33.02	85.66	117.03	2.59	0.68
Candidate IS (00/588)	302.87	<b>100</b>	259.43	354.45	7.85	2.06
First Paul Ehrlich Primary standard	116.75	38.55	<b>100</b>	136.63	3.03	0.79
Current Paul Ehrlich Inst. standard	85.45	28.21	73.19	<b>100</b>	2.21	0.58
AFSSAPS standard	3859.27	1274.24	3305.71	4516.59	<b>100</b>	26.2
Abbott Standard	14730.56	4863.68	12617.67	17239.50	381.69	<b>100</b>

**Table 9** Percentage GCVs for each preparation against each standard

Standard	%GCV for preparation					
	Int Std	Candidate IS (00/588)	PEI original	PEI current	French	Abbott
International Standard – IU	N/A	30	45	38	45	39
Candidate IS (00/588) Not assigned	30	N/A	16	18	22	21
First Paul Ehrlich Primary standard	45	16	N/A	21	20	29
Current Paul Ehrlich Inst. standard	38	18	21	N/A	19	25
AFSSAPS standard	45	22	20	19	N/A	23
Abbott Standard	39	21	29	25	23	N/A

**Table 10** Relationship of the different units in terms of each other

Unit	Unitage equivalent to 1 IU	IU equivalent to 1 unit of each reference preparation	Potency of the Candidate IS (00/588) in each unitage
International Unit	1.0	1.0	33
PEI units primary	0.584	1.713	19.3
PEI units current	0.427	2.341	14.1
French ng	1.931	0.518	63.7
Abbott ng	5.587	0.179	184.4

**Table 11 Potencies in IU of panel members A-D**

Panel member	Predicted IU HBsAg/vial
A	8.25
B	2.06
C	0.52
D	0.13

**Table 12 Phase 2 -Assay of panel in a range of EIA and other assay kits**

Manufacturer	Kit	Panel member					
		Cand IS	A	B	C	D	E#
Green Cross Life Sciences Corp	EIA	R	R	R	R	R/NR	NR
Asan Pharmaceutical Co Ltd	EIA	R	R	R	R	NR	NR
Dong-A Pharm	EIA	R	R	R	R	R	NR
LG Chem	EIA	R	R	R	R	R/NR	NR
ThermoLab systems, Helsinki	EIA	R	R	R	R	NR	NR
J Mitra&Co, New Delhi	EIA	R	R	R	R	NR	NR
In house	RIA	R	R	R/NR	NR	NR	NR
Shanghai KEHUA Biotech	EIA	R	R	R	NR	NR	NR
Ortho-Clinical Diagnostics	Ab to HBsAg ELISA (test system 3 code 931802)	R	R	R	R	NR	NR
Abbott	IMX V2	R	R	R	R	R	NR
Fujirebio	Lumipulse HBsAg (CLIA)	R	R	R	R	R	NR
Fujirebio	ESPLINE HBsAg (Immunochromatography)	R	R	R	R	NR	NR
Asan Pharmaceutical Co Ltd	Asan Easy test HBs	R	R	NR	NR	NR	NR
Green Cross Life Sciences Corp	Genedia HBsAg rapid	R	R/NR	NR	NR	NR	NR
Orchid Biomedical Systemsd, Goa	Virucheck	R	R	NR	NR	NR	NR
Trinity Biotech, Ireland	Unigold	R	R	NR	NR	NR	NR
Dr Reddy's Laboratory, Hyderabad	Fast Forward	R	R	NR	NR	NR	NR
Equipar SRI Italy	Dipstick, rapid	R	NR	NR	NR	NR	NR
Standard Diagnostics	Bioline	R	R/NR	NR	NR	NR	NR
Standard Diagnostics	Bioline HBsAg	R	R	R/NR	NR	NR	NR

# Panel member E = negative control

R reactive; NR non-reactive





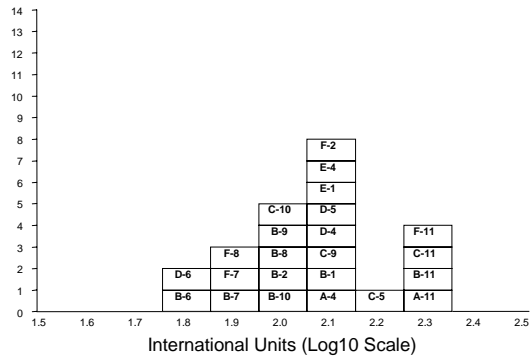
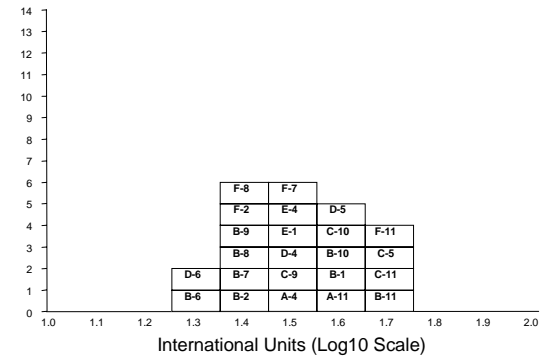
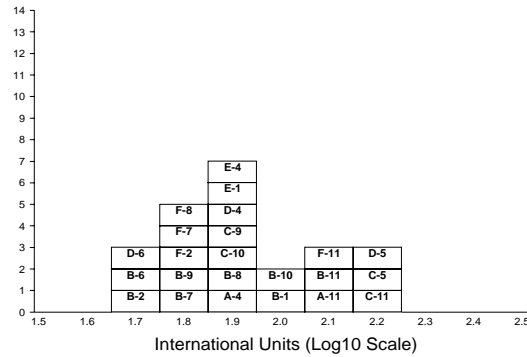


Figure 1  
 Potency against the IS

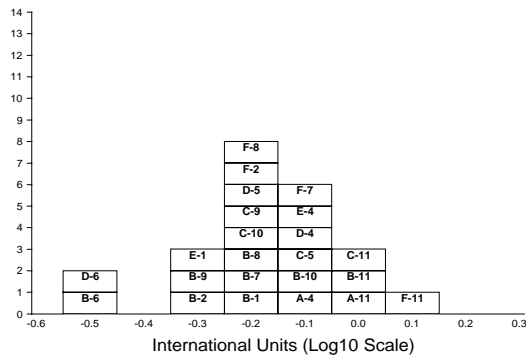
Potency of Candidate IS (IU)



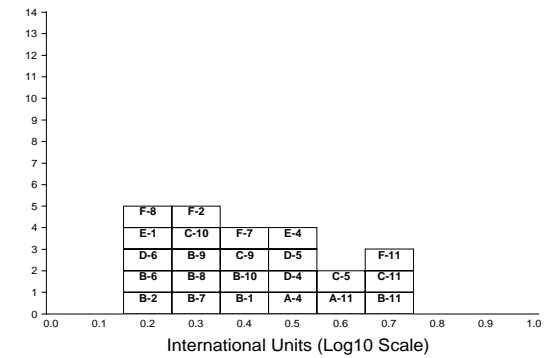
Potency of PEI Primary Standard (IU)



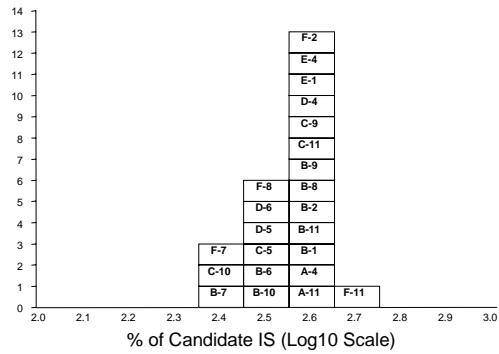
Potency of Abbott Standard (IU)



Potency of AFSSAP Standard (IU)



Potency of PEI Current relative to Candidate IS (%)



Potency of PEI Primary relative to Candidate IS (%)

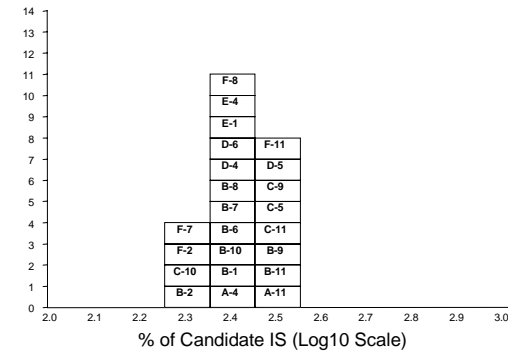
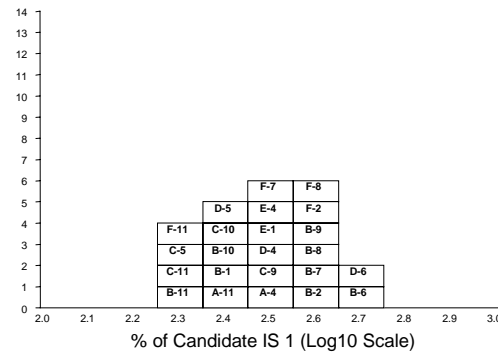
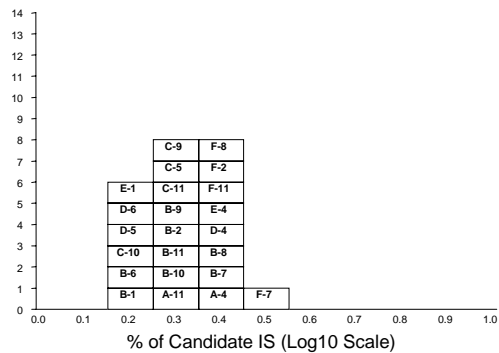


Figure 2  
Potency against the  
candidate IS

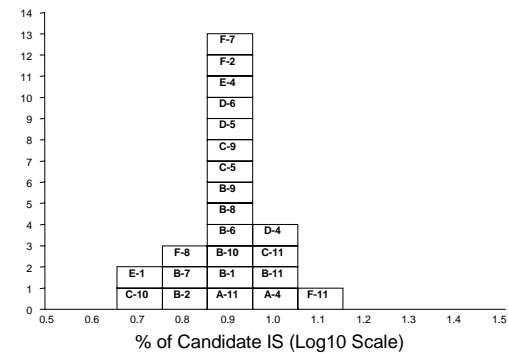
Potency of IS relative to Candidate IS (%)



Potency of Abbott Standard relative to Candidate IS (%)



Potency of AFFSAP Standard relative to Candidate IS (%)



Potency of PEI Current relative to PEI Primary (%)

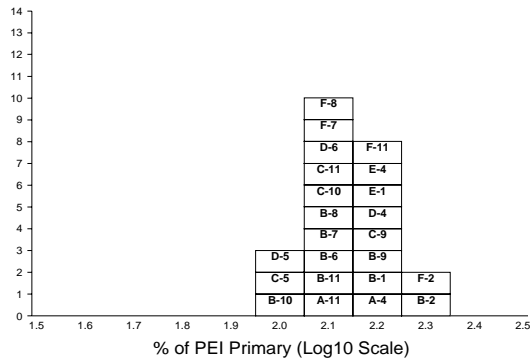
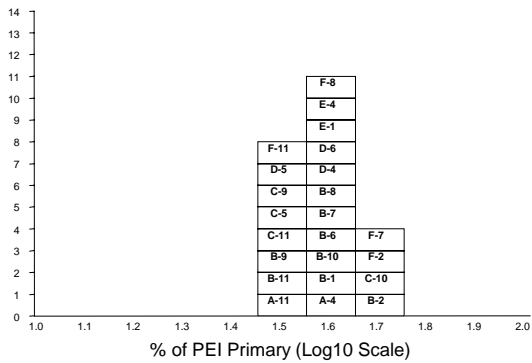
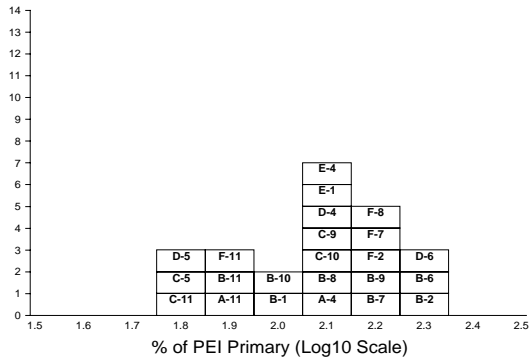


Figure 3  
Potency against the primary PEI standard

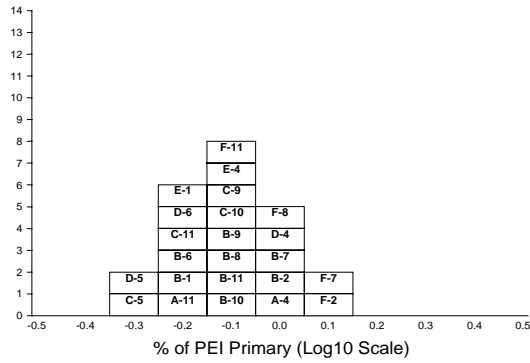
Potency of Candidate IS relative to PEI Primary (%)



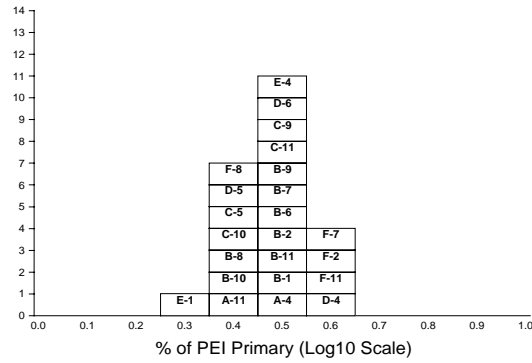
Potency of IS relative to PEI Primary (%)



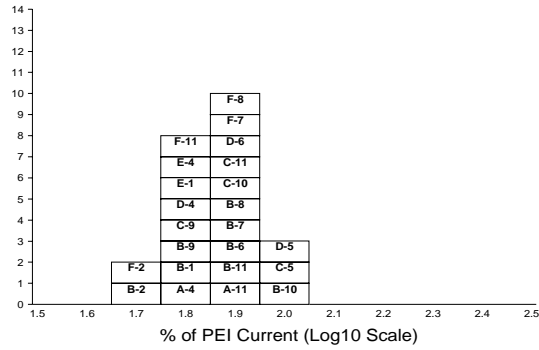
Potency of Abbott Standard relative to PEI Primary (%)



Potency of AFFSAP Standard relative to PEI Primary (%)



Potency of PEI Primary relative to PEI Current (%)



Potency of Candidate IS relative to PEI Current (%)

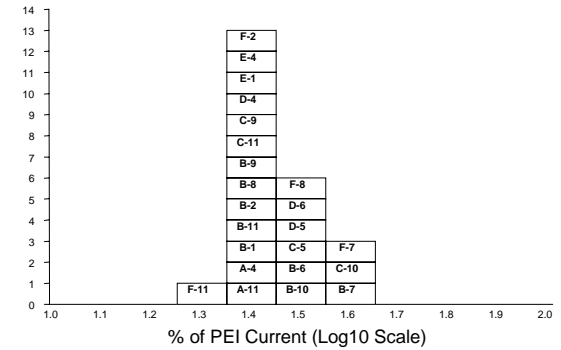
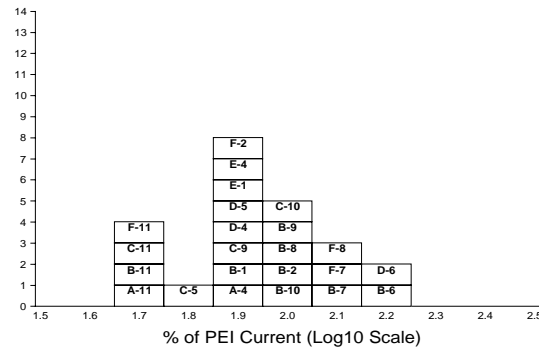
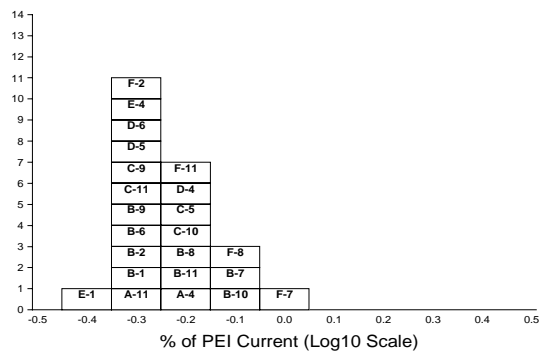


Figure 4  
Potency against the current PEI standard

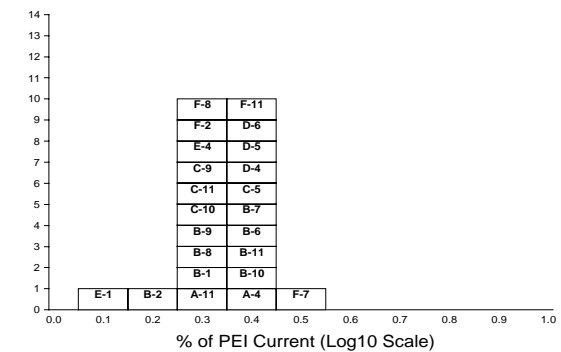
Potency of IS relative to PEI Current (%)



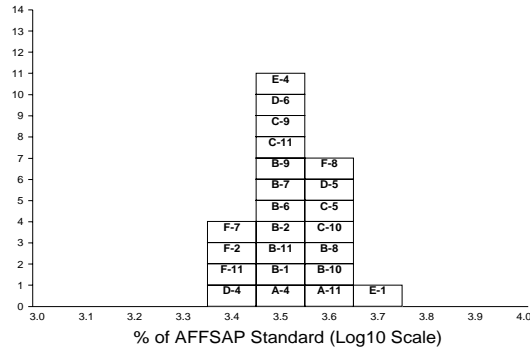
Potency of Abbott Standard relative to PEI Current (%)



Potency of AFFSAP Standard relative to PEI Current (%)



Potency of PEI Primary relative to AFFSAP Standard (%)



Potency of Candidate IS relative to AFFSAP Standard (%)

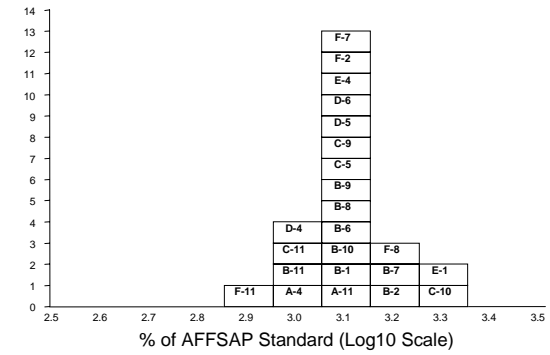
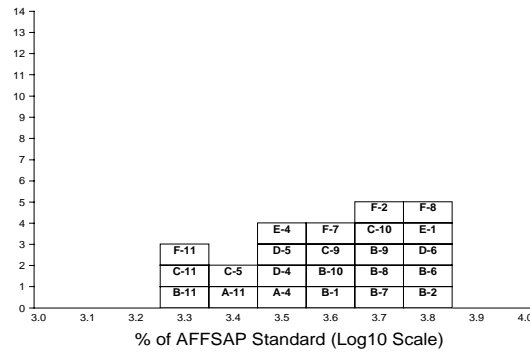
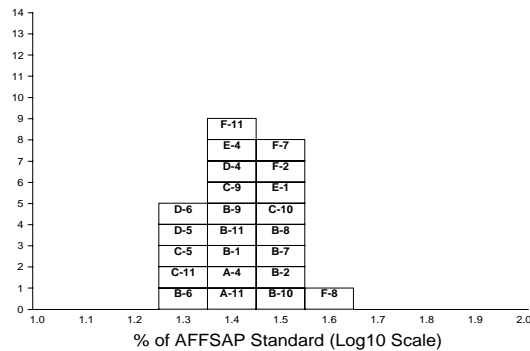


Figure 5  
Potency against the  
French standard

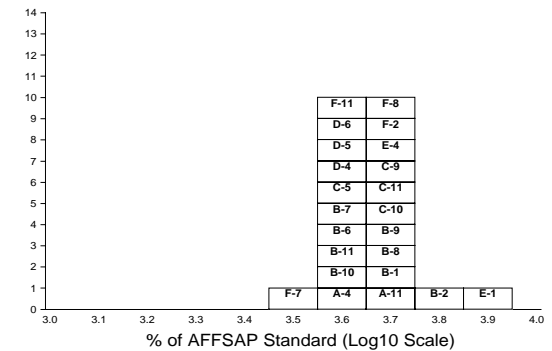
Potency of IS relative to AFFSAP Standard (%)



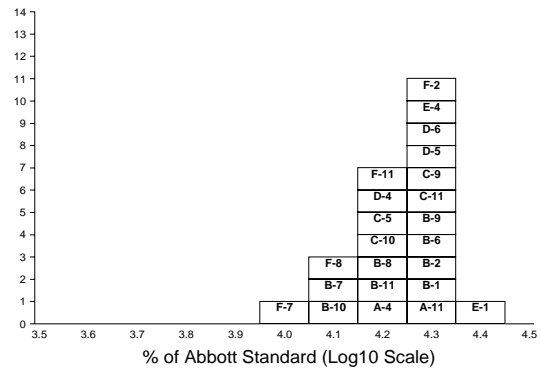
Potency of Abbott Standard relative to AFFSAP Standard (%)



Potency of PEI Current relative to AFFSAP Standard (%)



Potency of PEI Current relative to Abbott Standard (%)



Potency of Candidate IS relative to Abbott Standard (%)

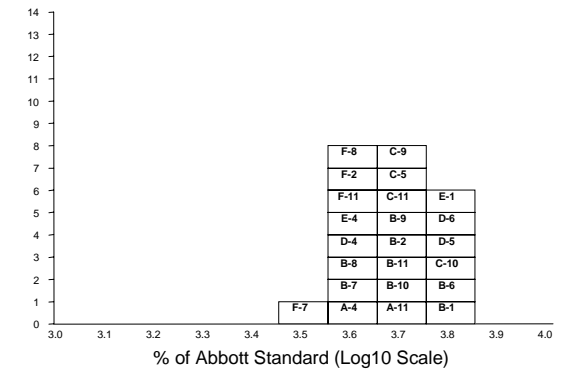
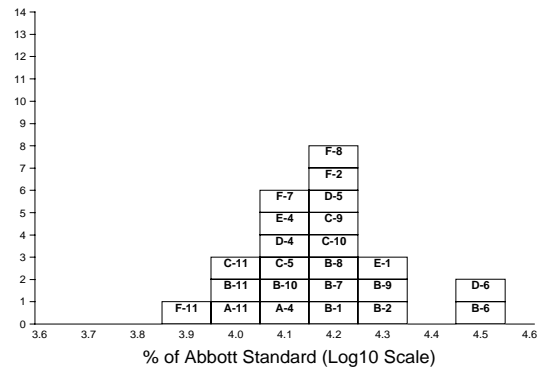
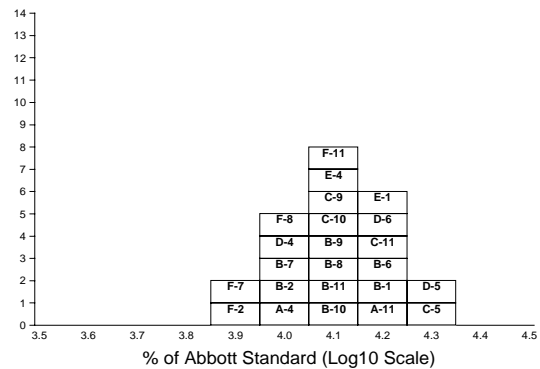


Figure 6  
Potency against the  
Abbott standard

Potency of IS relative to Abbott Standard (%)



Potency of PEI Primary relative to Abbott Standard (%)



Potency of AFSSAP Standard relative to Abbott Standard (%)

