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The prevalence of hepatitis B surface antigen in commercially prepared plasma products

PTH Plasma Products Adverse Effect HBsAg HBV Paid donor

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Commercially available lots of plasma derivatives prepared between 1957 and 1975 were tested for hepatitis B surface antigen (HB_sAg) by radioimmunoassay. In all, 69 per cent of lots of plasma protein fraction, 40 per cent of factor IX concentrate, 20 per cent of normal serum albumin, 13 per cent of antihemophilic factor, 3 per cent of fibrinogen, and 0.7 per cent of immune serum globulin lots tested were HB_sAg-positive. There was great variation in the prevalence of HB_sAg-positive lots of each product among the different manufacturers, reflecting not only differences in methods of processing plasma, but also differences in donor populations. Those manufacturers relying upon volunteer donor plasma or placental source material demonstrated lower rates of HB_sAg-positive lots of final products than those relying upon commercial donor plasma. There was a marked decrease in the prevalence of positive lots during the period 1971 to 1973, coincident with the onset of routine plasma donor screening for HB_sAg. However, current requirements for plasma screening have not resulted in totally HB_sAg-free plasma products. Use of more sensitive and more reliable tests for HB_sAg will probably reduce contamination of plasma pools with HB_sAg to undetectable levels. Despite HB_sAg-status, however, the "high-risk" plasma products (fibrinogen, antihemophilic factor, factor IX concentrate) must still be considered capable of transmitting hepatitis and used only with the strictest indications.

The pooled, human plasma derivatives were found to transmit viral hepatitis shortly after their first use over twenty-five years ago.[1] Subsequent advances in protein component purification have not always been accompanied by advances in the protection of recipients of these products from hepatitis. With an increase in demand and use of pooled plasma products, measures to reduce the hepatitis risk are high in priority.

Based upon the relative risk of transmitting viral hepatitis, the pooled human plasma derivatives can be categorized as either "high-risk" or "low-risk" plasma products. At the present time, six major plasma products are in wide-scale use in the United States. Three of these are "high-risk" plasma products: fibrinogen (Cohn Fraction I), antihemophilic factor, and factor II, VII, IX, and X concentrate. Use of these products in susceptible patients results in hepatitis in a high percentage of cases.[2-9] The other three commonly used plasma derivatives are "low-risk" plasma products: immune serum globulin (Cohn Fraction II), normal serum albumin (Cohn Fraction V), and plasma protein fraction (Cohn

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Table I. HB_sAg in fibrinogen

	Per cent of lots HB_sAg positive by radioimmunoassay						
Manufacturer	*1967-70 (%)*	*1971 (%)*	*1972 (%)*	*1973 (%)*	*1974 (%)*	*1975 (%)*	*Total (%)*
A	7 (4/55)*	5 (1/21)	11 (3/28)	0 (0/14)	0 (0/30)	0 (0/12)	5 (8/160)
B	20 (3/15)	—	0 (0/2)	0 (0/11)	0 (0/6)	—	9 (3/34)
C	3 (3/114)	2.5 (1/41)	0 (0/51)	0 (0/35)	0 (0/25)	0 (0/17)	1.4 (4/283)
D	0 (0/21)	0 (0/15)	0 (0/2)		0 (0/4)	0 (0/5)	0 (0/47)
E		0 (0/5)					0 (0/5)
Total	5 (10/205)	2.5 (2/82)	3.6 (3/83)	0 (0/60)	0 (0/65)	0 (0/34)	3 (15/529)

*Numbers in parentheses indicate number positive/number tested.

Table II. HB_sAg in antihemophilic factor

	Per cent of lots HB_sAg positive by radioimmunoassay						
Manufacturer	*1966-1970 (%)*	*1971 (%)*	*1972 (%)*	*1973 (%)*	*1974 (%)*	*1975 (%)*	*Total (%)*
A	—	—	—	—	0 (0/18)*	0 (0/13)	0 (0/31)
B	8 (7/82)	6 (1/18)	4 (1/27)	4 (3/74)	0 (0/99)	0 (0/45)	3 (12/345)
E	0 (0/1)	25 (1/4)	11 (4/37)	0 (0/37)	5 (3/60)	0 (0/3)	6 (8/142)
F	31 (8/26)	5 (2/39)	7 (7/94)	7 (4/56)	5 (1/222)	0 (0/94)	4 (22/531)
G	—	32 (6/19)	47 (14/30)	27 (20/73)	35 (6/17)	0 (0/59)	23 (46/198)
H	—	70 (23/33)	0 (0/13)	40 (38/95)	30 (31/104)	40 (23/58)	38 (115/303)
Total	14 (15/109)	29 (33/113)	13 (26/201)	19 (65/335)	8 (41/520)	8 (23/272)	13 (203/1,550)

*Numbers in parentheses indicate number positive/number tested.

Table III. HB_sAg in factor IX concentrate

	Per cent of lots HB_sAg positive by radioimmunoassay					
Manufacturer	*1971 (%)*	*1972 (%)*	*1973 (%)*	*1974 (%)*	*1975 (%)*	*Total (%)*
A	—	0 (0/1)*	0 (0/13)	0 (0/22)	0 (0/3)	0 (0/39)
B	0 (0/3)	80 (8/10)	55 (29/53)	72 (41/57)	8 (3/37)	50 (81/160)
Total	0	73 (8/11)	44 (29/66)	52 (41/79)	7.5 (3/40)	40 (81/199)

*Numbers in parentheses indicate number positive/number tested.

Fractions IV-4 + V). Use of these products appears to be without risk of transmitting viral hepatitis.[10–13]

The discovery and characterization of the hepatitis B surface antigen (HB_sAg, Australia antigen) promised to provide a simple and practical means of detecting the hepatitis B virus in plasma which might significantly reduce the risk of clinical hepatitis associated with blood transfusion.[14, 15] United States Federal regulations, effective in July 1972, required that all donor blood be tested for HB_sAg by methods at least as sensitive as

Table IV. HB_sAg in normal serum albumin

Manufacturer	Per cent of lots HB_sAg positive by radioimmunoassay		
	1957-1960 (%)	1961-1965 (%)	1966-1970 (%)
A	7 (2/30)*	33 (17/52)	70 (38/54)
B	0 (0/30	3 (3/92)	57 (48/84)
C	40 (20/50)	75 (72/96)	95 (88/92)
D	0 (0/5)	0 (0/52)	0 (0/76)
F	0 (0/33)	0 (0/75)	23 (19/81)
G	0 (0/64)	0 (0/20)	13 (6/46)
J	0 (0/26)	0 (0/67)	6 (5/79)
Others	0 (0/33)	0 (0/16)	12 (14/116)
Total	8 (22/271)	19 (92/470)	35 (218/628)

*Numbers in parentheses indicate number positive/number tested.

Table V. HB_sAg in plasma protein fraction

Manufacturer	Per cent of lots HB_sAg positive by radioimmunoassay		
	1958-1960 (%)	1961-1965 (%)	1966-1970 (%)
A	100 (34/34)*	98 (257/261)	98 (290/296)
B	—	51 (75/147)	78 (128/165)
F	—	—	80 (33/41)
G	—	—	80 (4/5)
J	—	—	0 (0/5)
K	—	—	7 (1/15)
Total	100 (34/34)	81 (332/408)	87 (456/527)

*Numbers in parentheses indicate number positive/number tested.

counterelectrophoresis (CEP).[16] Estimates from prospective studies of whole blood transfusion (an "intermediate risk" product) suggested that elimination of units positive for HB_sAg by CEP would reduce the incidence of post-transfusion hepatitis by about 25 per cent.[17] Whether a similar decrease would occur in the incidence of hepatitis following the use of "high-risk" plasma products has not been studied. One means of assessing the overall contamination of plasma derivatives with hepatitis B virus is to assay the final product for the presence of HB_sAg by a sensitive method. The availability of a solid-phase

1971 (%)	*1972 (%)*	*1973 (%)*	*1974 (%)*	*1975 (%)*	*Total (%)*
29	0	0	0	0	32
(6/21)	(0/18)	(0/2)	(0/8)	(0/10)	(63/195)
65	0	0	13	0	21
(13/20)	(0/21)	(0/22)	(7/55)	(0/10)	(71/334)
100	35	0	4	0	64
(7/7)	(6/17)	(0/8)	(1/24)	(0/10)	(194/304)
0		0	0	0	0
(0/11)	—	(0/5)	(0/9)	(0/4)	(0/162)
0	0	0	0	0	6
(0/20)	(0/20)	(0/13)	(0/40)	(0/10)	(19/292)
30	32	50	83	0	15
(6/20)	(9/28)	(5/10)	(5/6)	(0/10)	(31/204)
0	6				3
(0/10)	(1/16)	—	—	—	(5/198)
5	4	0	14	12	8
(2/39)	(2/52)	(0/31)	(9/63)	(3/25)	(30/375)
20	10	6	11	4	20
(34/148)	(18/172)	(5/91)	(22/205)	(3/79)	(414/2,064)

1971 (%)	*1972 (%)*	*1973 (%)*	*1974 (%)*	*1975 (%)*	*Total (%)*
100	0	13	0	0	83
(7/7)	(0/20)	(4/30)	(0/58)	(0/10)	(592/716)
83	45	75	58	10	63
(5/6)	(9/20)	(15/20)	(32/55)	(1/10)	(265/423)
50	24	14	11	0	39
(9/18)	(5/21)	(1/7)	(4/35)	(0/10)	(52/132)
100	97	89	86	—	91
(6/6)	(21/22)	(23/26)	(6/7)		(60/66)
—	0	—	0	0	0
	(0/13)		(0/21)	(0/11)	(0/50)
—	—	—	—	—	7
					(1/15)
73	36	52	24	2	69
(27/37)	(35/96)	(43/83)	(42/176)	(1/41)	(970/1,402)

radioimmunoassay (RIA) for detecting HB$_s$Ag which is approximately 100 times as sensitive as CEP has made such testing of final products possible.[18] Sgouris[19] has presented data showing that lots of antihemophilic factor tested by the RIA method often gave positive results and that the use of volunteer donor plasma and CEP screening of donors has decreased the frequency with which HB$_s$Ag could be detected in this product. The present study was undertaken to assess the overall prevalence of HB$_s$Ag-positivity in commercially available lots of plasma derivatives distributed in the United States over the past 18 years.

Table VI. Prevalence of HB_sAg in plasma derivatives

Plasma product	*Quantitative distribution of HB_sAg**	*Per cent positive*		
		Before 1972	*Since 1972*	*Infectivity*
Antihemophilic factor	0.5-1.2%	25%	11.4%	+
Fraction I (fibrinogen)	0.6%	4.2%	0%	+
Fraction II (ISG)	<0.01%	0.8%	0%	−
Fraction III (thrombin)	9%	—†	—†	+
Fraction IV-4 (PPF)	8-11%	84%	29%	−‡
Fraction V (NSA)	1-5%	24%	8%	−‡
Factor IX (supernate I)	<0.1%	0%	0%	+
(fraction IV)	Unknown	70%	50%	+

*Calculated from references 24-26.
†Thrombin is no longer used as a biologic.
‡PPF and NSA are not infectious after heating at 60° C. for 10 hours.

Materials and methods

Samples of every lot of commercial plasma derivatives produced by licensed manufacturers in the United States are submitted to the Bureau of Biologics prior to distribution for testing for potency, safety, and purity. Residual samples of these products, which have been stored at 4° C., were available for testing for HB_sAg. Data available on each lot consisted of manufacturer, lot number, date of submission to the Bureau of Biologics, and source (whether plasma or placenta derived). Six products were investigated: fibrinogen, antihemophilic factor (AHF), factor IX concentrate, immune serum globulin (ISG), normal serum albumin (NSA), and plasma protein fraction (PPF). Because of the large number of lots of ISG, NSA, and PPF available, a representative collection of these products was chosen consisting of approximately twenty lots of each product from each manufacturer for each year. The final collection tested represented approximately 20 per cent of all ISG lots, 25 per cent of NSA lots, and 40 per cent of PPF lots submitted during this time span. In the case of AHF, factor IX, and fibrinogen not all lots submitted before 1974 were still available for testing. Since January 1974, however, all lots of AHF, factor IX, and fibrinogen submitted to the Bureau of Biologics have been tested for HB_sAg. This report summarizes data on testing of products submitted through June 1975.

Samples were tested for HB_sAg by solid-phase RIA: those dated prior to January 1975 were tested by Ausria-I (Abbott) those dated after that time by the slightly more sensitive Ausria-II (Abbott). Samples were considered positive if they yielded reproducible counts per minute (c.p.m.) at least 2.1 times the negative control mean and could be confirmed as true specific positives by inhibition with human antibody to HB_sAg.[20] Samples positive by RIA were tested by CEP.[21] A few selected samples were also tested by reversed passive hemagglutination.[22]

Fibrinogen. A total of 529 lots of fibrinogen, representing products from five manufacturers submitted between 1966 and June 1975, were tested for HB_sAg. The results are tabulated in Table I by manufacturer and date of submission to the Bureau of Biologics. Fifteen of the 529 lots (3 per cent) were specifically reactive for HB_sAg by RIA. Only weakly positive reactions were observed: none was reactive on CEP and all 15 samples yielded counts per minute less than nine times the negative control mean counts per minute on RIA. A considerable variation was seen among the five manufacturers in the prevalence of HB_sAg positive fibrinogen lots, ranging from 0 per cent for two manufacturers (D and E) that rely predominantly upon volunteer donor plasma to 1.4 per cent, 5 per cent, and 9 per cent for three that rely heavily upon plasma from commercial sources. The prevalence of HB_sAg reactive lots of fibrinogen decreased in 1971-1972, in that 4.2 per cent of lots (12/289) submitted before 1972 were found to be HB_sAg positive, but no lot submitted since then has been HB_sAg reactive.

Antihemophilic factor (AHF). A total of 1,550 lots of AHF submitted by six manufacturers from 1966 to 1975 were tested for HB_sAg. Table II summarizes the results

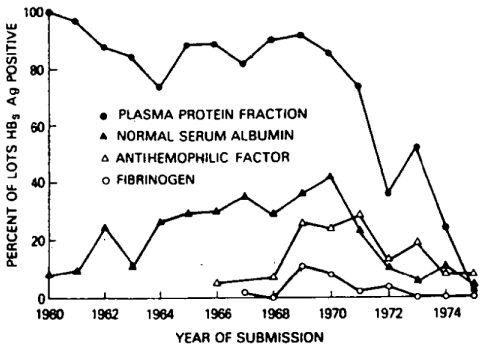

Fig. 1. The prevalence of hepatitis B surface antigen (HB_sAg) in commercially prepared lots of plasma products by year of submission to the Bureau of Biologics.

has been repeatedly shown to be infectious,[2-5] a fact that has rightly limited its use to few clinical situations. Obviously, the HB_sAg status of fibrinogen does not adequately reflect its infectivity. More dramatic is the situation with the two types of factor IX concentrate. Manufacturer A prepares factor IX-rich concentrates by a DEAE cellulose adsorption from the supernate of Cohn fraction I. This results in very little HB_sAg in the eluate and to date, no commercially available factor IX lot prepared in this manner has been found HB_sAg-positive. Manufacturer B prepares factor IX from fraction III, a fraction that receives a high percentage of HB_sAg reactivity. As would be expected 50 per cent of lots prepared in this manner have been found HB_sAg-positive. However, factor IX concentrates made by both methods have been shown to be infectious in an alarming percentage of patients.[6-9] Furthermore, the infectivity of HB_sAg-positive and negative lots of both of these products has been documented by inoculation of susceptible gibbons and chimpanzees (unpublished data). This discrepancy between HB_sAg reactivity and infectivity could be explained as either a "masking" of the HB_sAg by antibody in the pooled plasma, or perhaps by the presence of naked virus nucleocapsid core material. Most likely, however, this discrepancy is merely a matter of dilution of the HB_sAg to levels below the detectability of present immunologic tests for this viral envelope antigen. The RIA is an extremely sensitive method which can detect as little as 1 ng. per milliliter of HB_sAg. Nevertheless, dilution studies in chimpanzees have shown that HB_sAg-positive sera that are reactive on RIA to a dilution of 10^{-4} to 10^{-5} were still infectious at dilutions of 10^{-7}.[27] Thus, infectivity is 100 to 1,000 times as sensitive an indicator for hepatitis B virus as is the RIA for hepatitis B antigen. If a single unit of HB_sAg positive plasma was diluted in a pool of 1,000 units of antigen-negative plasma, and the antigen titer was reduced 10- to 100-fold further by fractionation or by random addition of plasma containing anti-HB_s, the final product would be HB_sAg negative by the most sensitive present methods. However, as many as 10,000 infectious particles could still be present in every milliliter of starting plasma.

Because PPF receives most of the HB_sAg reactivity present in plasma pools and thus is most frequently positive, this fraction serves as the best indicator of the overall contamination of plasma pools with HB_sAg reactive units. The prevalence of HB_sAg in PPF preparations can thus be used to suggest contamination of other plasma products that are

prepared from the same plasma pools, but which receive only a low percentage of HB_sAg reactivity. In the case of factor IX concentrate, none of the lots prepared by Manufacturer A was HB_sAg positive. However, 83 per cent of PPF lots prepared by this manufacturer were reactive. This again suggests that these factor IX preparations although HB_sAg negative, were probably prepared from HB_sAg-containing plasma pools and harbored low levels of infectious virus.

Differences among manufacturers. For each of the plasma products investigated, there were significant differences in HB_sAg positivity among the various manufacturers. Rates of HB_sAg positivity ranged from a low as 0 per cent for each product to as high as 9 per cent for fibrinogen, 38 per cent for AHF, 50 per cent for factor IX, 66 per cent for NSA, and 91 per cent for PPF. Some of these differences could be attributed to methods of fractionation and processing which might differ from manufacturer to manufacturer. As already mentioned, this was especially true for factor IX concentrate. This could also be true for AHF, since the methods of preparation of AHF vary considerably among manufacturers, and quite different levels of purity of factor VIII are achieved. Unfortunately, these processing differences cannot be assessed by the type of prevalence data presented here. However, what can be assessed and what does stand out as a determinant of HB_sAg-status of these plasma derivatives is the nature of the donor population; whether volunteer or commercial. Volunteer donor populations characteristically have lower prevalences of HB_sAg than commercial donor populations.[28] It would be expected that plasma products made from volunteer donor plasma would be reactive for HB_sAg less frequently than those prepared from commercial plasma. This appeared to be the case. The comparable rates of HB_sAg positivity for products prepared from volunteer and commercial plasma were 0 per cent (0/52) versus 3 per cent (15/477) for fibrinogen, 1 per cent (6/429) versus 25 per cent (405/1,556) for NSA, and 0 per cent (0/50) versus 72 per cent (970/1,352) for PPF. The effect of volunteer donor population was also seen in NSA lots prepared from placental material. Those manufacturers relying on placental material for preparing NSA demonstrated low rates of HB_sAg positivity for this product (7 per cent as compared to the average of 20 per cent). However, since placental plasma requires processing steps not necessary for fractionating venous plasma, it is possible that differences in processing methods as well as differences in HB_sAg prevalence in the starting material contribute to the prevalence of HB_sAg reactivity of the final products.

Effect of donor plasma testing. Analysis of the prevalence of HB_sAg positive lots of plasma derivatives by year of submission to the Bureau of Biologics has demonstrated a sharp decrease in that prevalence beginning in 1972 (Fig. 1). This decrease coincided with HB_sAg screening of donors and elimination of positive units, a practice that began in 1971 and became a federal requirement in July 1972.[16] This decrease in HB_sAg prevalence was seen for each of the six products tested (Table VI).

Using PPF as an indicator of overall contamination with HB_sAg, it can be seen that before 1972 over 80 per cent of lots were HB_sAg reactive. This suggests that most if not all large, commercial plasma pools made at that time were contaminated with hepatitis B virus and explains why the unheated, "high-risk" products (fibrinogen, antihemophilic factor, and factor IX concentrate) made from these plasma pools were so frequently found to be infectious. Starting in 1972, the prevalence of HB_sAg positive lots of PPF began to decrease. However, the requirement for mandatory testing has not resulted in totally HB_sAg-free products, and some manufacturers have been less successful than others in

eliminating HB_sAg contamination. The reasons for this are probably a combination of the poor sensitivity and proficiency of tests for HB_sAg as performed by some plasma suppliers[29] and the inclusion of plasma obtained and partially processed before the federal requirement for HB_sAg testing. Until recently, most donor screening was done by CEP. In semi-annual proficiency panels distributed by the Bureau of Biologics to all licensed blood banks and plasmapheresis establishments, CEP has consistently failed to detect 16 per cent to 37 per cent of CEP-reactive specimens.[29] Thus, in the best of circumstances about 25 per cent of CEP positive plasma would not be eliminated by routine CEP screening. Indeed, if CEP testing were performed with maximum proficiency, it is doubtful that any of the final products would be HB_sAg reactive, even by RIA. RIA testing, on the other hand, is generally not only more sensitive but also more reliable than CEP testing. In these same proficiency panels, the number of RIA-positive samples that went undetected by this test method have been low, ranging from 0 per cent to 3 per cent. Those manufacturers that have now turned to RIA screening of all plasma have demonstrated a total lack of detectable HB_sAg in the final plasma products submitted. For these and other reasons the Bureau of Biologics has required that test methods more sensitive and more reliable than CEP be used in screening all blood and plasma for HB_sAg.[30]

While the achievement of totally HB_sAg-negative "high-risk" plasma products is certainly a desirable goal, it should not be then concluded that these products are safe from transmitting viral hepatitis. While testing blood for HB_sAg by RIA will eliminate most units that are infectious for type B hepatitis, it will not eliminate all.[31–32] Post-transfusion type B hepatitis continues to occur in recipients of blood all of which is screened by RIA,[31] and it can be expected that even with RIA testing this type of unit will continue to be included in large commercial plasma pools and continue to cause the"high-risk" products to be infectious. There is no doubt, however, that RIA screening can eliminate many if not most of the hepatitis B virus-containing units and, thus, will result in a significant lowering of hepatitis B virus titer in these products. It remains to be seen whether the factors of processing, fractionation, and pooling with antibody-containing units will be such that this lower titer of virus is neutralized or "diluted-out" to the point that these are no longer infectious for type B hepatitis.

One final consideration makes it important to stress the infectivity of these "high-risk" plasma products despite their lack of HB_sAg reactivity. Recently, it has been shown that not all post-transfusion hepatitis can be classified as type B hepatitis.[31–33] More startling was the finding that the "non-B," post-transfusion hepatitis could not be classified as type A hepatitis.[33] This has led investigators to postulate the existence of a third human hepatitis virus, a virus which also appears to be harbored in blood. It is possible that this third hepatitis virus can withstand the pooling and fractionation procedure (unlike the Epstein-Barr Virus or cytomegalovirus) and that it is reponsible for some cases of hepatitis following the use of "high-risk" plasma products. At present there are no markers and no means of detecting this virus in blood. Until the nature of this virus and its disease is elucidated, it is important to consider human blood and pooled plasma products as potentially infectious. At the present time, fibrinogen, AHF and Factor IX concentrates remain "high-risk" plasma products (despite their HB_sAg status) which must be considered likely to produce overt hepatitis in susceptible recipients.

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