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ADVISORY GROUP ON TESTING FOR THE PRESENCE OF HEPATITIS B SURFACE ANTIGEN AND ITS ANTIBODY

H/1523/94 K

1ST MEETING : 7 DECEMBER 1978

ENCLOSURES:

1. List of Members

2. Agenda for 1st Meeting and Papers

   AGHB P1.1
   AGHB P1.2
   AGHB P1.3
   AGHB P1.4

3. Admission Pass to Hannibal House

ADVISORY GROUP ON THE TESTING FOR THE PRESENCE OF HEPATITIS B SURFACE ANTIGEN AND ITS ANTIBODY

1ST MEETING 7 DECEMBER 1978

LIST OF MEMBERS

Dr W J Jenkins — Chairman
Dr G W G Bird
Dr C M Patricia Bradstreet
Dr C J Burrell
Dr J D Cash
Dr D S Dane
Dr T H Flewett
Dr R S Lane
Professor Dame Sheila Sherlock
Dr G H Tovey
Dr Elise M Vandervelde
Professor A J Zuckerman

Scottish Home and Health Department

Dr A E Bell

DHSS Secretaries

Mr T Dutton 01-703 6380 Ext 3574
Dr Sheila L Waiter 01-703 6380 Ext 3487

ADVISORY GROUP ON TESTING FOR THE PRESENCE OF HEPATITIS B SURFACE ANTIGEN AND ITS ANTIBODY

1ST MEETING

DATE: 7 December 1978
TIME: 11.00 am
VENUE: Room 918 DHSS Hannibal House, London SE1

Coffee available from 10.45 am
Lunch will be provided

AGENDA

1. Chairman's Introduction
2. Terms of Reference
3. Revision of Second Report of the Advisory Group on Testing for the Presence of Hepatitis B Surface Antigen and its Antibody AGHB(78)P1.1
4. Families of patients suffering from haemophilia and related diseases as blood donors AGHB(78)P1.2
5. Papers submitted
   i) STOP-GAP PROVISION FOR PLASMA FRACTIONATION AT BPL, ELSTREE AGHB(78)P1.3
   ii) COMPARATIVE STUDY OF A NUMBER OF TESTS FOR HEPATITIS B SURFACE ANTIGEN AND AN EVALUATION OF THE 'HEPANOSTIKA' (MICROELISA) TEST AGHB(78)P1.4
6. Any Other Business
7. Date of Next Meeting

Copies of the 'Revised Report' (1972) and 'Second Report' (1976) have been sent to members.

T E DUTTON
DR SHEILA L WAITER

NOVEMBER 1978

REVISION OF SECOND REPORT OF THE ADVISORY GROUP ON THE TESTING FOR THE PRESENCE OF HEPATITIS B SURFACE ANTIGEN AND ITS ANTIBODY

Since this report was issued, (it updated the earlier report 'Revised Report of the Advisory Group ON THE TESTING FOR THE PRESENCE OF AUSTRALIA (HEPATITIS ASSOCIATED) ANTIGEN AND ANTIBODY') considerable progress has been made in the field of viral hepatitis. This Advisory Group has been reconvened to consider a number of questions which have been raised and to revise the 1976 report. Among the questions raised are:-

1. The possible introduction of other and more sensitive techniques for screening blood donors for hepatitis B surface antigen such as radioimmunoassay, enzyme immunoassay.

2. To consider the value of tests for hepatitis B core antibody as a marker of infectivity with this virus.

3. To examine the merits of routine screening of blood donors for transaminases.

4. Non-A, non-B hepatitis, its relevance in transfusion of blood and blood products.

5. To consider whether Hepatitis B surface antigen positive donors should be re-examined and whether they should (if negative) be re-admitted to the donor panel.

6. Responsibility to detected HBsAg carriers.

T DUTTON
DR SHEILA L WAITER

NOVEMBER 1978

FAMILIES OF PATIENTS SUFFERING FROM HAEMOPHILIA AND RELATED DISEASES AS BLOOD DONORS

At the meeting of Regional Transfusion Directors held on 4 October 1978, the question was raised whether families of patients suffering from haemophilia and related diseases should be accepted as blood donors. It was suggested that the question should be put before the reconvened Advisory Group.

The advice of this Committee is sought.

STOP-GAP PROVISION FOR PLASMA FRACTIONATION AT BPL

Paper submitted by Dr R S Lane Director,
Blood Products Laboratory, Elstree

(2)

STOP-GAP PROVISION FOR PLASMA FRACTIONATION AT BPL

A review of the effects of proposed changes in plasma collection on the fractionation process with particular attention to testing plasma for hepatitis virus (HBsAg).

Planned developments at BPL are based on two premises:

1. The best source material for protein fractionation is obtainable as single donations of fresh frozen plasma (FFP).

2. All FFP used for factor VIII production must be tested for the presence of HBsAg by radioimmune assay (RIA).

In reviewing the situation at BPL, emphasis must be placed on the condition of frozen fresh plasma for use in factor VIII production and possibly in the future for freeze-dried cryoprecipitate supernatant: pure albumin and PPF solutions are heat-treated to bring about destruction of the hepatitis virus.

At present all FFP is tested by RIA at BPL unless this has been done already at the Regional Transfusion Centre. This approach is facilitated at present by FFP collection in 5L packs (approx. 30 donations) so reducing the number of tests carried out at BPL. Plans to abolish FFP collection in 5L packs and accept single donations of FFP, mean that the requirement for hepatitis-testing at the Regional Transfusion Centres or BPL must change.

Reasons for abolishing FFP collection in 5L packs are stated in the accompanying report prepared for presentation at the RTD's Meeting (July 1978), but other aspects of this change in procedure should be considered as they might affect Regional Centres and BPL.

At many RTCs the 5L plasma pooling process takes a secondary position behind the daily priorities of separation of blood for platelets, cryoprecipitates and FFP for the region: thus time-limits place constraints on the pooling of fresh plasma which may influence its optimal freshness and the maximum volume of FFP collected for BPL. Collection of FFP in single packs should simplify the routine work-flow in RTCs and permit an increase in quality and quantity of FFP available for production of AHG concentrate. As the proportion of FFP increases in relation to the number of whole blood donations, utilization of the residual fresh whole blood in the regions should be more complete; time-expired blood will tend to be in the form of red-cell concentrates and there should follow an equivalent reduction in the availability of time-expired plasma as a source material.

For factor VIII production and Cohn fractionation of albumin, a predominance of source material as FFP is highly desirable. At BPL, as factor VIII production rises, starting material for albumin fractionation will increasingly become cryoprecipitate supernatant. Decrease in time-expired plasma as a source material would not become absolute in the foreseeable future, thus small pool dried plasma production could continue in the absence of an alternative being developed, e.g. freeze-dried cryoprecipitate supernatant.

Control of HBsAg

The control of HBsAg transmission in plasma fractions depends on constant surveillance of whole human plasma so that detectable antigen-positive material is excluded from processing. Various assays are currently available which vary

considerably in their ability to detect antigen; the most sensitive test (radioimmune assay RIA) does not exclude the possibility of hepatitis transmission by injection of the tested material, but does provide the highest level of confidence currently available in the safety of a plasma fraction.

The primary source of plasma for HBsAg testing will become single FFP donations obtained in less than 18h from blood donation. At present, these will have been tested at RTCs by one of the alternative forms of assay for HBsAg, but not necessarily RIA. Before use at BPL, all FFP will need to be tested by RIA either at RTCs or on arrival at BPL. General policy at present does not advocate the extension of RIA testing for HBsAg into all 14 RTCs, a policy partly influenced by the high cost of RIA per test using commercial reagents and it is suggested that donor selection, coupled with a second generation test for HBsAg, may provide an adequate cost-effective scrutiny of donors at the regional level. In practice, hepatitis testing at RTCs varies at present between RIA, RPH, Haemagglutination-inhibition and a combination of RIA for new donors and RPH for old donors.

The requirements for regions and BPL need reappraisal and rationalization in the face of new procedures for increased FFP collection. A programme, currently pursued jointly by BPL, RTC Edgware and the Middlesex Hospital Virology Department, has been developed to provide an economic, controlled RIA-test for HBsAg which could be utilised by the NBTS.

The problem of protein fractionation from large plasma pools

Limitation of HBsAg transmission in coagulation factor concentrates is part of an active approach to maintain the highest possible safety of products fractionated at BPL and PFL (Oxford). In haemophilia A and B, the patient, at commencement of replacement therapy, is now committed to many years exposure by blood products and it is essential that the risk of infection by hepatitis is minimised or else, for the patient, chronic liver disease may become a greater problem than haemophilia management. Increasing sporadic use of other coagulation factors, II, IX, X and VII, in the control of poorly defined haemorrhagic diatheses reinforces this need for care in plasma component production.

Effective control of HBsAg transmission by coagulation factor concentrates is limited to screening plasma by RIA, and strict control on plasma pool size during fractionation; the latter is a compromise between the increasing risk in large pools and the efficiency of the fractionation process. In spite of precautions, cases of hepatitis B arising from the use of these products continue to be reported.

The above problems arise only in plasma fractionation units and are at variance with the terms of reference and therefore outside the recommendations of the second report of the Advisory Group on Testing for the Presence of HBs Antigens and Antibody. The Advisory Group advocated RPH as the minimum standard of testing for use in RTCs. Outright acceptance of this recommendation ignores the fact that where fresh plasma collection for BPL is concerned, the function of BPL and the 14 RTCs is a collective one to which the particular requirements of BPL must apply.

The problems are twofold; first, if Hepatitis-testing by RIA is not to be duplicated in BPL and RTCs, then a choice between them must be made; second, if RIA-testing is to be carried out in RTCs, then the approach to HBsAg-testing in RTCs needs reassessment. A policy at RTCs of RIA-testing FFP for BPL and

RPH testing the remainder of blood products for hospitals invites criticism of a system having two standards in hepatitis detection. In any event, a single approach to HBsAg-testing in a Transfusion Centre is simpler to implement.

Both problems are more readily solved by provision of a centrally organised and controlled RIA-test which can be made available to the regions. Such is the objective of the joint programme involving BPL, RTC Edgware and the Middlesex Hospital, Department of Virology.

Provision of Radioimmune Assay for HBsAg

Present experience indicates that existing facilities and skills are sufficient to provide this service (assuming extension of BPL into buildings on the present Lister Estate).

RIA performed at BPL uses AUSRIA II modified by extra purification and diluted to permit more efficient and economic use without any loss of sensitivity. In fact, using AUSRIA controls, positive/negative ratios are obtained six times in excess of those stipulated by ABBOTT for their test; lower cut off values are found to be more acceptable with a higher potential for safety and purification of the AUSRIA reagent improves the binding efficiency of the iodinated antibody to HBsAg.

Preparation of Immune Serum to HBsAg

A pool of 60L of crude serum from HBsAg 'ad' and 'ay' specific immune horses and goats is available for testing, selection and further refinement. Additionally, the animals (2 horses and 6 goats) are being made available for further boosting, testing and venesection. Current tests indicate that antiserum from several of these animals has a high probability of being satisfactory for RIA. Initially, the assay will be set up and jointly controlled by the three laboratories, but provision and control of the test will progressively become the responsibility of the hepatitis-testing unit at BPL, backed up when necessary by the other laboratories.

Introduction of the Assay

The primary need is to complete the RIA-testing service at present used at RTC Edgware, to replace commercial reagents used at BPL and provide sufficient testing services to cover the introduction of single packs of FFP to BPL during Stop-Gap and until the RTCs can take over the responsibility of RIA-testing the plasma at source.

The secondary objective will be to extend the provision of RIA to the regions.

Cost

Following preparation of the antisera and proof that a viable, controlled test is available and in use, the capital and revenue costs generated by production and control of the assay could be accommodated within a modest increment to the BPL budget. Provision to the regions at a later date could be without charge.

Approximate costing of this programme is not a simple matter while the future of BPL at Elstree remains unsettled. Taken from another angle, present cost of RPH-testing in the transfusion service is about £200,000 per annum based on the cost of Hepatest at 10p per test.

Allowing for the capital expenditure needed at BPL to adapt new laboratory space for safe working with HBsAg, it is improbable that the annual revenue cost of providing an RIA-service to the RTCs would exceed £40,000 per annum, i.e. 20% of the current cost of Hepatest. This would provide an RIA test at between 1p and 2p per test.

Implementation of the Programme

Arrangements for producing an RIA test at BPL are now well advanced. The main problems for resolution are those concerning introduction of this test into RTCs. For this purpose reconstitution of the 'Advisory Group on Testing for HBsAg and its Antibody' would be welcome so that problems raised in this paper can be taken into consideration.

28th July, 1978.

R.S. LANE.
Director Designate
Blood Products Laboratory.

COMPARATIVE STUDY OF A NUMBER OF TESTS FOR HEPATITIS B SURFACE ANTIGEN AND AN EVALUATION OF THE 'HEPANOSTIKA' (MICROELISA) TEST

Papers submitted by Dr T H Flewett

SAMPLES FOR ELISA TESTING

CODE	CLINICAL CONDITION	PHA Titre	ELISA	RIA	RIA COUNT RATIO
A	Fading jaundice	-	~~++~~	+	137
B	Haemophilia	-	~~++~~	+	57
C	Recovering from Hep B Infe	1:8	+	+	2.3
D	Hepatoma	1:2	+	+	22
E	Recovering	1:8	~~+++~~	+	15
F	Recovering	? +	~~++++~~	+	41
G	Recovering	1:32	~~+++~~	+	50
H	Recovering	+	-	+	4.3
I	Hepatoma	1:8	~~+++~~	+	33
J	Hepatoma (same case as I)	1:8	~~++~~	+	4
K	Hepatitis	1:8	~~++~~	+	44
L	Recovery	-	-	+	3.1
M	Acupuncture Hepatitis case	1:16	~~++~~	+	24
N	Acupuncture Hepatitis case	1:8	~~+++~~	+	5.2
O	Hepatitis titre never high	1:16	~~++~~	+	27
P	Ante-natal + ve	1:8	-	+	50
Q	Recovering	1:16	~~+++~~	+	8.0
R	as for O	-	~~+++~~	+	9.2
S	Ante-natal + ve	1:4	~~+++~~	+	8.4
T	Recovering	1:8	~~+++~~	+	15
U	Baby (cord)	-	-	+	2.1
V	Baby (cord)	-	-	+	4.8
W	Baby (cord)	-	-	+	5.9
X	Recovering	1:4	~~+++~~	+	6.4

~~+++~~ means three pluses etc.

Hepanostika (Organon Teknika)

Hepanostika is a "microelisa" test for Hepatitis B surface antigen (HB_sAg) and is produced by Organon Teknika. "Elisa" stands for enzyme linked immuno sorbent assay. The principle of the test is outlined in the diagram (Fig. 1) and it is claimed that the sensitivity of the test approaches that of Radioimmunoassay (RIA). Organon Teknika have asked me to consider purchasing this test for the purpose of assessing, 1) the sensitivity of the test, and 2) its suitability as a routine screening test for use in diagnostic or blood transfusion laboratories.

A test of this level of sensitivity is not required for routine screening of diagnostic samples and for this reason I have not carried out a full scale assessment of 'Hepanostika'. However, by arrangement with the Central Public Health Laboratory at Colindale, London, I have had some of my own samples tested by Hepanostika and travelled down to London to see the technique demonstrated and to read the results.

Method

Antibody to HB_s is coated on the surface of the wells of 'V' bottomed microtitre plates. 0.1 ml sample is added and incubated for 2 hours at $37^{o}C$. If any HB_s is present in the sample it will bind to the antibody in the plate; excess sample is removed and the wells washed four times by an automatic dispenser and washer specifically designed for use with microelisa tests. 0.1 ml horse radish peroxidase enzyme-antibody complex is added, using a multi-channel dispenser; another incubation (2 hrs/at 37^{o}) follows. Unbound material is again removed by washing. The enzyme substrate (which may present some carcinogenic hazard) is added and the final incubation takes place in the dark at room temperature for 50 mins, during which time a colour change develops. The reaction is stopped by the addition of sulphuric acid. A yellow colour indicates a positive sample, but very strong positives can develop a bright orange or almost red colouration. The

contd.

colour change could be read spectrophotometrically by passing the contents of the well through a continuous flow spectrophotometer which gives a digital read out.

Results

The test was much easier to read than I had expected; this is partly because at the end of the reaction there is a fairly large volume of liquid in the microtitre well giving a reasonable depth of colour. I had 24 samples tested by this method; they were all positive by RIA, but many were negative by Hepatest.

Method:	Hepatest	Elisa	RIA
Number Positive	16	18*	24
Number Negative	8	6	0
	24	24	24

*The samples which were negative by Elisa were repeated using an overnight incubation: an extra two samples were found to be positive.

The positive samples which were missed by Elisa all had count ratios less than 5.9 (4.3, 3.1, 5.0, 2.1, 4.8, 5.9) i.e. all were low positives by RIA. All samples positive by Hepatest were positive by Elisa.

Conclusions

The test is simple to carry out, easy to read, and has a greater sensitivity than Hepatest, although not quite as sensitive as RIA. Its possible advantage over RIA as a screening method may be that it does not require expensive counting equipment, but the hazard of working with radioactivity has been replaced by the hazard of working with possible carcinogens.

Discussion

Organon Teknika are very keen that Blood Transfusion Centres should look at their test. In order to demonstrate its increased sensitivity

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over Hepatest, not less than 10,000 samples should be tested in parallel by each test. Oxford and Cardiff, and possibly Bristol, are already looking at this test and for the moment it would be advisable to wait for the results of this study. However, it would be a good idea for someone from Birmingham BTS to go down to Oxford (as that is probably the nearest centre) to see the test first-hand and discuss the practical problems of large scale screening with them.

Problem

Post-transfusion hepatitis due to hepatitis B can occur for the following reasons:

(1) Present screening tests are not sensitive enough to detect low titre donors.

(2) Operator error - tests misread, samples mislabelled, etc.

(3) Donor incubating hepatitis at the time of donation, may be infectious, but HB_sAg level too low to be detected, even by the most sensitive test.

(4) Infectious carriers in whom no HB_sAg is detectable.

Unfortunately, we do not know the answers to these questions, but I intend to make a much fuller study of the samples referred to us as HAA(J) donors and have asked for fuller details of all the cases of post-transfusion hepatitis and the donors involved in these cases. Until we understand more fully the reasons why post-transfusion hepatitis occurs, we may not be able to prevent it by simply using a more sensitive test.

Cost of Hepanostika

Standard price £325 for a 500 test kit.

Automatic washer £3,600

Multi-channel pipetter £245.

Discount prices (basis of 250,000 tests per year)

If equipment supplied free - 29½p per test.

If equipment bought at discount prices of

Washer £2,500

Pipetter £245

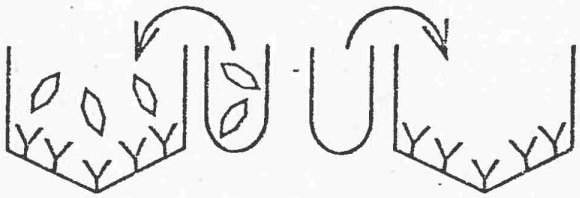
Cost per test = 28½p.

General principle and procedure of HEPANOSTIKA:

figure 1

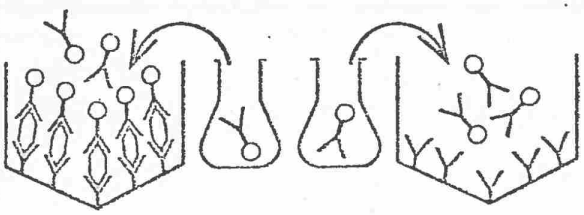
1. MICROELISA testplate pre-coated with sheep antibody to HB_s-Ag.

2. Addition of 0.10 ml. of plasma or serum sample.



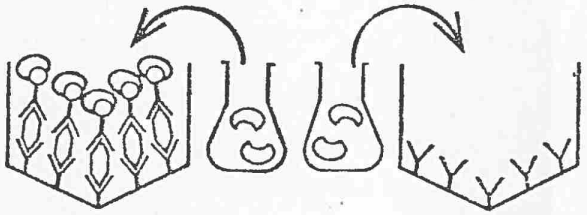
3. After incubation (room temperature overnight or 2 hours at 37°C) unbound material is removed by washing.

4. Addition of enzyme labelled sheep antibody to HB_s-Ag.



5. After two hours incubation at 37°C unbound material is removed by washing

6. Addition of the enzyme substrate.



7. Following exactly 50 minutes incubation at room temperature in the dark, the enzyme colour reaction is stopped by adding 4 N H_2SO_4.

8. Yellow coloured wells indicate screening positive results. Non coloured wells screening negatives.