STANDARDIZATION OF THE NATIONAL REFERENCE ANTI-HBs IMMUNOGLOBULIN BY RADIOIMMUNOASSAY

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SUMMARY: The method for assaying potency of anti-HBs immunoglobulin (HBIG) developed in our Department was applied to the standardization of the Japanese national reference HBIG preparation which is specified in the Minimum Requirements for Biological Products. The method was studied for utility in routine anti-HBs determination. The reference HBIG preparation proved to be satisfactorily stable.

INTRODUCTION

Although there is an agreement on the effectiveness of anti-HBs immunoglobulin (HBIG) in such post-exposure prevention of hepatitis B as one after accidental needle pricks, further study will be needed to see if its efficacy in other occasions of hepatitis B infection can be established as well. There may be such a wide range in the infectivity of the source of infection, on the one hand, and a level of exact quantitation does not seem to have been attained by everyone in expressing anti-HBs antibody content of HBIG, on the other.

In a previous report (Yasuda et al., 1979), we described a method for potency determination of HBIG by radioimmunoassay (RIA) by applying the parallel line assay method (Finney, 1964). As the International Reference Preparation of HBIG (hereafter referred to as IRHBIG) was not available when we started our work, comparison was then made with the Reference Preparation Lot 2 supplied by the Bureau of Biologics, U.S. Food and Drug Administration (hereafter referred to as Bob-2) from which IRHBIG is said to have been prepared (WHO/BS/77.1164).

Although it is theoretically possible to calculate the potency of Bob-2 from the units assigned to IRHBIG, Lot 26-1-77 (501U/ampoule reconstituted to 0.5 ml) by multiplying by 3.667, which is the protein concentration ratio of the former to the latter (16.5/4.5), there has been no official information concerning the potency in international units of the former. Therefore, we tried direct comparison between IRHBIG and our proposed National Reference HBIG (hereafter referred to as NRHBIG) to which international units had to
be assigned. As the outcome of our work in this direction, the Monograph for HBIG along with the designation of NRHBIG was added to the Minimum Requirements for Biological Products (MRBP) (Ministry of Health and Welfare, 1979) in 1980.

In this report, the results of our experiments in the process of standardization of NRHBIG will be presented. It is also attempted to demonstrate the utility of NRHBIG in potency testing of commercial HBIG preparations and to assure its stability.

**Materials and Methods**

*HBIG preparations:* 1) *IRHBIG*, lot 26-1-77. A lyophilized preparation containing 50 International Units (IU) per ampoule according to the label and to a WHO document (Expert Committee, 1978). From another WHO document (WHO/BS/77.1164), each ampoule was filled with 0.5 ml of a 9% protein solution of a bulk material of the HBIG preparation. On experiment, the total content was dissolved in one ml of the diluent described below, so that the reconstituted solution contained 4.5% protein and 100 IU/ml.

2) *BoB-2*. A liquid product kindly supplied by the courtesy of Dr. E. B. Seligmann, BoB, is a 16.5% protein solution of the HBIG preparation from which IRHBIG was prepared (WHO/BS/77.1164). Therefore, the concentration ratio of IRHBIG to BoB-2 is calculated at 16.5/4.5=3.667.

3) *NRHBIG*. A liquid product described as Japanese Red Cross lot 5 (abbreviated as JRC-5) in our previous paper (Yasuda et al., 1979). This was obtained from the Japanese Red Cross Central Blood Center, Tokyo. Though most of data in the present paper were obtained while current NRHBIG was still a candidate, the term “NRHBIG” will be used throughout the present report to avoid possible confusion.

4) *Seventeen liquid products prepared by four manufacturers*. Two of the manufacturers are Japanese and the remaining two are foreign. Some of the products were used in clinical trials by the Project Team for the Development of HBIG sponsored by the Ministry of Health and Welfare (Chairman: Prof. F. Ichida, Niigata Univ. School Med.).

*RIA:* The method for RIA is as described in the previous paper (Yasuda et al., 1979). Briefly it is as follows: The AUSAB kit (Abbott Lab.) for the solid phase RIA technique was used. The directions for use were followed excluding the instructions to use negative controls. For dilution of test samples, physiological saline containing human serum albumin conforming to MRBP in 0.02% was used (Saito et al., 1979). Each of the double dilution steps was tested with three or more beads, and cpm was read with each of the beads.

*Statistical analyses:* $\log_{10}$ net cpm of the test specimen, without calculating the ratio of cpm of the test specimen to the cut-off value, and the $\log_{10}$ dose (dilution factor) were used for statistical analyses. The parallel line assay method (Finney, 1964) was applied to the validity test of assay results and in estimation
of the relative potency (Yasuda et al., 1979).

**RESULTS**

*Standardization of NRHBIG*

Anti-HBs antibody content of NRHBIG together with IRHBIG and BoB-2 was determined by RIA. As shown in Fig. 1, no significant deviation from parallism was found among the three dose-response regression lines. The statistical analyses of the data in Fig. 1 are presented in Table I. The relative potencies (R) and their upper and lower confidence limits (R_U, R_L) at the 0.95 level of NRHBIG and BoB-2 to IRHBIG are summarized in Table II.

The IU of these preparations were calculated from the above relative potencies at $50 \times 2.020 = 101.00$ IU/ml for NRHBIG and $50 \times 3.581 = 179.05$ IU/ml for BoB-2. The difference between the potency ratio of IRHBIG to BoB-2 (3.667) calculated from their protein concentrations and the one (3.581) experimentally obtained (Table II) was only 0.086. The experimentally obtained value of the relative potency was very close to the one calculated from the protein concentration. Furthermore, we have indirect information on the relative

![Fig. 1. Log dose-log response regression lines of IRHBIG, NRHBIG and BoB-2.](image-url)
potency of NRHBIG to IRHBIG. Prior to the experiment presented in Fig. 1 and Tables I and II, we performed several experiments in which NRHBIG and BoB-2 were assayed in parallel. Thus, we could calculate the relative value of NRHBIG to IRHBIG from the experimentally obtained relative potency of NRHBIG to BoB-2. The relative potencies in international units of NRHBIG to BoB-2 thus calculated are shown in Table III as experiments 2, 3, and 4, whereas the same calculation applied to the experimental data presented in
TABLE IV  
International Units of HBIG preparations

<table>
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<tr>
<th>Manufacturer</th>
<th>Lot no.</th>
<th>Relative potency</th>
<th>IU/ml</th>
<th>Confidence limits of IU</th>
<th>PHA titer*</th>
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<td></td>
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<td>R/**</td>
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* The value stated by each of the manufacturers.
** The value determined in comparison with NRHBIG.

Fig. 1 is given as exp. 1. Though the values distributed from 0.564 to 0.629, the difference between the highest and the lowest values was not statistically significant. Therefore, the weighted mean and its confidence limits were calculated at 0.587 (0.599, 0.573).

Let the potency of BoB-2 be $50 \times 3.667 = 183.35$, then by using this value the potency of NRHBIG may be calculated at $0.587 \times 183.35 = 107.63$ IU/ml. We assigned, by rounding, 108.00 IU/ml as the potency of NRHBIG. This value was adopted in MRBP. Its confidence limits were 109.83 and 105.06.

*Potencies of Several HBIG Preparations*

The potencies of 17 preparations submitted by four manufacturers were estimated in terms of IU in several separated experiments. NRHBIG was used as the reference in every experiment and the potencies of preparations were calculated always from the relative potencies to NRHBIG to which 108 IU/ml was assigned as stated above (Table IV). The variance analyses demonstrated no significant deviations either from linearity or parallelism of the dose-response lines of all the preparations, including NRHBIG.

Table IV shows also anti-HBs antibody titers determined by passive hemagglutination (PHA) as stated by each of the manufacturers. There may be
differences among the manufacturers in regards to the correlation of their PHA titers to our RIA values. Though the overall correlation coefficient was 0.713, when a pair of isolated values (see Fig. 2) was excluded, the coefficient became 0.345. It must be noted that, with preparations of a fixed PHA titer of, e.g. 16,000, the RIA values distributed from about 100 IU/ml to nearly 400 IU/ml, and with another group with a PHA titer of 32,000, from about 450 to about 710 IU/ml.
Stability of NRHBIG

There was no tendency of reduction in the potency of NRHBIG while stored at 2–10°C for 5 years. Even heating at 60°C for 6 hr did not exert any detectable effect on the potency of NRHBIG, though it became somewhat opalescent on inspection.

DISCUSSION

Parallel line assay for anti-HBs antibody determination was first attempted by B.o.B., U.S. Food and Drug Administration, using solid phase radioimmunoassay (Barker et al., 1978). Use of this method is said to have allowed precise and reproducible estimates of the potencies of HBIG preparations (Barker et al., 1978).

In publications so far available, however, the anti-HBs antibody contents of HBIG and of Normal Immunoglobulin (NIG) preparations used in clinical trials have been almost exclusively expressed in PHA titers as summarized by Scheiermann and Kuwert (1980). As the PHA titer was usually determined from the end point of twofold dilution series and the performance of the test was not always on the same level, there might have been fairly large experimental deviations (WHO/BS/77.1164), thus making it difficult to compare the anti-HBs titers in PHA from one preparation to another. Poor stability of certain HBIG preparations may have added another problem in evaluating their efficacy. Prince (1978) described an example of high-titer HBIG incidentally containing proteases which decreased the potency during the course of a trial.

The method used in this report is in several aspects different from the one elaborated by B.o.B., as pointed out in our previous report and in “Materials and Methods” of this report under “radioimmunoassay” and “statistical analyses”. Its common error variance of 10 test trials ranged from 0.00022 to 0.00086 with an average of 0.00045, which corresponds to 0.211 in terms of standard deviation. This value is about one-third of the pooled standard deviation obtained in a cooperative study (Barker et al., 1978).

Apart from the reports by Barker et al. (1978) and ours (1979), there are only few reports considering the parallel line assay method or the use of a reference preparation in anti-HBs antibody determination. The procedure used by Uthermann (1981), though declared to have adopted a parallel line assay method, seems to be different from the one established by Finney (1964), as mentioned by the author (Uthermann, 1981). Stamm, Gerlich and Thomssen (1980), having probably used parallel line assay according to the B.o.B. method, made quantitation of anti-HBs antibody in sera from blood donors and convalescents and in NIG and HBIG preparations. They expressed the potency in IU. The authors compared the binding capacities of test samples measured by their electroimmunodiffusion technique with the results of their parallel line assay, but the error variance by the latter method was fairly large.
The PHA titers obtained by individual manufacturers did not well correlate to the IU values obtained by us by the parallel line assay method using RIA. Though there may be several possible interpretations, the data so far available are not large enough to draw any conclusion. We are by no means insisting upon that only RIA can be used in the potency test of HBIG. To apply a parallel line assay method to PHA, some new device would be necessary. The device used by Uemura et al. (1980) is an example, in which the size of agglutinated red cell mass formed in the bottom of the tube or the well was taken as a graded response (Takahashi et al., 1975), though the precision of the estimate was somewhat lower than those of ours and of Barker et al. (1978). Such techniques as enzyme immunoassay and single radial immunodiffusion may provide additional methods for the potency test of HBIG to which parallel line assay may also be applied.

In the past, a series of experiments was carried out by a Project Team on the stability of equine diphtheria-, tetanus-, and gas gangrene antitoxin preparations in an experimental design based on the accelerated degradation test (Jerne and Perry, 1956; Greif and Rightsel, 1965). The half life of the potency at 5°C was calculated according to the hypothesis that Arrhenius' theory can be applied to the degradation process of test materials. Then, the half life of the antitoxin preparations tested was estimated to be longer than several thousand years since they had shown 10–20% loss in potency upon heating at 60°C for 10 min (Murata et al., personal communication). In comparison with these data of antitoxins, our NRHBIG appears to be satisfactorily stable, since its potency was unaffected by heating at 60°C for 6 hr.

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References


