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Subject Interest
VIROLOGY/BLOOD TRANSFUSION

HBsAg detection—results of comparative large scale testing of blood donations

A. BARR, B. C. DOW AND I. MACVARISH

Glasgow and West of Scotland Blood Transfusion Service, At Law Hospital, Carluke, Lanarkshire ML8 5ES, Scotland

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At present in the U.K. reversed passive haemagglutination (RPHA) is recommended for total screening of blood donations for the presence of hepatitis B surface antigen (HBsAg). A comparison was made between RPHA enzyme immunoassay (EIA) and radioimmunoassay (RIA) with a view to assessing their suitability for routine screening of large numbers of samples. RIA was shown to be the most sensitive and specific method, followed closely by EIA, whilst the RPHA methods varied greatly in their degree of sensitivity and specificity.

Introduction

The discovery by Blumberg in 1965[1] of the Australia Antigen and the subsequent realization that this antigen, now universally known as the hepatitis B surface antigen (HBsAg), was a specific marker of the agent of viral hepatitis type B,[2] resulted in the recommendation that all blood donations should be tested for its presence.[3, 4] This recommendation has led to a proliferation of techniques for the detection of HBsAg, many of which are listed in the World Health Organization Report.[5]

Currently, both the National Health Service Advisory Group[6] and the WHO report[7] recommend that techniques at least as sensitive as reversed passive haemagglutination (RPHA) should be used to screen blood donors. The former report, whilst recognizing the greater sensitivity of radioimmunoassay (RIA) techniques over RPHA, suggests that this extra degree of sensitivity is outweighed by the considerable advantages of RPHA, while the latter report further recommends that 'sensitive tests' (presumably more sensitive than RPHA) should be used to screen plasma destined for the preparation of plasma protein fractions.

Since 1970, all blood donations in the West of Scotland have been screened for the presence of HBsAg. In the first 5 years, the method of testing was counter-immunoelectrophoresis (CIEP). During this period several cases of proven viral hepatitis type B were notified. On retrospective testing of stored samples from donors implicated in such cases, HBsAg was detected by the more sensitive techniques of RPHA and/or RIA.[8] These findings prompted us to introduce more sensitive techniques for total screening of donors, and to evaluate other techniques as they became available.

This paper presents the result of this work, and shows that the techniques for the detection of HBsAg vary considerably in both specificity and sensitivity.

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Materials and Methods

Techniques

The method of Milne & Barr[9] was used for CIEP. The RPHA tests were those of Wellcome Reagents Ltd. (Hepatest),[10] Organon Teknika (Hepanosticon)[11] and Abbott Laboratories (Auscell).[12] Two EIA methods were investigated, one being Organon Teknika (Hepanostika)[13] using visual reading, the other being Abbott Laboratories (Auszyme)[14] utilizing a spectrophotometer to obtain results. Only one RIA technique, Abbott Laboratories (Ausria-II),[15] was available and tests were carried out by the standard procedure A (Incubation: 2 h at 45°C; 1 h at 45°C). Positive reactions were defined as those with counts above a cut-off value of 1.5 times the negative mean. All these commercial methods were performed to manufacturers' instructions. The long incubation RIA method was a modification of Ausria-II procedure B with an extended second incubation of 4 h at 45°C. All confirmatory tests were also carried out to manufacturers' recommendations.

Samples

Where possible, blood donor sera were used in all tests, although in some it was necessary to use plasma. Plasma, however, tends to give false positive reactions by the RIA method and certain RPHA methods. In these tests plasma was treated where necessary following the instructions of the manufacturers.

Results

Total screening of donations

(a) *RIA*. In 1975 total screening of donations for HBsAg by RIA (Ausria-II) was introduced for one year. In this period 140,696 blood donations were screened, 32,776 of them for the first time. Thirty-nine out of this group had HBs antigenaemia, an incidence of 1 : 841. Of the donors previously tested by CIEP (73,778) or RIA (34,142), 19 and 3 respectively were found to be HBsAg positive at a subsequent donation (Table 1). Previous experience with CIEP suggests that the majority of the 19 samples now found to be RIA positive would have been HBsAg positive at their earlier donations.[16] The three HBsAg positive donors who had been previously tested by RIA had all donated within the past six months. Examination of the preserved sera from the previous donations of these three donors verified the original RIA results.

TABLE 1
One year screening of donors using RIA (Ausria-II)

	New donors	Previous donors negative by CIEP	Previous donors negative by RIA	Total
Number tested	32,776	73,778	34,142	140,696
HBsAg	39 (1 : 841)	19	3	61

Of the 61 new examples of HBsAg detected by the RIA screening procedure, only 49 were capable of being detected by RPHA (Hepatest), whilst CIEP only detected 35.

(b) *RPHA*. The following year RPHA (Hepatest) was used to screen all blood donations. In the light of our earlier experience it was felt that antigens would be missed by RPHA. It

was therefore decided that plasma destined for fractionation should be made into small pools of 10 and the samples from these pools further tested by a long incubation RIA (Ausria-II).

A total of 222,375 donations were screened by RPHA (Hepatest) over a period of 19 months. This group consisted of 47,873 new donors, 18,511 previously found to be negative by CIEP, 71,782 previously negative by RIA, and 84,209 previously negative by RPHA (Table 2). Fifty HBs antigens were detected among new donors (an incidence of 1 : 957), 14 among the samples previously negative by CIEP, and only one in the group which had been previously tested by RPHA. A stored sample was available from the donor who had been previously tested by RPHA and found negative. Examination of this sample by RPHA and RIA failed to demonstrate the presence of HBsAg.

TABLE 2
Nineteen months screening of donors using RPHA (Hepatest)

		Previous donors negative by			
	New donors	CIEP	RIA	RPHA	Total
Number tested	47,873	18,511	71,782	84,209	222,375
HBsAg	50 (1 : 957)	14	0	1	65

In the last 12 months of this period 8589 plasma pools were tested by the long incubation RIA method, and four positive results were obtained. Four HBsAg carriers were detected and confirmed when the 40 individual samples constituting these pools were examined. These four examples of HBsAg were RPHA negative and RIA positive. It is possible that this is an underestimate, as factors such as neutralization and dilution of HBsAg in the plasma pools could have influenced the number of positive results obtained.

Comparison of RPHA, EIA and RIA

As several HBs antigens had now been found which were RPHA negative and RIA positive, these conveniently constituted the basis of panels prepared as new test systems became available. These panels varied in both size and composition, depending on the quantity of reagents available. Three panels illustrate the results of evaluations over the last three years.

Panel 1

This consisted of 200 HBs antigens, of which 160 were blood donors, three were proficiency panel samples, two from patients suffering from viral hepatitis type B, 24 from plasma pools, and 11 others. One hundred HBsAg negative samples were included in the panel. One of these HBsAg negative samples was from a proficiency panel, the rest being blood donors.

Table 3 shows the number of samples in which the presence of HBsAg was confirmed by the respective techniques. Of the 200 HBsAg positive samples six were not detected by the original EIA (Hepanostika) kit, while 22 were not detected by RPHA (Auscell), and 27 by both RPHA (Hepanosticon) and RPHA (Hepatest). The HBsAg positive sample not detected by EIA in the donor group is of special interest because this specimen was from a donor who had been involved in a fatal case of post-transfusion hepatitis.

TABLE 3
Results of testing Panel 1 by RIA, EIA and three RPHA methods

	RIA (Ausria-II)	EIA (Hepanostika)	RPHA (Auscell)	RPHA (Hepanosticon)	RPHA (Hepatest)
Donors	160	159	156	153	155
Panel	3	2	2	2	2
Hepatitis patients	2	2	2	2	2
Plasma pools	24	20	7	5	3
Others	11	11	11	11	11
Total	200	194	178	173	173

Panel 2

This panel was composed of 90 samples, of which 66 were HBsAg positive. Fifty of the HBsAg samples were from blood donors, 12 from hepatitis patients, and four were commercial positive controls. All 24 negative samples were from blood donors.

TABLE 4
Results of testing Panel 2 by RIA, EIA and RPHA

	RIA (Ausria-II)	EIA (Hepanostika)	RPHA (Auscell)	RPHA (Hepatest)
Donors	50	50	49	48
Hepatitis patients	12	10	11	5
Commercial controls	4	4	4	2
Total	66	64	64	55

Table 4 shows the results of testing the samples. With two exceptions EIA (Hepanostika) was able to confirm the presence of HBsAg. RPHA (Auscell) also failed to detect two HBs antigens. However, RPHA (Hepatest) failed to detect 11 HBs antigens.

Panel 3

A further panel was prepared consisting of 70 samples, of which 54 were HBs antigens. Fifty of the positive samples were from blood donors and four were commercial positive controls. The 16 HBsAg negative samples consisted of one commercial negative control and the remainder were all blood donor samples.

TABLE 5
Results of testing Panel 3 by RIA, EIA and RPHA

	RIA (Ausria-II)	EIA (Auszyme)	RPHA (Auscell)	RPHA (Hepatest)
Donors	50	46	47	44
Commercial controls	4	4	3	0
Total	54	50	50	44

The results of testing this panel by various techniques is shown in Table 5. EIA (Auszyme) failed to detect four HBs antigens; RPHA (Auscell) four HBs antigens and RPHA (Hepatest) 10 HBs antigens.

Specificity

Table 6 represents the results of large scale testing of the various tests with regard to the incidence of false positives. RIA (Ausria-II) had the lowest incidence (0.034%), but even using a cut-off level of 1.5 times the negative mean only 0.12% of results were false positives. EIA (Hepanostika) (i) had the highest false positive rate (2.17%) but it should be noted that the test was still under development at this stage. More recent experience of this test (ii) showed a reduced level of false positives (0.68%). The three RPHA methods varied considerably in their incidence of false positives from 0.69% for RPHA (Auscell), 1.01% for RPHA (Hepanosticon) to 1.94% for RPHA (Hepatest).

TABLE 6
Specificity of the various techniques in screening large numbers of donors

Test	Number tested	Percentage of false positives
RIA (Ausria-II)	140,696	0.034 (using 2.1 cut-off)
		0.12 (using 1.5 cut-off)
EIA (Hepanostika) (i)	7,000	2.17
EIA (Hepanostika) (ii)	439	0.68
RPHA (Auscell)	3,475	0.69
RPHA (Hepanosticon)	12,000	1.01
RPHA (Hepatest)	222,375	1.94

Discussion

Sensitivity and specificity are important aspects of any screening test for HBsAg. These aspects of RPHA, EIA and RIA were studied in relation to each other.

The RPHA systems tested showed remarkable variation in both sensitivity and specificity. Of the three techniques investigated RPHA (Auscell) appeared to be the most sensitive and specific, though it did fail to detect some antigens present in blood donors (Tables 3 to 6). From our results of one year of testing using RIA, 12 of the 61 antigens detected failed to give a positive reaction when tested by RPHA (Hepatest). When testing plasma pools whose individual constituents were negative by RPHA (Hepatest), four HBs antigens were identified by RIA. Platelets from one of these latter donors were transfused, and the recipient subsequently developed HBs antigenaemia. This finding of RPHA negative RIA positive HBsAg carriers therefore appears not to be a rare event. It is likely that a considerable number of such donors exist and can transmit type B hepatitis. Archer[17] suggested a modification to RPHA (Hepatest) claiming an increase in sensitivity. Our examination of this modification verified his results but unfortunately the improvement in sensitivity was associated with a considerable loss of specificity (6.6% false positives). Essentially similar results to ours were obtained by Reesink *et al.*[18]

Various authors[19, 20] have claimed EIA to be as sensitive as RIA. Our experience, however, shows EIA to be slightly less sensitive than RIA (Tables 3, 4 and 5). One HBsAg which was consistently negative by EIA was of special interest as it was from a donor who was involved in a fatal case of post-transfusion hepatitis (Table 3). EIA (Hepanostika) was first seen by us in 1976 when a large number of false positives were found (2.17%). Upon recent re-examination of this test, as now presented by the manufacturers, the number proved acceptable (0.68%). Only a small number of samples were investigated using EIA (Auszyme). Both kits, however, seemed comparable.

RIA was shown to be the most sensitive and specific method of testing for HBsAg (Tables 1, 3 to 6). In one year of screening blood donors by this method, no confirmed HBsAg post-transfusion cases were reported to us.

The adoption of RPHA to screen every blood donation for the presence of HBsAg in U.K. has been recommended.[21] Using this method of screening, a minimum of 200 antigens are likely to go undetected annually. Burrell *et al.*[22] noted that 8 out of 35 haemophiliacs receiving Factor VIII showed antibody responses consistent with exposure to HBsAg, and that one other haemophiliac developed clinical hepatitis. The WHO report[23] recommends that 'sensitive tests should be applied to ensure that only plasma units that are nonreactive for HBsAg are used for the preparation of plasma protein fractions'. This recommendation, viewed along with our findings, suggests that at present RIA is the most acceptable method of testing such units. As the majority of blood donations are now processed for fractionation, the institution of total RIA screening of donations for HBsAg should reduce the exposure of patients and staff to the hepatitis B virus.

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