HBsAg Testing in Commercial Plasmapheresis

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ABSTRACT

Since 1971, the introduction of routine testing of individual units of plasma or blood for the presence of hepatitis B surface antigen has significantly increased the safety of biologic products prepared from blood. Third-generation testing techniques have resulted in donor reactive rates in well-run, paid plasmapheresis programs essentially the same as found in volunteer whole blood donor programs. It is particularly important that high risk blood products, such as Factor VIII concentrates, be prepared from donor populations with a low incidence of hepatitis B surface antigen.

INTRODUCTION

The potential for posttransfusion hepatitis B transmission has been, and still remains, a potential risk in blood transfusions and in blood component therapy. However, since 1971 the introduction of routine testing of individual units of plasma or blood for the presence of hepatitis B surface antigen (HBsAg) has significantly increased the safety of biologic products prepared from blood, with respect to both hepatitis B transmission[1-5] and other types of posttransfusion hepatitis.[3,4]

The presence of HBsAg is now well regarded as an indicator of hepatitis B infection, whether in the acute, chronic, or chronic-carrier state. HBsAg is a marker for potential hepatitis B infectivity[6-8] and may also be indicative of other potential posttransfusion hepatitis infectivities.[9,10] Although it has been stated by some researchers that the blood or plasma from commercial donors has a higher risk of hepatitis transmission than that from volunteer donors,[2] this conclusion is subject to debate. Most of the opinion about high hepatitis B incidence in commercial donors is based on pre–third-generation testing, i.e., pre-March, 1976. Table I summarizes the published data relating the incidence of HBsAg in the donor population, the incidence of posttransfusion hepatitis from unscreened blood, and the type of donor (paid or volunteer). The data indicate that: 1) there is no correlation between the percentage of paid donor blood and the incidence of posttransfusion hepatitis ($r = 0.01$); 2) there is no correlation between the percentage of paid donor blood and the incidence of HBsAg antigenemia in the donor population ($r = 0.24$); 3) there is a good correlation between the incidence of posttransfusion hepatitis and the incidence of HBsAg antigenemia within the donor population ($r = 0.97$)

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Table I. Infectivity of commercial versus volunteer blood.

Donor Type				
Paid (%)	Volunteer (%)	HBsAg (%)	Posttransfusion Hepatitis (%)*	Reference
3	97	—	0	Taswell et al.[37]
0	100	0.06	0.5	Grady and Bennett[38]
0	100	—	0.6	
0	100	—	2.0	
0	100	—	2.1	
100	0	—	2.3	
24	76	0.28	2.5	
0	100	0.22	2.1	
36	64	—	2.2	
0	100	—	2.7	
44	56	—	3.4	
0	100	0.66	3.6	
38	62	1.47	8.5	
57	43	1.08	—	
0	100	—	0.05	Grady et al.[39]
0	100	—	0.07	
29	71	—	0.28	
0	100	—	0.03	
40	60	—	0.33	
0	100	0.15	—	Gocke[6]
100	0	0.65	—	
100	0	1.6	—	Cherubin and Prince[10]
100	0	0.4	—	
100	0	0	—	
100	0	1.3	—	
100	0	2.6	—	
0	100	0.09	—	
0	100	0.125	0.37	Alter et al.[3]
0	100	0.07	—	Kliman[41]
100	0	0.09	—	Miller[42]
0	100	0.18	—	
0	100	0.16	—	
25	75	—	0.29	Cohen and Dougherty[43]
100	0	—	0.27	Allen[44]
0	100	—	0.05	
100	0	—	0.68	Kunin[45]
100	0	—	0.14	
100	0	—	0.19	
0	100	—	0.23	
0	100	—	0.18	
15	85	—	0.05	Alsever and Van Schoonhoven[46]
4	96	—	0.05	
70	30	—	0.04	
95	5	—	0.04	
66	34	—	0.04	
95	5	—	0.04	
61	39	—	0.03	
48	52	—	0.02	
38	62	—	0.01	
0	100	0.22	4.7	Senior et al.[1]
73	27	1.72	17.9	

*Determined by countermmunoelectrophoresis or complement fixation.

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Thus the HBsAg incidence in the donor population, rather than the type of donor population (paid versus volunteer), appears to be the primary factor involved with the hazard of posttransfusion hepatitis.

HBsAg ASSAY SYSTEMS

The importance of HBsAg screening of blood has led to the development of increasingly sensitive assay systems. Currently, three "generations" of tests for HBsAg are recognized in the United States, based upon the ability of the test method to detect weakly reactive HBsAg samples within a standardized panel. Table II summarizes representative methods of each generation, comparing their relative sensitivity, ease of performance, relative cost, and time required for assay completion.

The first-generation tests are characterized by low sensitivity but high specificity for antigen subtypes; the most common of these tests is agar gel diffusion. The agar gel diffusion test is simple to perform and inexpensive, but requires one to three days for completion.

The second-generation tests, including counterimmunoelectrophoresis, complement fixation, and passive hemagglutination inhibition, provide moderately increased sensitivity. The ease of performance and short time for completion have made counterimmunoelectrophoresis a popular screening test in the past.

The third-generation tests, such as radioimmunoassay, radioimmunoprecipitation, enzyme-linked immunoassay and reverse passive hemagglutination inhibition, are orders of magnitude more sensitive than either the first- or second-generation tests. The first third-generation test became commercially available in August 1972. Although the first licensed third-generation radioimmunoassay test, AusRIA-I®, was 100 to 200 times more sensitive than the second-generation test methods, various modifications were introduced to increase the test sensitivity and specificity. The first modification of the original AusRIA-I test, introduced in June 1973, added normal guinea pig serum to the second-stage antibody. A second modification of the AusRIA-I technique, announced in November 1973, involved shorter incubation periods at elevated temperatures. A further modification of the radioimmunoassay technique, AusRIA-II®, introduced in December 1974, involved a polystyrene bead support for the first-phase antibody and a heterologous second-phase antibody. This modification appears to be more sensitive and specific than the three earlier versions of AusRIA-I.[12]

In an effort to provide the highest degree of safety for its biologic products, the plasmapheresis centers owned by Alpha Therapeutic Corporation have been routinely screening all plasma donations for HBsAg since January 1971. Since the initiation of HBsAg screening, a system involving numerous double checks has been utilized to assure that HBsAg reactive plasma units are excluded in the manufacture of biologic products and that HBsAg reactive donors are immediately excluded from the regular plasmapheresis program.

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Table II. Techniques for measuring HBsAg.

Technique	Relative Sensitivity	Minimum HBsAG Particles per ml of Serum Required for Detection	Ease of Performance	Relative Cost	Time Required for Completion (Hours)
First Generation					
Agar gel diffusion	1	10^{11}	Simple	Inexpensive	24–74
Second Generation					
Counterimmuno-electrophoresis	2 to 10	10^{12}	Simple	Moderate	2
Rheophoresis			Simple	Moderate	2
Complement fixation			Moderate	Inexpensive	2–24
Reversed passive latex agglutination			Moderate	Expensive	2
Passive hemagglutination inhibition			Moderate	Expensive	2
Third Generation					
Radioimmunoassay	100 to 10,000	10^{9}	Complex	Expensive	4–24
Enzyme-linked immunosorbent assay			Complex	Expensive	4–24
Radioimmuno-precipitation			Complex	Expensive	4–24
Reversed passive latex agglutination			Complex	Expensive	4–24
Reverse passive hemagglutination inhibition			Complex	Expensive	2–24

Originally, the screening was performed using the second-generation counterimmunoelectrophoresis technique, AUS-tect®. It should be noted that screening by a second-generation method was not required by the Bureau of Biologics until 1972.

In April 1973, Alpha Therapeutic Corporation replaced the counterimmunoelectrophoresis testing in all its donor centers with the more sensitive AusRIA-I technique for routine hepatitis B surface antigen testing. Modifications of this test were implemented as soon as they became commercially available. The most sensitive current radioimmunoassay technique, AusRIA-II, has been used routinely since February 1975. HBsAg screening by a third-generation method was required by

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the Bureau of Biologics in March 1976.

The results of Alpha Therapeutic Corporation's HBsAg screening over a six-year period are presented in Figure 1. The reactive rate per 1,000 donations is plotted on a monthly basis over the five-year period; in addition, chronological events within this testing period have been noted.

The first period of counterimmunoelectrophoresis testing during 1971 and 1972 indicated a relatively stabilized donor population. Transition from the counterimmunoelectrophoresis test to the radioimmunoassay technique, in April 1973, was marked by an enormous increase in the number of antigen-positive donations detected. Consistent with increased sensitivity of the radioimmunoassay technique, a substantial number of donors were rejected in the ensuing five months. A subsequent slight rise in reactive rate during the first quarter of 1974 was attributed to the introduction of a modified version of the AusRIA test; as noted previously, the test modification, involving shorter incubation periods at elevated temperature, greatly increased the test specificity. Again, after the first few months of test introduction, during which time previously unidentified HBsAg positive donors were excluded from the plasmapheresis program, the incidence of hepatitis B antigenemia in the donor population stabilized. The introduction of the AusRIA-II test, in February 1975, has allowed the most sensitive detection of antigen-positive donations.

DISCUSSION

In reviewing the reactive donation rate, it is essential to take into account the test turn-around time. Until the use of a courier service was introduced in September 1975, there was a one-week turn-around time in the radioimmunoassay testing program. Because a plasmapheresis donor may contribute plasma twice weekly, the HBsAg-reactive donor contributed an average of 1.7 donations before being excluded from the plasmapheresis program (as noted previously, all reactive donations are excluded from the manufacturing process). To allow comparison of plasmapheresis reactive donation rates with those of whole blood programs, in which a donor may contribute only one time every ten weeks and is excluded from the blood program before contributing a second reactive donation, the plasmapheresis reactive rate data must be corrected for the multiple donation factor. A summary of the corrected and uncorrected reactive rates for the testing experience of Alpha Therapeutic Corporation's plasmapheresis program is given in Table III.

The stabilization of reactive rates is apparent in both Figure 1 and Table III, which summarize the reactive rates over different time periods and different test procedures. During the period of counterimmunoelectrophoresis testing, the average annual reactive rates per 1,000 donations were 2.1 (January through December 1971) and 2.4 (January through March 1973). From April through December 1973, concomitant with the introduction of radioimmunoassay testing, the reactive rate increased to an annual average of 9.0 per 1,000 donations, reflecting a peak of 31.7 per 1,000, stabilizing to 4.0 per 1,000 by the last third of the year. This

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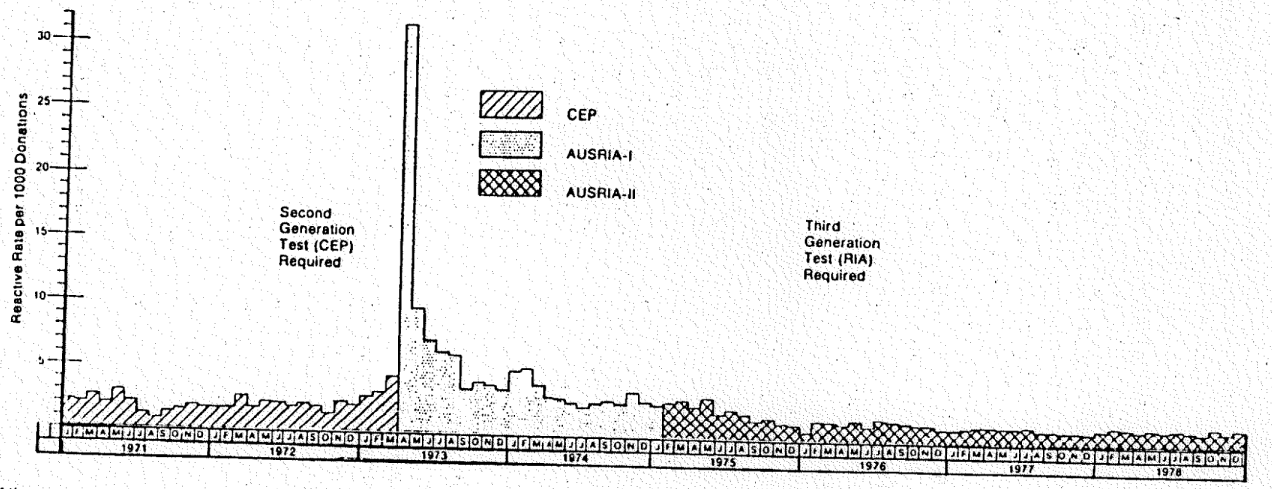

Figure 1. Results of eight years of HBsAg testing experience in commercial plasmapheresis.

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Table III. HBsAg testing of Alpha Therapeutic Corporation's plasmapheresis donations.

Time Period	Test Procedure	Comment	Reactive Rate Per 1,000 Donations	
			Uncorrected	Corrected
Jan.–Dec., 1971	CEP		2.1	
Jan.–Dec., 1972	CEP		2.4	
Jan.–Mar., 1973				
Apr.–Dec., 1973	AusRia-I	Introduction of new testing technology	9.0	
Jan.–Mar., 1974	AusRIA-I	Introduction of modified testing technology	5.0	
Apr.–Dec., 1974	AusRIA-I		2.9	1.7
Jan.–Aug., 1975	AusRIA-II		2.7	1.6
Sept.–Dec., 1975	AusRIA-II	Implementation of faster test turn-around	1.4	1.4
Jan.–Dec., 1976	AusRIA-II		1.2	1.2
Jan.–Dec., 1977	AusRIA-II		1.3	1.3
Jan.–Dec., 1978	AusRIA-II		1.2	1.2

marked increase represents the detection of formerly unidentified HBsAg reactive donors in addition to antigen-reactive new donors. Since the implementation of the modified AusRIA procedures, the corrected annual reactive rates per 1,000 donations have stabilized at 1.2 to 1.4.

In good agreement with the new donor reactive rate observed in community service volunteer blood centers,[12] nearly 90% of Alpha Therapeutic Corporation's reactive donations are attributed to new donors. These donations were never used in the manufacture of plasma products and, as mentioned previously, these donors were summarily excluded from the regular plasmapheresis program.* Thus the reactive rate within Alpha Therapeutic Corporation's continually tested plasmapheresis donor population may be estimated as 0.12 to 0.14 per 1,000.

The reactive donation rate of 1.2 to 1.4 per 1,000, which the Alpha Thera-

*Selected HBsAg reactive donors are plasmapheresed in special programs; their plasma is used for production of diagnostics, such as HBsAg-positive controls, and for vaccine development work.

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peutic Corporation's plasmapheresis program has maintained since April 1974, is comparable to the rate of 1.5 per 1,000 reported for a volunteer whole blood donor program.[13] Holley et al.[14] obtained similar results and conclusions at Fitzsimmons Army Medical Center in 1975. Thus the comparative reactive donor rates indicate that in a well-run commercial plasmapheresis program the incidence of HBsAg reactive donors is not significantly different from that reported in a volunteer whole blood donation program.

Hemophilia

That the Alpha Therapeutic Corporation's HBsAg reactive donor rate is comparable to that reported for a volunteer whole blood donor program is a most important factor in Profilate®, a freeze-dried Factor VIII concentrate for the treatment of hemophilia A. The introduction of freeze-dried Factor VIII concentrates, such as Profilate, has revolutionized the treatment of hemophilia A. The greater activity, stability and predictability of action of the concentrates have made home care practicable and have greatly facilitated the performance of major operations on hemophilic patients. Yet hepatitis B remains a major hazard in the management of hemophilia.[15]

Hemophiliacs are the group of multitransfused patients potentially exposed most frequently, and for the longest period of time, to the agent(s) implicated in posttransfusion hepatitis. Historically, hemophiliacs have a low incidence of acute hepatitis, but frequently demonstrate abnormal liver function tests.[18-21] In addition, hemophiliacs demonstrate a high prevalence rate of antibody to the hepatitis B surface antigen, which is considered a reliable index of past exposure to the hepatitis B virus.[22-25] Factor VIII concentrates prepared from plasma units tested for HBsAg by less sensitive, second-generation techniques have been implicated in transmitting hepatitis B to hemophilic recipients, even though the final Factor VIII concentrates were nonreactive by radioimmunoassay.[16,17] Apparently, third-generation testing of only the final product contributes little to product safety because of the dilution factor involved in a large pool product.

A recent study compared the hepatitis risk to hemophiliacs treated with single donor volunteer blood or plasma derivatives with that of hemophiliacs treated with commercial concentrates prepared from large pools of donors.[26] In the study, the commercial plasma units had been individually screened for HBsAg by a third-generation method, and the commercial donor population had an incidence of HBsAg antigenemia comparable to that of the volunteer donors used in the single donor products. The study showed no significant difference in hepatitis risks between the single donor volunteer products and the commercial concentrates prepared from large pools of low HBsAg antigenemia rate paid donor plasma. Thus it is suggested that users of Factor VIII concentrates ask their suppliers to verify that the products being sold have been prepared from donor populations with a low incidence of HBsAg antigenemia.

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