



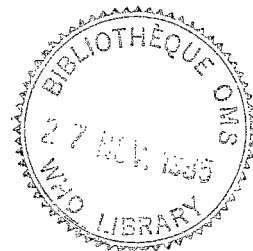
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PROPOSED INTERNATIONAL REFERENCE PREPARATION FOR
HEPATITIS B SURFACE ANTIGEN

by

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SUMMARY

An international collaborative study was carried out to investigate the suitability of a preparation to serve as a standard for hepatitis B surface antigen (HBsAg). Twelve laboratories from three countries assayed seven coded samples of HBsAg and the proposed standard; the assay methods used were all modifications of solid phase radioimmunoassay. Good agreement was found between estimates of potency relative to the proposed standard obtained by the different laboratories. Furthermore, results from accelerated degradation tests indicates that the stability of the proposed standard was satisfactory.

On the basis of results from the study, in 1982 the material was established as the first British Standard for HBsAg with an assigned potency of 100 British units per ampoule. This material is now proposed as the International Standard for HBsAg.

INTRODUCTION

Sensitive tests for the presence of hepatitis B surface antigen (HBsAg) are essential for safety testing of blood used for transfusion and in the preparation of specialized blood products. A standard for HBsAg was needed to provide a basis for standardization of HBsAg assays and to enable comparisons of results obtained in different laboratories to be made. Accordingly the Hepatitis Advisory Group of the Department of Health and Social Security (DHSS) collaborated with the National Institute for Biological Standards and Control (NIBSC) to prepare and establish a standard for HBsAg. The British Standard was prepared from a hepatitis B positive serum of subtype ad.

This report describes the results of an international collaborative study which was undertaken to assess the suitability of a preparation to serve as the British and International Standard for HBsAg. This was done by comparing the behaviour of the proposed standard with that of other preparations containing HBsAg. An interim report of the study was published (1).

MATERIALS

Seven samples coded A to G, and the proposed standard coded 80/549, were included in the study; details are summarized in Table 1.

Proposed standard for HBsAg (80/549)

In 1980 Dr T. D. Davies (North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex HA8 93D) kindly provided a hepatitis B positive serum which was obtained in February 1978 from a clinically healthy donor who had been an antigen carrier for at least seven years; the donation was positive for anti-HBe by immunodiffusion. The serum was diluted 1 in 4 in water, heated at 60°C for 10 hours in order to reduce any possible infectivity, and further diluted (1 in 75) in phosphate buffered saline with 5% bovine serum

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albumin and 0.1% sodium azide by Dr C. H. Cameron of the Middlesex Hospital Medical School (London W1). The material was sent to Glaxo Operations (UK) Ltd (Liverpool) where it was distributed in volumes of 1 ml into ampoules and then freeze dried. The ampoules were then sent to NIBSC and stored at -20°C .

Samples coded A to G

Samples coded A to D were from HBsAg positive donors; samples E and F were 1 in 200 dilutions in normal human serum of the German Reference Preparations - E the ay subtype and F the ad subtype; and sample G was a coded sample of the proposed standard. The German Reference Preparations were supplied by Professor R. Thomssen of the Institute of Hygiene of the University of Göttingen; they were stored at -70°C in liquid form. The reference antigens had been prepared from donations of human plasma with high antigen content of the corresponding subtypes. The plasma had been recalcified but the serum had not been purified or concentrated, and no other additives or preservatives were added.

THE COLLABORATIVE STUDY

Participants

Twelve laboratories from three countries participated in the study. Throughout this report laboratories are identified by a code number, which does not necessarily correspond with the order of listing in the Appendix.

Design of study

Participants were requested to assay the proposed standard and the seven coded samples using their own preferred method of testing, and to perform three assays. In each assay, freshly opened ampoules of the freeze-dried preparations (80/549 and G) were to be used, and at least three dose levels for each of the eight preparations were to be tested.

Statistical analysis

Assay data were analysed using the parallel line method (2) which includes validity tests for deviations of the log dose-response lines from linearity and parallelism. Potencies were calculated for each sample relative to the proposed standard.

Potency estimates were combined by taking their geometric mean and the variation between them was expressed as the geometric coefficient of variation (3).

RESULTS

Twelve laboratories contributed a total of 31 assays. Assay methods used by the participants were all modifications of solid phase radioimmunoassay. Six participants used a commercial kit, Ausria II (4), two used a modification of it (5), and the remaining four used other methods of radioimmunoassay. Data were received from a total of 31 assays. Laboratory 3 did not test sample G. One assay from Laboratory 1 was judged to be unsatisfactory for technical reasons. Examination of the slopes for the proposed British Standard and sample G, identical preparations apart from their codes, and those of the liquid samples gave no evidence that the log dose-response relationships for 80/549 and the liquid samples were not parallel.

For each sample the geometric mean potency ratio obtained for individual laboratories were found, and the frequency distribution of these values was plotted (Fig. 1). For each sample the overall geometric means and coefficients of variation of combined potency estimates are given in Table 2. There was considerably closer agreement between laboratories for the potency estimate of sample G, a coded sample of 80/549 (geometric coefficient of variation: 19%) than for the other preparations (geometric coefficients of variation: 53%-100%).

Accelerated degradation studies to determine the stability of the proposed standard were carried out at NIBSC. The contents of ampoules stored at temperatures above 20°C were difficult to reconstitute, so were excluded from the tests. After 20 months, samples stored at 4°C , and at 20°C had respectively 95% (95% confidence interval: 91-99) and 84% (95%

confidence interval: 80-87) of activity remaining relative to material stored at -20°C). The predicted loss per year in the material was estimated (6) to be 0.6% at -20°C , 4% at 4°C and 11% at 20°C .

DISCUSSION

There were no obvious differences between potency estimates obtained by different methods of solid phase radioimmunoassay (Fig. 1); laboratories using the commercial kit gave similar results to those using a modification of it and to those using their local methods.

It was encouraging to find that for sample G - a coded sample of the proposed standard - the estimates of relative potency were very close to unity, the known value. There was also remarkably close agreement between laboratories' estimates for sample G - much better than for the other samples. One possible reason for this was that the proposed standard and sample G were supplied freeze dried, whereas the other samples were supplied as liquids. This might have led to the proposed standard and sample G being treated in a manner similar to each other but different from the other preparations.

There may well have been other factors (e.g. qualitative differences between the preparations) which might have led to the different assay methods and reagents used by the laboratories having given differences in estimates of relative potency. However, the differences found between estimates of potency in terms of the proposed standard obtained by laboratories using various modifications of solid phase radioimmunoassay were considered to be relatively small in practical terms.

CONCLUSION

There was reasonable agreement between laboratories in their estimations of potency relative to the proposed standard of the coded samples containing HBsAg. Furthermore, the stability of the proposed standard was satisfactory. On the basis of results from this study, in 1982 the material (80/549) was established as the first British Standard for Hepatitis B Surface Antigen with an assigned potency of 100 British units per ampoule. This material is proposed as international standard for hepatitis B surface antigen with an assigned potency of 100 International Units per ampoule.

REFERENCES

1. Seagroatt Valerie et al. Preliminary evaluation of a collaborative study of the proposed British Standard for Hepatitis B Surface Antigen. Medical Laboratory Sciences, 1981, 38, 335-339
2. Finney, D. J. Statistical Method in Biological Assay, 3rd edition, London, Griffin & Son, 1978
3. Kirkwood, T. B. L. Geometric means and measures of dispersion. Biometrics, 1979, 35, 908-909
4. Abbott Laboratories Ltd., Diagnostic Division, Brighton Hill Parade, Basingstoke, Hampshire RC22 4EH, England
5. Hopkins, R. et al. Improved economies of HBsAg screening with commercial radioimmunoassay reagents. J. Clin. Pathol., 1980, 33, 19-23
6. Kirkwood, T. B. L. Predicting the stability of biological standards and products. Biometrics, 1977, 33, 736-742

TABLE 1. PREPARATIONS USED IN THE COLLABORATIVE STUDY

Preparation	Subtype	HBeAg/ anti-HBe	Serum/ plasma	State
Proposed standard	ad	Anti-HBe	Serum	Freeze dried
A	ad	HBeAg	Serum	Liquid
B	ad	Anti-HBe	Plasma	Liquid
C	ay	HBeAg	Serum	Liquid
D	ay	Anti-HBe	Serum	Liquid
E	ay	HBeAg	Serum	Liquid
F	ad	HBeAg	Serum	Liquid
G*	ad	Anti-HBe	Serum	Freeze dried

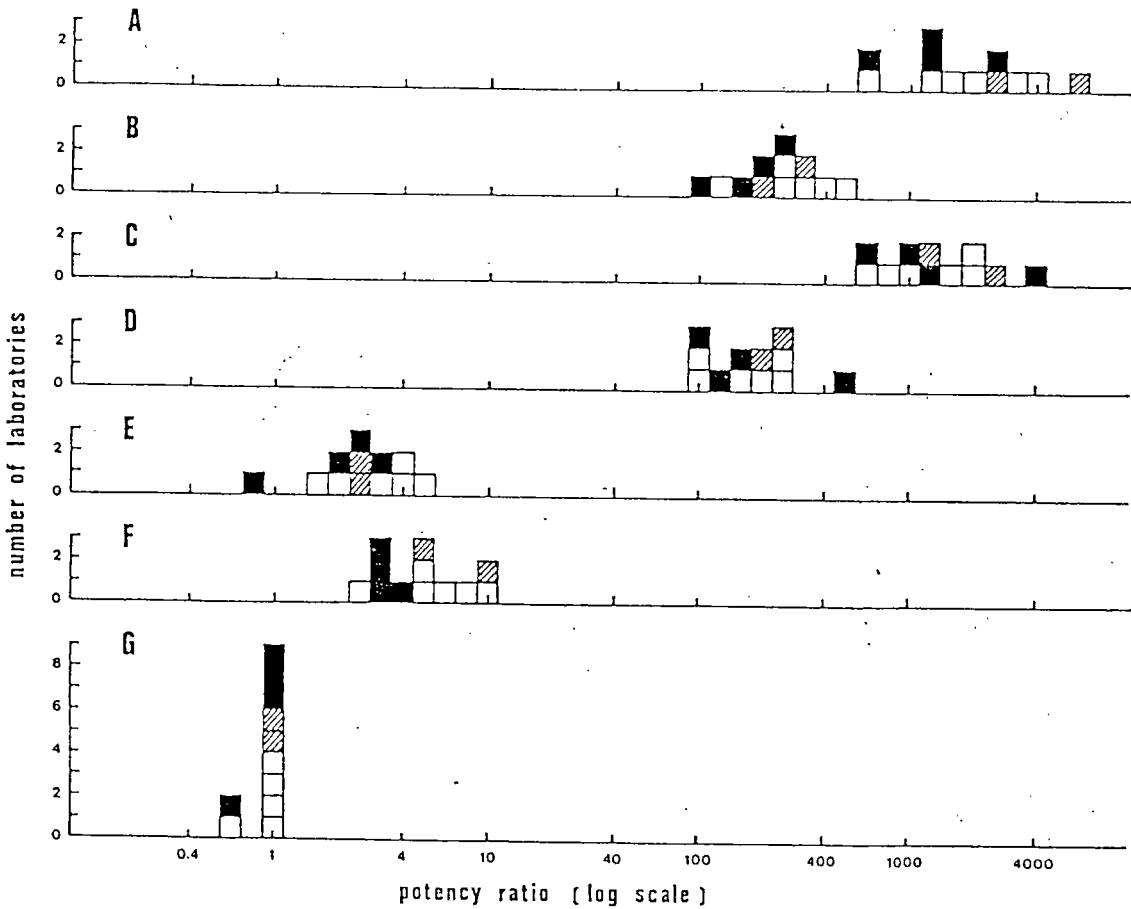
* Coded duplicate of the proposed standard.

TABLE 2. FOR EACH PREPARATION THE OVERALL GEOMETRIC MEAN (GM) AND COEFFICIENT OF VARIATION (GCV) OF COMBINED POTENCY RATIOS OF LABORATORIES EXPRESSED RELATIVE TO THE PROPOSED STANDARD

Preparation	GM potency ratio	GCV
A	1790	100%
B	240	62%
C	1310	75%
D	170	63%
E	2.56	59%
F	4.36	53%
G*	0.95	19%

* Coded duplicate of proposed standard

FIG. 1. FREQUENCY DISTRIBUTIONS OF POTENCY ESTIMATES FOR
SAMPLES A TO G IN TERMS OF THE PROPOSED STANDARD



Each box denotes the mean potency obtained by one laboratory:

□ potency obtained by laboratory using commercial kit, AUSRIA II⁴

▨ potency obtained by laboratory using a modification of AUSRIA II⁵

■ potency obtained by laboratory using other methods

APPENDIX

PARTICIPANTS IN THE STUDY

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