WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

WHO/BS/77.1164

ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL
STANDARDIZATION

INDEXED

Geneva, 6-12 December 1977

STUDY OF A PROPOSED INTERNATIONAL
REFERENCE PREPARATION FOR ANTI-HEPATITIS B IMMUNOGLOBULIN

by

L. F. Barker, D. Lorenz, S. C. Rastogi,
J. S. Finlayson and E. B. Seligmann
Bureau of Biologics, FDA, Bethesda, Md
United States of America

1. INTRODUCTION

Tests for antibodies against hepatitis B surface antigen (anti-HBs) have been used to select high-titre plasma for the manufacture of anti-hepatitis B immunoglobulin (HBIG) and clinical trials of a number of batches have shown these preparations to be effective in hepatitis B prophylaxis in several situations (Wld Hlth Org. techn. Rep. Ser., 1977, No. 602). Although the optimum or minimum effective doses have not been clearly delineated by the studies to date, a biological standard for anti-hepatitis B immunoglobulin is essential to ascertain whether batches meet potency requirements for this product imposed by national control authorities. Accordingly a preparation of anti-hepatitis B immunoglobulin was freeze-dried in two separate runs in 1977 in ampoules labelled Hepatitis B Immunogl., Dried 26-1-77 and 17-2-77, Proposed WHO Intern. Reference Preparation, for evaluation as candidate international reference materials.

This report describes an international study to evaluate the use of this material to assay the potency of anti-hepatitis B immunoglobulin and to compare results obtained by a variety of assay methods.

Aims of the collaborative study

The principal aim of this study was to assess the suitability of the freeze-dried preparations to serve as international reference materials. This assessment was made primarily by comparative assays of six coded materials against each other. The study also provided an opportunity to compare the precision and accuracy of several different laboratory methods for testing anti-hepatitis B immunoglobulin and also of one common method used in multiple laboratories. Some of the collaborating laboratories provided the results of additional serologic tests on the coded materials. Finally, physical and chemical studies and accelerated stability studies are in progress in our laboratory.

2. MATERIALS

Participants were sent the materials coded with numbers only, as follows:

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Code
number

1. Proposed WHO International Reference Preparation of HBIG, Lot 1-77, freeze-dried on 26 January 1977, 0.5 ml in heat-sealed glass ampoules (referred to subsequently as PRP-1).

2. Bureau of Biologics, United States of America, Reference HBIG, Lot 2, liquid, 0.5 ml in rubber stoppered vials.

3. Commercial HBIG, Lot 14989, Abbott Laboratories, United States of America, diluted 1:3 in 0.9 M NaCl, liquid, 0.5 ml in rubber stoppered vials.

4. Bureau of Biologics, United States of America, Reference HBIG, Lot 2 (code number 2), diluted 1:4 in 0.9 M saline, liquid, 0.5 ml in rubber stoppered vials.

5. Proposed WHO International Reference Preparation HBIG, Lot 2-77, the same bulk material used for Lot 1-77 (code 1 and 6) freeze-dried on 17 February 1977, 0.5 ml in heat-sealed glass ampoules (subsequently referred to as PRP-2).

6. Proposed WHO International Reference Preparation HBIG, Lot 1-77, freeze-dried on 26 January 1977, 0.5 ml in heat-sealed glass ampoules (duplicate of code number 1).

It should be noted that the United States Reference HBIG, Lot 2, is a 16.5% protein solution from which the proposed WHO reference materials were made by diluting a portion of the same liquid bulk material to a 9% protein solution. This dilution and the filling, drying and sealing of the ampoules were done by Dr J. H. de Bruijn at the Rijks Instituut voor de Volksgezondheid, Bilthoven, Netherlands (see Appendix II).

3. STUDY DESIGN

Sixteen laboratories in eleven countries participated in the study. The names of the participants are listed in Appendix I; in this report they are identified by letters which are not related to the order of listing in Appendix I.

The laboratories provided information describing the assay method or methods to be used. All laboratories were sent a description of the assay being used for national control purposes in the United States of America and data reporting forms for this and other methods with three vials of each of the six coded materials described above. The test materials were shipped frozen with instructions to store them frozen until use.

Each laboratory was asked to do at least three independent assays, each of which is defined as a comparison of the six coded materials, by making dilutions from a discrete set of individual ampoules and testing them at one time, under identical conditions and with the same reagents.

Where possible, participants were asked to test the materials by a common method, that currently used in the United States of America, as well as by methods established in their own laboratories. Descriptions of the procedures used were returned to the Bureau of Biologics with report forms, which were provided to record the raw data, for the overall analysis of the results.

4. METHODS

(i) Radioimmunoassay, parallel line bioassay method

A commercially available solid-phase radioimmunoassay kit (Ausab, Abbott Laboratories, United States of America) was used by eight laboratories following a common protocol to test the coded samples. The solid-phase radioimmunoassay system for anti-HBs uses the sandwich principle in which plastic beads, coated with hepatitis B surface antigen (HBsAg), are

incubated with dilutions of the test material. The sample is then removed and the beads are washed prior to the addition of ^{125}I-labelled HBsAg. Following a second incubation and a second washing step, the radiolabelled HBsAg remaining attached is detected in a gamma counter. The test sample counts are compared with counts produced by a negative control serum, which is used to establish a cut-off point above which the samples are considered to contain anti-HBs. Over an appropriately selected range of dilutions a linear relationship exists between the amount of anti-HBs present in the dilutions of the sample and the amount of radiolabelled HBsAg bound by the sample dilution in this method. Further details of this method and the statistical method for analysis of the results to determine relative potency are to be published elsewhere (Rastogi, Lorenz & Barker, manuscript in preparation).

(ii) Radioimmunoassay, end-point titre methods

Eleven laboratories used the solid-phase radioimmunoassay method mentioned above to provide end-point titre values for the six coded samples. End-point titre values obtained by five additional radioimmunoassay methods, both solid-phase and liquid, were submitted by four laboratories.

(iii) Passive haemagglutination

Eight laboratories used the methods which they normally employed to determine end-point titres by passive haemagglutination. Six of the eight laboratories used red blood cells coated with HBsAg from the same commercial source (Electro-Nucleonics, Inc., Bethesda, MD, United States of America), although they used a number of different reagent batches for this testing. These laboratories determined titres separately against red cells coated with the *adw* and the *ayw* subtypes of HBsAg. The other two laboratories used their own preparations of HBsAg coated red blood cells; in one case the cells were coated with the *adw* subtype and in the other with a mixture of the *adw* and *ayw* subtypes.

(iv) Counterelectrophoresis

Nine laboratories used the methods which they normally employed to determine end-point titres by counterelectrophoresis. Three of these laboratories tested the materials against both the *adw* and the *ayw* subtypes of HBsAg, one each tested against only the *adw* or the *ayw* subtypes, and four tested against a mixture of these two subtypes.

(v) Methods used for physical and chemical characterization of the reference material

Protein determination was done spectrophotometrically. Samples (after reconstitution if dry) are diluted with 0.15 M NaCl and measured at 279 nm in a 1-cm cuvette, $\underline{a}_{279} = 1.45$.

pH is measured at ambient temperature, usually 24°C, after dilution to 1% (w/v) protein with 0.15 M NaCl. Meter is always calibrated with standard buffer.

Stability (i.e. heat stability). After the sample (reconstituted if dry) is heated at 57°C for four hours, it is examined for evidence of gelation or flocculation according to United States regulations for biological products, 21 CFR 640.111(a).

Purity. Electrophoresis is performed at pH 8.6, ionic strength 0.075, room temperature, by using barbital/sodium barbital buffer and cellulose acetate membranes in a Beckman Microzone^{R} apparatus according to the procedure in the manufacturer's manual. Staining is done with Ponceau S dye.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) is carried out on non-reduced samples of the HBIG according to the method of Weber & Osborn (*J. Biol. Chem.*, 244, 4406-4412, 1969) as applied by Young, Aronson & Finlayson (*J. Biol. Standardization*, 5, in press, 1977). Both 5% and 7.5% gels are used in each test; the former reveals components larger than monomeric IgG; the latter yields sharper bands of components smaller than monomeric IgG. Most runs are carried out for four hours at room temperature with a current of 6 milliamperes per gel. Staining is done with Coomassie Blue R 250.

Ultracentrifugation for monitoring the stability during storage was done at 20°C in a Spinco model E ultracentrifuge. Samples were diluted to 1% (w/v) protein in phosphate-buffered saline, pH 7.2, ionic strength 0.154 (composition given in Art & Finlayson, Vox Sang., 17, 419-433, 1969) and centrifuged at 59 780 rpm. A schlieren optical system was used. Areas under schlieren peaks were determined by planimetry after dropping perpendiculars. No correction was made for radial dilution.

Residual moisture determined by the gravimetric procedure according to United States regulations for biological products, 21 CFR 610.13(a).

Sterility tested by the United States procedure for biological products, 21CFR 610.12 and passed.

Results

(i) Radioimmunoassay, parallel line bioassay method

HBIG Panel code number 1, or the proposed WHO Reference HBIG, Lot 1-77 (PRP-1), has been assigned a value of 1.00 and used as the reference point for determining the relative potencies of the other members of the panel by this and each of the other methods. Expected relative potency values presented in parentheses in the tables are approximations based on the material used for each of the other panel members (vide supra, Materials) and, in the case of code number 3, which was from an HBIG lot unrelated to the proposed reference preparation, on preliminary testing prior to distribution of the panel.

The 18 independent assays reported by eight laboratories using this method provided 87 individual comparisons with PRP-1 (code number 1). Nine of the 87 were excluded from further analysis because they fell outside the two standard deviation limits obtained when they were included in the analysis. Parallel line bioassay values from two laboratories were excluded because they were not obtained in triplicate with a single lot of reagents on the same day as required by the protocol.

The geometric means and two standard deviation limits for relative potencies of code numbers 2, 3, 4, 5 and 6 show excellent agreement with the expected relative potency values in parentheses (Table 1). As code number 6 is a duplicate of PRP-1, we would expect the individual comparisons of the relative potency of these two samples to be evenly distributed above and below one. Of 17 such relative potencies, nine fell below one and eight are above one, as expected. The frequency distribution of the relative potencies of each of the other five panel members with respect to PRP-1 are presented in Fig. 1.

One laboratory provided parallel line bioassay results using their own ay and ad subtype reagents in addition to the commercial reagents. The average results from five independent assays using each of these three systems were quite comparable (Table 2).

(ii) Radioimmunoassay, end-point titre methods

Forty-three independent assays reported by 12 laboratories provided 215 individual comparisons with PRP-1. In seven instances where no end-point was determined or the end-point was expressed as equal to or greater than the highest dilution tested, the results were excluded from further analysis. The geometric means for relative potencies of code numbers 2, 3, 4, 5 and 6 show good agreement with the expected relative potency values and also with the relative potency values obtained by the parallel line bioassay method (Table 3).

The range of titres by radioimmunoassay using the same commercial method was quite wide for each of the panel members (Table 4). For example, in the case of PRP-1 (code number 1) the titres ranged from 1:4000 to 1:100 000, with a median of 1:20 000 and a geometric mean of 1:19 000.

(iii) Passive haemagglutination

Eight laboratories reported 34 independent assays of the six coded materials by passive haemagglutination, providing a total of 170 individual comparisons with PRP-1 (code number 1).

The geometric mean relative potency values for each laboratory and for all laboratories for each panel member are presented in Table 5 with two standard deviation limits on the geometric mean values for each panel member. The limits are quite wide; no reported values were excluded from the analysis of these results. The geometric mean results agree with those found by the radioimmunoassay methods and with the expected values.

As seen in Table 6, the titre ranges were extremely wide by passive haemagglutination. In the case of PRP-1 the range is from 1:8000 to 1:400 000 with a median of 1:63 000 and a geometric mean of 1:44 000 when tested against cells coated with the ad subtype; the range is 1:8000 to 1:8 192 000 with a median of 1:20 000 and a geometric mean titre of 1:48 000 when tested against cells coated with the ay subtype.

(iv) Counterelectrophoresis

Nine laboratories employed counterelectrophoresis to obtain end-point titres in 26 independent assays of the six coded materials, providing a total of 130 individual comparisons with PRP-1 (code number 1). The results presented in Table 7 demonstrate good agreement with expected values and values obtained by other methods for the relative potencies of the other panel members with respect to PRP-1. The end-point titres by this relatively insensitive method varied over a much narrower range than did the titres by the more sensitive radioimmunoassay and passive haemagglutination methods. In the case of PRP-1, the titre ranged from 1:8 to 1:16 with a median of 1:8 and a geometric mean of 1:9 when the ad subtype of HBsAg was used in the test, and the titre ranged from 1:4 to 1:16 with a median of 1:8 and a geometric mean of 1:7 when the ay subtype was used.

(v) Comparison of results by four test methods

The relative potencies of the remaining five panel members with respect to PRP-1 (code number 1) were converted to $\log_{10}$ for comparison of the results obtained by the radioimmunoassay, parallel line bioassay and end-point titre methods, the passive haemagglutination and the counterelectrophoresis methods (Table 9). Comparison of the standard deviations indicates that the parallel line bioassay method is clearly the most precise method and that the other methods in order of decreasing precision are the radioimmunoassay end-point titre method, the counterelectrophoresis method and the passive haemagglutination method. For each of the five samples the means of the relative potencies expressed as $\log_{10}$ are not significantly different. The same comparisons made on the geometric mean relative potencies with two standard deviation limits are presented graphically in Fig. 2. It can be seen by both methods of analysis that the potency ratios obtained by these four methods are in good agreement with each other for each of the other five panel members with respect to PRP-1.

(vi) Other tests for anti-HBs

Two laboratories reported data bearing on the subtype specificity of the panel members. Laboratory D performed quantitative subtyping by a solid phase radioimmunoassay employing subtype specific reagents for absorption of the panel members and for the reactants in the test system. The results showed removal of more than 90% of the anti-HBs activity by absorption with either the ad or the ay subtype. These were interpreted as meaning that most of the activity is directed against the group-specific antigen, anti-a, and that only low levels of anti-d or anti-y may be present.

Laboratory B tested code numbers 2 and 3 for subtype specificities by agar gel diffusion and found evidence of anti-d and anti-w, in both materials; anti-y was not detected.

Laboratory P tested all of the coded samples for anti-HBs by agar gel diffusion and obtained titres of 1:8 for each material by this relatively insensitive method.

The binding capacities of the six coded materials were determined by two laboratories. Laboratory D used a quantitative immunoelectrophoresis method to measure the micrograms of

HBsAg protein bound per millilitre of sample. Laboratory H used a radioimmunoprecipitation technique to measure the amount of purified HBsAg protein with which 1 ml of sample material would combine at the 50% binding capacity point (Table 10). The relative potencies determined in terms of binding capacities of the test materials determined by these two methods are consistent with the expected values and the values obtained by other methods (Table 10).

(vii) Test for other antibodies

Three laboratories (B, D and K) tested the six materials for antibodies to hepatitis B core antigen (anti-HBc) by three different methods (counterelectrophoresis, a radioimmunoassay blocking method and indirect immunofluorescence). Each of the laboratories found variable levels of anti-HBc in the materials (Table 11).

Laboratory O tested the materials for hepatitis B e antigen and antibody by immunodiffusion and obtained negative results on all six materials. This laboratory also tested all of the materials for antibodies to smooth muscle, gastric parietal cells, mitochondria and nuclei. Code number 2 contained detectable antibodies to smooth muscle; the rest of the results were negative.

6. STABILITY

Physical and chemical as well as serologic tests have been conducted to assess the stability of PRP-1 and PRP-2, and the freeze-dried materials have been compared with the liquid United States Reference HBIG (code number 2) as they all came from the same liquid bulk lot. The results of these studies at the time of the distribution of the materials for the collaborative serological testing are presented in Table 12. After eight and 16 weeks of storage at 56°C the freeze-dried materials (PRP-1 and PRP-2) showed a considerable increase in 10S material and appearance of 12S material by ultracentrifugation; the liquid preparation developed gelation at 56°C, but at eight and 16 weeks storage at 37°C it showed appearance of 2.0% and 2.8% 3.5S material, respectively.

Studies of the potency of these preparations are in progress; to date there is a fall in relative potency of the freeze-dried materials after 16 weeks storage at 56°C by the parallel line bioassay method and a similar fall in relative potency of the liquid material after 16 weeks storage at 37°C with respect to materials stored at -70°C. These studies are continuing and will be available in more detail after six months storage at multiple temperatures.

7. CONCLUSIONS

The results of this international collaborative study of anti-hepatitis B immunoglobulin potency assays involving sixteen laboratories in eleven countries showed that four major assay methods, two radioimmunoassay methods, passive haemagglutination and counterelectrophoresis, all gave similar relative potencies for the six coded materials. Stability studies in progress have shown loss of potency of the freeze-dried materials after 16 weeks at 56°C, but they appear stable so far after 16 weeks storage at 37°C or frozen at -70°C.

All of the participants were asked to choose between four unitages (10, 20, 50, 100) to assign to vials of PRP-1, recognizing that this selection must be somewhat arbitrary at this time. Of 14 respondents, nine preferred 50 units per 0.5 ml vial, or 100 units/ml; three preferred 10 units per vial and one each preferred 20 and 100 units per vial.

ACKNOWLEDGEMENTS

We are most grateful to the participating laboratories for their willing and excellent cooperation in the performance of this study. The study received help from many other contributors; we would particularly like to thank the National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD for donating the anti-hepatitis B immunoglobulin used for the proposed reference preparation, Cutter Laboratories, Inc., for manufacturing and shipping this material, Abbott Laboratories for providing an independent lot of globulin for inclusion in the collaborative study, and Dr J. H. de Bruijn for formulating the final bulk solution, ampouling and freeze-drying the proposed reference material.

REFERENCES

General

Viral hepatitis and tests for the Australia (hepatitis-associated) antigen and antibody, Bull. Wld Hlth Org., 1970, 42, 957-992

Viral hepatitis, Wld Hlth Org. techn. Rep. Ser., 1973, No. 512

Viral hepatitis, Wld Hlth Org. techn. Rep. Ser., 1975, No. 570

Advances in viral hepatitis, Wld Hlth Org. techn. Rep. Ser., 1977, No. 602

Requirements for Human Immunoglobulin, Wld Hlth Org. techn. Rep. Ser., 1967, No. 361

Code of Federal Regulations, Title 21, Parts 610, General biological products standards and 640, Additional standards for human blood and blood products, U.S. Government Printing Office, 1977

Specific

Amouch, P. J. & Drouet, J. (1971) Dosage radio-immunologique de l'antigene Australie, Nouvelle Revue Française d'Hematologie, 11, 656-668

Brzosko, W. J., Madalinski, K., Krawczynski, K & Nowoslawski, A. (1973) Duality of hepatitis B antigen and its antibody. I. Immunofluorescence studies, J. Infect. Dis., 127, 424-428

Burrell, C. J., Parker, A. C., Ramsay, D. M. & Proudfoot, E. (1974) Antibody to hepatitis B antigen in haemophiliacs and their household contacts, J. Clin. Path., 27, 323-325

Duimel, W. J. & Brummelhuis, H. G. J. (1975) A new solid phase radioimmunoassay (CLB-RIA) for the detection of hepatitis-B antigen and antibody, Vox Sang, 29, 1-14

Schober, A., Biswas, R. M. & Thomssen, R. (1975) Direct solid-phase radioimmunoassay (dSP-RIA) for detection of antibody to hepatitis B surface (HBs) antigen, Devel. Biol. Stand, 30, 93-102

Soulier, J. P. & Courouce-Pauty, A. M. (1973) New determinants of hepatitis B antigen (Au or HB antigen), Vox Sang, 25, 212-234

APPENDIX I

PARTICIPANTS

Dr H. G. J. Brummelhuis, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service, Plesmanlaan 125, Amsterdam, Netherlands

Dr C. J. Burrell, University of Edinburgh, Department of Bacteriology, Tevoit Place, Edinburgh, EH8 9AG Scotland

Professor R. Butler, Chef Diagnostic Department, Swiss Red Cross, Wankdorfstrasse 10, 3000 Bern 22, Switzerland

B. J. Cohen, P. P. Mortimer, M.D., E. Vandervelde, M.D., Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England

Professor D. S. Dane, Virus Laboratory, School of Pathology, Middlesex Hospital Medical School, London W1P 7LD, United Kingdom

Robert J. Gerety, M.D., Ph.D., Director, Hepatitis Branch, Division of Blood and Blood Products, Bureau of Biologics, NIH, Bldg. 29, Rm. 311, 8800 Rockville Pike, Bethesda, MD 20014, United States of America

Dr Ian Gust, Virus Laboratory, Fairfield Hospital for Communicable Diseases, Varra Bend Road, Fairfield, Victoria 3078, Australia

Svante Hermodsson, M.D., Sten Iwarson, M.D., Departments of Virology and of Infectious Diseases, University of Goteborg, East Hospital, S-416 85 Goteborg, Sweden

James E. Maynard, M.D., Ph.D., Director, Hepatitis Laboratories Division, Bureau of Epidemiology, Center for Disease Control, 4402 North Seventh Street, Phoenix, AZ 85014

Dr Alfred Prince, The Community Blood Council of Greater New York, Inc., The New York Blood Center, 310 East 67th Street, New York, NY 10021

Dr Robert H. Purcell, NIAID Bldg. 7, Rm. 329, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20014

Professor Dr Halina Seyfried, Dr Adam Nowoslawski, Department of Serology, Institute of Hematology and National Institute of Hygiene, Warsaw, 00791 Chocimska 5, Poland

Professor J. P. Soulier, Centre National de Transfusion Sanguine, 6 Rue Alexandre Cabanel, Paris 15, France

Patricia E. Taylor, Ph.D., Department of National Health and Welfare, Canadian Communicable Disease Centre, Ottawa KIA OK9, Ontario, Canada

Professor Dr R. Thomssen, Dr Br. Stamm, Dr W. Gerlich, Hygiene-Institute der Universität, Göttingen, Lehrstuhl Medizinische Mikrobiologie, Kreuzbergring 57, D-34, Göttingen, West Germany

Professor Arie J. Zuckerman, M.D., D.Sc., Director, Department of Medical Microbiology and WHO Collaborating Centre for Reference and Research on Viral Hepatitis, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WCIE 7HT, United Kingdom

APPENDIX II

PREPARATION OF THE FREEZE-DRIED PROPOSED INTERNATIONAL REFERENCE PREPARATION OF HBIG, Lots 1-77 and 2-77

Plasma from individuals found to have naturally occurring high titres of antibody to HBsAg was pooled and fractionated by the cold ethanol method of Cohn by Cutter Laboratories, Inc., Berkeley, CA, United States of America, under contract with the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Bethesda, MD, United States of America. The bulk solution (Lot PR2527) was tested and found by the manufacturer to meet United States requirements for immune globulin products. This material was contributed to the Bureau of Biologics by NIAID for use as reference material.

In November 1976, approximately 1.5 litres of the bulk material was shipped to Dr J. H. de Bruijn at the Rijks Instituut voor de Volksgezondheid, Bilthoven, The Netherlands, where it was stored at 4°C and sample dryings were made at 16.5%, 13.0%, 11.0%, 9.0%, 7.0% and 5.0% protein concentrations. On the basis of serological and physical evaluation of these sample dryings, a 9% protein concentration was selected as optimal. On 25 January 1977, 654.5 ml of the 16.5% bulk hepatitis B immunoglobulin solution was diluted with 545.5 ml of 0.28 M glycine in 0.9% NaCl solution. The resulting 1200 ml of 9% solution was filled in 0.5 ml amounts into 2368 ampoules. After pre-freezing under rotation at -70°C, freeze-drying was carried out over 20 1/2 hours at condenser temperatures of -56°C at the beginning to -65°C at the end. Subsequently, the ampoules were temporarily closed at 15 micron Hg with butyl rubber stoppers and sealed within the next six hours. On 17 February 1977 the same procedure was carried out with 685 ml of immunoglobulin bulk diluted with 570.8 ml of 0.28 M glycine in 0.9% NaCl solution, and again 2368 ampoules were filled with 0.5 ml each, this being the maximum number of ampoules which can be freeze-dried in one run. Both batches of freeze-dried ampoules are stored at 4°C. Ampoules were shipped to the Bureau of Biologics in March and April 1977, for final container testing and distribution as PRP-1 and PRP-2 in the collaborative study.

Appendix II

TABLE 1. PARALLEL LINE BIOASSAY METHOD USING RADIOIMMUNOASSAY[1]
AVERAGE RELATIVE POTENCY OF HBIG PANEL WITH RESPECT TO PRP-1

Lab code	No. of tests	HBIG panel code					
		1 (1.0)[2]	2 (2.0)	3 (4.0)	4 (0.5)	5 (1.0)	6 (1.0)
E	1	1.00	2.41	4.63	0.52	0.97	0.99
B	1	1.00	1.47	4.19	ID[3]	0.78	0.90
M	3	1.00	2.14	3.86	0.67	0.99	1.08
J	3	1.00	2.06	4.95	ID	0.97	0.95
G	1	1.00	2.30	4.06	0.47	0.80	0.84
D	5	1.00	2.20	5.57	0.56	0.95	0.97
I	1	1.00	1.79	4.54	0.48	0.91	0.93
N	3	1.00	5.61[4]	13.11[4]	1.16[4]	1.06	1.08
Geometric mean			2.03	4.51	0.54	0.92	0.96
2-S.D. Limits	lower		1.44	3.51	0.40	0.75	0.81
	upper		2.85	5.80	0.71	1.14	1.15

[1] Ausab, Abbott Laboratories, Chicago, Ill., United States of America.

[2] Expected relative potency values.

[3] Insufficient data.

[4] Excluded from GM and limits calculations.

TABLE 2. RELATIVE POTENCIES WITH RESPECT TO PRP-1 BY PARALLEL LINE BIOASSAY METHOD CONDUCTED WITH THREE DIFFERENT SETS OF REAGENTS

HBIG code	Ausab	Relative potency ay Goe	ad Goe
1	1.00	1.00	1.00
2	2.22	2.22	2.17
3	5.64	4.57	4.26
4	0.55	0.53	0.54
5	0.95	0.94	0.93
6	0.99	0.99	1.00

Appendix II

TABLE 3. RADIOIMMUNOASSAY END-POINT TITRE METHOD
GEOMETRIC MEAN RELATIVE POTENCY OF HBIG PANEL WITH RESPECT TO PRP-1

Lab code	Test method	No. of tests	1 (1.0)[1]	2 (2.0)	3 (4.0)	4 (0.5)	5 (1.0)	6 (1.0)
A	A	3	1.00	1.60	8.00	0.40	0.80	0.80
M	A	3	1.00	0.97	2.88	0.26	0.41	0.59
B	A	3	1.00	1.67	4.17	0.33	0.67	1.00
G	A	3	1.00	2.25	4.45	0.50	0.82	0.90
E	A	3	1.00	3.20	4.10	0.50	0.80	0.80
J	A	3	1.00	3.20	7.90	0.50	1.00	1.00
L	A	1	1.00	2.00	4.00	0.50	1.00	1.00
K	A	3	1.00	3.70	3.70	0.47	0.59	1.00
N	A	3	1.00	8.23	NEP[2]	1.45	1.45	1.45
P	A	4	1.00	1.78	5.06	0.42	0.50	0.83
O	A	1	1.00	2.00	4.00	0.50	1.00	2.00
E	B	3	1.00	1.56	3.13	0.31	1.00	1.00
E	C	3	1.00	3.91	3.91	0.63	1.26	1.59
G	D	3	1.00	2.50	3.50	0.50	0.50	1.17
O	E	1	1.00	2.13	4.89	0.80	1.42	1.42
F	F	3	1.00	2.14	4.62	0.49	1.01	1.11
Geometric mean				2.36	4.37	0.49	0.83	1.06
2-S.D. limits	lower			0.88	2.46	0.22	0.39	0.58
	upper			6.27	7.75	1.08	1.77	1.93

[1] Expected relative potency values.

[2] No end-point.

TABLE 4. RADIOIMMUNOASSAY

Reciprocals ($x10^3$) of all titres by a single method[1]

HBIG	N	Range lower	Range upper	Median	GMT	2 S.D. Limits lower	2 S.D. Limits upper
1	26	4	100	20	19	4	77
2	26	8	128	48	42	10	186
3	24	16	256	95	91	21	386
4	29	1	32	10	8	2	32
5	26	4	64	16	13	4	45
6	28	4	64	16	16	5	51

[1] Ausab, Abbott Laboratories.

Appendix II

TABLE 5. PASSIVE HAEMAGGLUTINATION END-POINT TITRE METHOD
GEOMETRIC MEAN RELATIVE POTENCY OF HBIG PANEL WITH RESPECT TO PRP-1

Lab code	Antigen subtype	No. of tests	1 (1.0)[1]	2 (2.0)	3 (4.0)	4 (0.5)	5 (1.0)	6 (1.0)
O	ad	1	1.00	2.00	8.00	0.50	1.00	1.00
B	ad	2	1.00	2.81	5.62	0.69	1.00	1.00
M	ad	3	1.00	0.93	1.59	0.20	0.46	0.46
A	ad	3	1.00	2.50	6.25	0.62	1.00	1.25
C	ad	12	1.00	1.51	2.82	0.43	0.68	0.68
L	ad	1	1.00	2.00	2.00	2.00	2.00	2.00
P	ad	5	1.00	1.08	2.19	0.35	0.57	1.08
O	ay	1	1.00	1.00	4.00	0.50	1.00	0.50
B	ay	2	1.00	3.91	1.96	1.00	0.69	1.39
M	ay	3	1.00	1.74	2.17	0.35	0.87	0.47
A	ay	3	1.00	2.00	8.00	0.60	1.00	1.00
L	ay	1	1.00	2.00	2.00	0.50	4.00	0.25
P	ay	5	1.00	1.96	7.81	0.38	1.54	1.77
G	ad + ay	2	1.00	2.00	2.00	0.36	1.42	0.50
Geometric mean				1.82	3.36	0.52	1.05	0.82
2-S.D. limits	lower			0.83	0.98	0.17	0.35	0.25
	upper			4.03	11.56	1.53	3.13	2.70

[1] Expected relative potency values.

TABLE 6. PASSIVE HAEMAGGLUTINATION
Reciprocals (x10^3) of all titres, ad subtype

HBIG	N	Range lower	Range upper	Median	GMT	2 S.D. limits lower	2 S.D. limits upper
1	27	8	400	63	44	6	291
2	24	16	500	72	70	13	382
3	25	32	1 260	128	139	29	673
4	25	4	158	20	20	3	123
5	27	8	128	32	32	7	144
6	27	10	256	40	40	6	269
		Reciprocals (x10^3) of all titres, ay subtype					
1	15	8	8 192	20	48	1	3052
2	13	16	256	40	44	8	240
3	13	32	1 024	80	88	16	494
4	13	4	32	10	11	2	53
5	15	8	512	20	30	2	438
6	15	4	512	16	25	2	362

Appendix II

TABLE 7. COUNTERELECTROPHORESIS END-POINT TITRE METHOD
GEOMETRIC MEAN RELATIVE POTENCY OF HBIG PANEL WITH RESPECT TO PRP-1

Lab code	Antigen subtype	No. of tests	1 (1.0)[1]	2 (2.0)	3 (4.0)	4 (0.5)	5 (1.0)	6 (1.0)
B	ad	1	1.00	2.00	4.00	0.50	1.00	1.00
M	ad	3	1.00	2.50	4.00	0.40	0.80	0.80
J	ad	3	1.00	1.25	4.00	0.50	1.00	1.00
C	ad	1	1.00	1.00	1.00	0.25	0.50	1.00
B	ay	1	1.00	2.00	4.00	0.50	1.00	1.00
M	ay	3	1.00	2.00	8.00	0.50	0.50	0.50
J	ay	3	1.00	1.33	5.33	0.50	0.67	0.67
P	ay	3	1.00	1.20	2.00	0.40	0.80	1.00
E	ad + ay	3	1.00	2.00	4.00	0.50	1.00	1.00
L	ad + ay	1	1.00	4.00	2.00	0.80	2.00	3.00
K	ad + ay	3	1.00	2.90	3.30	0.10	1.00	1.00
G	ad + ay	1[2]	1.00	1.50	2.00	0.62	1.00	1.00

		2	3	4	5	6
Geometric mean		1.83	3.20	0.42	0.88	0.98
2-S.D. limits	lower	0.82	1.06	0.15	0.42	0.43
	upper	4.09	9.67	1.21	1.84	2.25

[1] Expected relative potency values.

[2] Highest titre of three tests, according to laboratory director.

Appendix II

TABLE 8. COUNTERELECTROPHORESIS

Reciprocals of all titres, ad subtype

HBIG	N	Range lower	Range upper	Median	GMT	2 S.D. Limits lower	2 S.D. Limits upper
1	8	8	16	8	9	5	14
2	8	8	32	8	16	6	46
3	8	8	64	32	29	9	93
4	8	2	4	4	4	2	6
5	8	4	8	8	7	4	12
6	8	8	8	8	8	8	8
Reciprocals of all titres, ay subtype							
1	10	4	16	8	7	2	25
2	10	4	32	8	11	3	51
3	10	8	128	32	32	4	280
4	10	2	8	3	3	1	12
5	10	4	8	4	5	3	10
6	10	4	8	4	5	3	11
Reciprocals of all titres, ad + ay subtypes							
1	8	5	16	9	9	5	17
2	8	16	40	20	22	11	42
3	8	10	40	32	28	12	66
4	8	1	10	4	3	0	15
5	8	8	16	10	10	6	15
6	8	8	16	10	10	6	18

TABLE 9. MEANS AND STANDARD DEVIATIONS OF LOG_{10} RELATIVE POTENCIES WITH RESPECT TO PRP-1 FOR FOUR METHODS AND FIVE HBIG MATERIALS

HBIG	Parallel line bioassay		Radioimmunoassay end-point titre		Passive haemagglutination		Counter electrophoresis	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
2	0.307[1]	0.074	0.372	0.212	0.261	0.172	0.262	0.175
3	0.654[1]	0.055	0.640	0.124	0.526	0.268	0.505	0.240
4	-0.271[1]	0.062	-0.308	0.171	-0.288	0.237	-0.376	0.230
5	-0.034	0.046	-0.078	0.164	0.019	0.238	-0.056	0.161
6	-0.016	0.037	0.024	0.131	-0.087	0.259	-0.008	0.180

[1] Excluding the extreme outliers.

Appendix II

TABLE 10. BINDING CAPACITIES OF THE HBIG CODED MATERIALS

HBIG code	Binding capacity[1] (µg/ml)	Relative potency	Mean 50% binding capacity[2] (µg/ml)	Mean relative potency
1	85.5	1.00	560	1.00
2	185.8	2.17	1 323	2.32
3	312.2	3.65	2 666	4.63
4	42.0	0.49	311	0.54
5	91.0	1.06	673	1.18
6	99.0	1.16	748	1.34

[1] Tested by quantitative immunoelectrophoresis.

[2] Tested by radioimmunoprecipitation.

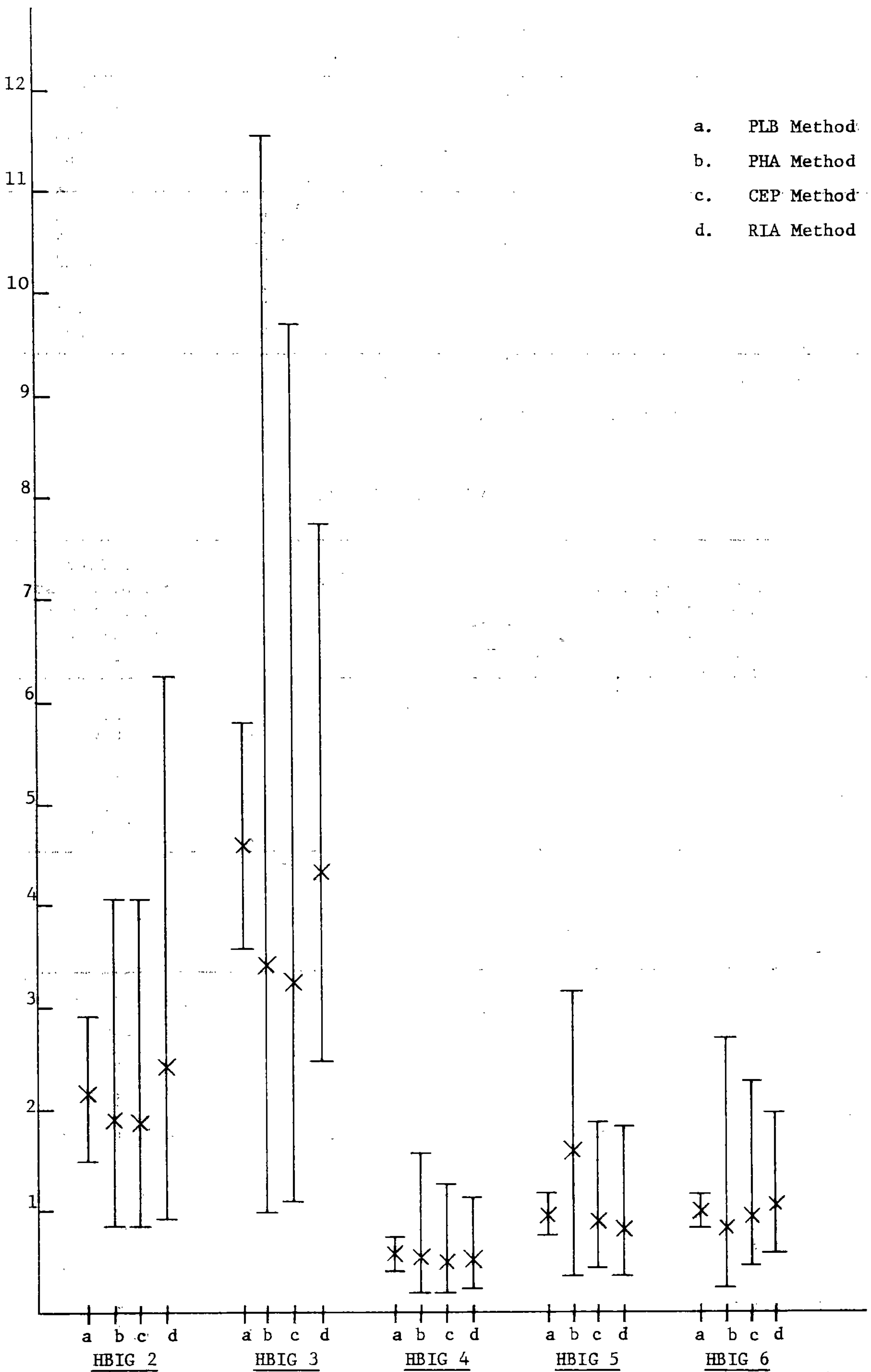
TABLE 11. ANTI-HBc ACTIVITY

Method → / HBIG code	Counterelectrophoresis (reciprocal titre)	Radioimmunoassay blocking (average relative potency)	Indirect immunofluorescence (reciprocal of geometric mean of 3 tests)
1	8	1.00	2.9
2	16	1.58	2.9
3	8	0.86	7.9
4	4	0.60	7.9
5	8	1.00	10.0
6	8	0.96	20.0

TABLE 12. CHARACTERISTICS OF ANTI-HEPATITIS B IMMUNOGLOBULIN REFERENCE MATERIALS

	PRP-1	PRP-2	U.S. Ref. 2
Residual moisture	0.84%	0.51%	liquid
Protein	9.2%	9.1%	16.4%
pH	6.60	6.60	6.65
Stability	satisfactory	satisfactory	satisfactory
Electrophoresis: Cellulose acetate	100%	100%	100%
Electrophoresis: polyacrylamide gel	major band 7S; no apparent 3.5S material	major band 7S; no apparent 3.5S material	major band 7S; no apparent 3.5S material
Ultracentrifugation	no 3.5S 88.9% 7S 10.2% 10S	no 3.5S 88.2% 7S 11.8% 10S	no 3.5S 94.6% 7S 5.4% 10S

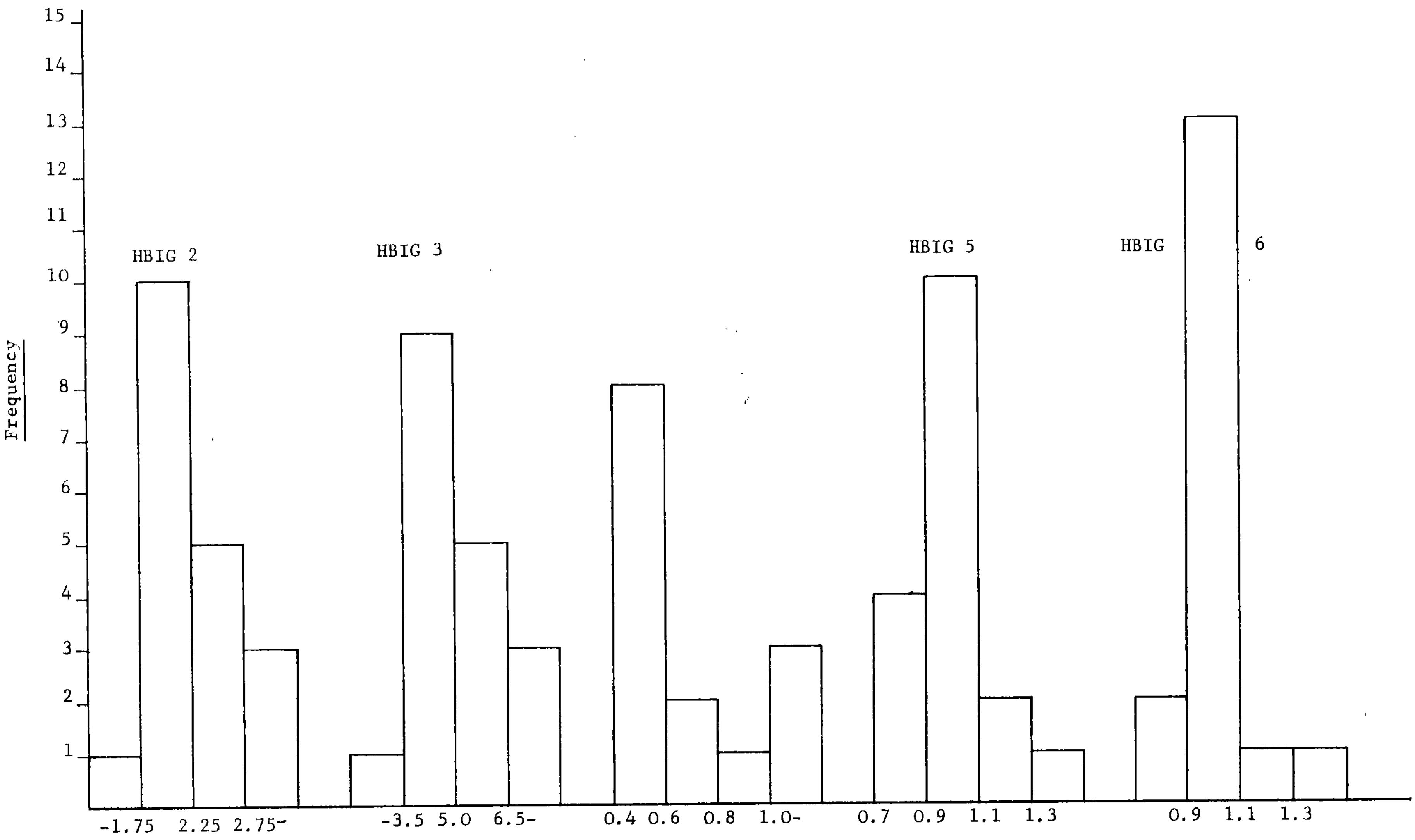
Appendix II



Appendix II

FIG. 1 HISTOGRAM OF RELATIVE POTENCIES

PARALLEL LINE BIOASSAY



* * *