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Report to the Chairman, Subcommittee on Human Resources, Committee on Government Reform and Oversight, House of Representatives

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BLOOD PLASMA SAFETY

Plasma Product Risks Are Low if Good Manufacturing Practices Are Followed

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Health, Education, and
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The Honorable Christopher Shays
Chairman, Subcommittee on Human Resources
Committee on Government Reform and Oversight
House of Representatives

Dear Mr. Chairman:

Each year, an estimated 1 million people in the United States receive products manufactured from human plasma. Many different components of plasma are used for medical treatment, ranging from treating the trauma of burns and surgery to replacing blood elements that are lacking as a result of disease, such as hemophilia. In the 1980s, before the mechanism of HIV transmission was understood, many hemophilia patients used plasma products made from donations by individuals infected with HIV, with 63 percent of all hemophilia patients in the United States becoming infected as a result. Many more contracted hepatitis B (HBV) and hepatitis C (HCV). While these diseases have been transmitted in many fewer cases since the introduction of antibody tests and viral inactivation and removal processes, some safety concerns remain.

One of these concerns relates to plasma donors, who are unpaid or paid. Through the volunteer sector, unpaid donors give whole blood, from which the plasma may be separated and sent for further manufacturing into plasma products. The commercial sector collects plasma from paid donors, known as source plasma, for manufacture into these same products. Some source plasma is also collected from unpaid donors. There has been a long-standing concern that the infectious disease rates among paid donors might be higher than those of volunteer donors because paid donors may have a financial incentive to conceal risk factors that would prevent them from donating. Concerns have also been raised about the number of donors to whom a recipient is exposed because the products are manufactured by pooling many donations. Further, the efficacy of viral clearance procedures used in manufacturing and the safety record of the manufacturers clearly affect the ultimate safety of the products.

In light of these concerns, you asked us to undertake a study to (1) compare the risk of incorporating an infectious unit of plasma into further manufacturing from volunteer versus paid plasma donors for HIV, HBV, and HCV; (2) examine the impacts on frequent and infrequent plasma users when pooling large numbers of plasma donations into manufactured

plasma products; (3) assess the safety of end products from plasma after they have undergone further manufacturing and inactivation steps to kill or remove viruses; and (4) examine the recent regulatory compliance history of plasma manufacturers.

In developing our information, we interviewed representatives of the volunteer and commercial plasma sector and officials of the Food and Drug Administration (FDA). We also attended technical conferences and examined the scientific literature on the collection and processing of plasma products and current good manufacturing practices within the plasma industry. We obtained data from industry representatives from which we calculated the chances of incorporating an infectious unit of plasma into further manufacturing from volunteer and paid donors. We did not independently verify data on infectious disease rates, but they are the most current and complete data available. We also obtained information on the effect of the number of donations that are used in manufacturing on the safety of the final products. We gathered information on the effect of viral inactivation and removal techniques used to further reduce the risk of viral transmission through plasma products. Finally, we obtained inspection reports of plasma-derived-product manufacturing facilities from the FDA that showed the agency's determination of whether these facilities were in compliance with current good manufacturing practices. We conducted our review from December 1997 to June 1998 in accordance with generally accepted government auditing standards. (See app. I for a further discussion of our methodology.)

Results in Brief

Viral clearance techniques have made the risks of receiving an infected plasma product extremely low when manufacturers follow all the procedures in place to ensure safety. While paid plasma donors are over one and a half times more likely to donate potentially infectious units (1 in every 3,834 units), a number of recent initiatives by the source plasma industry greatly reduce the chances of these units being pooled for manufacturing (to 1 in every 10,959 units). These initiatives include the use of only repeat donors (who have been found to have lower rates of viral infection than first-time donors) and a 60-day inventory hold on all units to allow manufacturers to retrieve units from donors who subsequently test positive or are otherwise disqualified. Even with these initiatives in place, the risks are still somewhat higher from plasma units donated by paid donors than from volunteer donors (where 1 in every 15,662 units are potentially infectious).

Limiting the number of donors whose plasma is pooled for production into plasma products helps to reduce the risks of viral transmission for those receiving these products. Presently, a 60,000-donor limit has been established for each individual plasma product. This effort has an impact on infrequent users by minimizing their exposure to a certain number of donors for the few times they would be infused with a plasma product. For frequent users of plasma products, such as severe hemophilia patients, this donor limit has a negligible impact because of the large number of infusions that they receive and, thus, the large number of pools that they would be exposed to in the course of their lifetime.

A more significant step in reducing risk of infection occurs in manufacturing—where all plasma products for intravenous use undergo viral removal, inactivation procedures, or both—which virtually eliminates enveloped viruses such as HIV, HBV, and HCV. This is supported by epidemiological data on the transmission of viruses through plasma products since the introduction of adequate viral removal and inactivation procedures in the late 1980s as well as laboratory data that characterize the effectiveness of viral clearance through these procedures. On the other hand, these processes have limited effectiveness against non-lipid enveloped viruses such as hepatitis A (HAV) and human parvovirus.

Certain advances—such as voluntary initiatives by the commercial plasma industry, increasingly sophisticated screening tests that close the "window period" between the time a donor becomes infected and the time a particular laboratory test becomes positive, and viral removal and inactivation procedures—are only effective if the processes used to produce finished plasma products adhere to current good manufacturing practices. This, however, has not been the case with all of the major manufacturing companies that produce plasma products. Recent FDA inspection reports highlight numerous instances of noncompliance with current good manufacturing practices. These problems have led to the imposition of consent decrees between FDA and two manufacturing companies, the temporary suspensions of production at one manufacturing facility, and shortages of some plasma products. Although there have been no known cases of transmission of HIV, HBV, or HCV from plasma products during the time these problems were identified by FDA, it is clear that there were numerous instances of noncompliance in the manufacture of plasma products. Without strict adherence to current good manufacturing practices related to the efficacy of viral removal and inactivation procedures, the safety of these plasma products could be compromised. Actions being taken by FDA and the plasma manufacturers

since these problems were identified should help to alleviate some of these concerns.

Background

Plasma is the liquid portion of blood, containing nutrients, electrolytes (dissolved salts), gases, albumin, clotting factors, hormones, and wastes. Many components of plasma are used, and include treatments for the trauma of burns and surgery and for replacing blood elements that are lacking as a result of disease, such as hemophilia. Table 1 lists the plasma components that are currently available in the United States and their primary uses.

Table 1: Plasma Components and Their Primary Uses

Component	Primary uses
Albumin	To restore plasma volume in treatment of shock, trauma, surgery, and burns
Alpha-1 proteinase inhibitor	To treat emphysema caused by genetic deficiency
Antihemophilic factor concentrate (factor VIII)	For prophylaxis and treatment of hemophilia A bleeding episodes
Anti-inhibitor coagulant complex	To treat bleeding episodes in the presence of factor VIII inhibitor
Antithrombin III	To prevent clotting and thromboembolism associated with liver disease, antithrombin III deficiency, and thromboembolism
Coagulation factor IX (human)	For prophylaxis and treatment of hemophilia B bleeding episodes and other bleeding disorders
Cytomegalovirus immune globulin	For passive immunization subsequent to exposure to cytomegalovirus
Factor IX complex	For prophylaxis and treatment of hemophilia B bleeding episodes and other bleeding disorders and for warfarin (anticoagulant) reversal
Hepatitis B immune globulin	For passive immunization subsequent to exposure to hepatitis B
Immune globulin: intravenous and intramuscular	To treat agamma- and hypogamma-globulinemia; for passive immunization for hepatitis A and measles
Plasma protein fraction	To restore plasma volume subsequent to shock, trauma, surgery, and burns
Rabies immune globulin	For passive immunization subsequent to exposure to rabies
Rho(D) immune globulin	To treat and prevent hemolytic disease of fetus and newborn infant stemming from Rh incompatibility and incompatible blood transfusions
Tetanus immune globulin	For passive immunization subsequent to exposure to tetanus
Vaccinia immune globulin	For passive immunization to laboratory exposure to smallpox or vaccinia
Varicella-zoster immune globulin	For passive immunization subsequent to exposure to chicken pox

Source: American Blood Resources Association, Basic Facts About the Commercial Plasma Industry.

The various plasma-derived products are purified from the plasma pool by a process known as fractionation. This process separates plasma proteins

based on the inherent differences of each protein. Fractionation involves changing the conditions of the pool (for example, the temperature or the acidity) so that proteins that are normally dissolved in the plasma fluid become insoluble, forming large clumps called precipitate. The insoluble protein can be collected by spinning the solution at high speeds or through filtration. One of the most effective ways for carrying out this process is the addition of alcohol to the plasma pool while simultaneously cooling the pool. For this reason, the process is sometimes called cold alcohol fractionation or ethanol fractionation. This procedure is carried out in a series of steps so that a single pool of plasma yields several different protein products such as albumin and immune globulins.[1]

It is estimated that each year, as many as a million patients rely on products manufactured from human plasma: more than 400,000 are given albumin, 15,000 to 18,000 are given factor VIII, 3,000 to 5,000 receive factor IX, greater than 20,000 receive immune globulin intravenous (IGIV), and an estimated 100,000 to 500,000 receive immune globulin intramuscular (IGIM). Additional patients receive a variety of hyperimmune globulins and other specialized products.

Plasma Donation

Plasma used for plasma-derived products manufactured and distributed in the United States can only be collected at facilities registered with the FDA. Centers require donors to provide proof that they are legally in the United States and have a local permanent residence. About 85 percent of plasma is collected from paid donors in a commercial setting and is known as source plasma. Through a process known as plasmapheresis, the plasma is removed and the red cells are reinfused into the donor. The remaining 15 percent of plasma is collected from volunteer donors and is known as recovered plasma. From the whole blood, plasma is "recovered"—that is, the red cells, platelets, and cryoprecipitate are separated for transfusion and the unused plasma is either transfused as plasma or sent for further manufacturing into plasma products. On the basis of a European Union policy position, many European countries are working toward self-sufficiency in plasma products using an all-volunteer system, although most countries continue to depend on U.S. products made from paid donors and on source plasma obtained from U.S. donations.

[1]It can take up to 7 months from the time plasma is collected until there is a final product release. Approximate times for steps in this process include collection and testing of the plasma (10 days), inventory hold (60 days), staging and internal quality control (10 days), pooling of the plasma (1 to 2 days), fractionation process (7 to 10 days), collection of intermediates and runs for internal quality control (20 days), preparation of final products (7 to 10 days), quality control testing prior to filling of final product (25 to 28 days), and manufacture and FDA testing and release of final product (60 days). Recently, this last step has been reduced to 2 weeks or less for most products.

Units of plasma collected as source plasma contain approximately 825 milliliters, while recovered plasma from whole blood donations contain approximately 250 milliliters. Thus, more than three times as many donated units of recovered plasma are required to make up a pool of equal volume to one made up of only source plasma.

Approximately 370 paid plasma collection centers annually collect about 11 million liters of plasma from 1.5 million donors, involving a total of approximately 13 million separate donations each year. The industry, through its trade organization, the American Blood Resources Association, maintains a limited national donor deferral registry that is checked for each first-time donor.[2] This is a list of known donors who are unsuitable for further donations because of positive test results. Repeat donors' records are checked at the plasmapheresis center where the plasma is removed. Most of these centers also ensure that donors are not migrating from one center to another over the 48-hour minimum donation interval.[3] The vast majority of source plasma is processed by four companies: Alpha Therapeutic Corporation, Baxter Healthcare Corporation, Bayer Corporation, and Centeon LLC.

An additional 1.8 million liters of plasma are collected annually from approximately 8 million volunteer (not paid) donors who contribute 12 to 13 million whole blood donations. Volunteer donors give blood at American Red Cross blood centers and independent blood centers represented by America's Blood Centers; the plasma is recovered for further manufacturing. Plasma collected by the American Red Cross is fractionated under contract by Baxter Healthcare and the Swiss Red Cross and returned to the American Red Cross for distribution. Plasma collected at member facilities of America's Blood Centers is currently sold only to the Swiss Red Cross, which manufacturers the various plasma products and sells them through U.S. distributors.

Paid donors typically receive between $15 and $20 for the 2 hours required to remove whole blood, separate the plasma from the cells and serum, and reinfuse the latter back into the donor. Source plasma donors may donate

[2]These 370 centers have been certified by the American Blood Resources Association. Under the association's Quality Plasma Program, collection centers are inspected by a third party for compliance to specific standards, such as facility maintenance, employee training, and donor screening. There are a total of 470 licensed plasma collection centers in the United States. The additional 100 centers include some whole blood facilities licensed to collect source plasma, centers that collect source plasma exclusively for export, and others. These centers do not provide source plasma to the four major fractionators for U.S. production.

[3]For example, centers may mark different fingers with florescent dye or use other methods to identify donors.

once every 48 hours but no more than twice a week. Whole blood donors can donate only once every 56 days since their red cells are not reinfused as is done with the paid donor.

Donor Screening

Donor screening is designed to prevent the donation of blood by persons who have known risk factors or other conditions such as low blood pressure. All prospective donors, both paid and volunteer, are screened for medical history and risk behaviors. High-risk donors, those whose blood or plasma may pose a health hazard, are encouraged to exclude themselves. Everyone who seeks to donate plasma must answer a series of behavioral and medical questions. If the answers indicate high risk, the prospective donor is deferred from donating. The screening requirements are completed before the donor is allowed to give plasma. Additionally, paid donors must pass an annual physical examination and a brief medical examination each time they donate. Similarly, volunteer donors undergo a brief medical examination each time they donate.

The American Blood Resources Association's National Donor Deferral Registry is one method by which the plasma industry has attempted to ensure that donors who are presenting to donate for the first time at a plasma center are checked for past deferrals at other centers. The American Red Cross has a similar system that is a national list of those deferred through their blood collection system. Each member facility of America's Blood Centers maintains its own donor deferral list against which donors are checked.

Testing of Donors

All donors are tested for certain viruses known to be transmissible through blood, including HBV, HCV, and HIV.[4] The specific screening tests check for the presence of hepatitis B surface antigen (HBsAg), antibodies to hepatitis C (anti-HCV), HIV-1 antigen (Ag) and antibodies to HIV types 1 and 2 (anti-HIV).[5] Donors with repeatedly reactive test results are rejected from further donations. (See app. II for more information on testing procedures.) For units found to be reactive on HIV tests, the positive units and all previously donated plasma units not pooled for manufacture in the preceding 6 months are retrieved, and those professional services who

[4]Additionally, paid donors are tested for syphilis every 4 months, while every volunteer donation is tested for syphilis. Testing for syphilis is performed, for the most part, as an indicator of high-risk behaviors. The value of this testing has been debated.

[5]Antibody tests detect antibodies that the human body produces in its immune response to a virus, whereas antigen tests detect a component of the actual virus. Because it takes time to develop antibodies, antigen tests detect infection earlier than antibody tests.

receive the plasma products are notified according to federal regulations (21 C.F.R. 610.46).[6]

All of the plasma fractionation companies have also received permission from the FDA to begin clinical trials of the polymerase chain reaction (PCR) technique, a more sensitive test that is now available, to detect viral material for HIV, HBV, and HCV. PCR is used to amplify the number of copies of a specific region of DNA or RNA in order to produce enough DNA or RNA to be adequately tested. This technique appears to be able to identify, with a very high probability, disease-causing viruses such as HIV, HBV, and HCV. Because PCR testing detects virus particles at the genetic level, infected donors can be identified days or even months sooner than if only traditional antibody or antigen testing is performed, thus shortening the window period. PCR testing is being investigated using minipools that can combine over 500 individual donations. All plasma used in the manufacturing process that undergoes PCR testing must be nonreactive for that specific test.[7]

Risk of Infectious Units Entering Plasma Pools Is Somewhat Higher for Paid Plasma Donors Than for Volunteer Donors

We calculated the risk of incorporating an infectious unit of plasma into a plasma pool for HIV, HBV, and HCV for both volunteer and paid plasma donors. Overall viral marker rates for HIV, HBV, and HCV are higher among individuals who present themselves to donate at paid plasma centers than among those who come to volunteer blood centers. This is due to higher HCV rates among paid donors. Units that test positive are excluded. The incidence rate of collecting infectious units from donors who are in the window period between the time they become infected and the time they test positive is much higher among paid plasma donors than among volunteer donors. However, a number of safety initiatives have been instituted by paid plasma centers that greatly reduce the likelihood of infectious units being pooled for manufacturing. Nevertheless, the final—or residual—risk of an infectious unit entering a plasma pool remains somewhat higher for paid donors than for volunteer donors.

[6]Additionally, tests are performed to examine the level of the liver enzyme alanine aminotransferase (ALT). An abnormal ALT may be an indicator of liver disease, a viral infection that causes liver disease, or both. Units with unacceptable ALT levels are not used. Whole blood donations are also tested for antibodies to human lymphotropic virus types I and II, but source plasma is not screened for this because it is cell associated and not found in plasma.

[7]Because of pooled sample testing, an individual unit still could be positive but only at a very low titer. Such a low-titer unit may not be detected using pooled PCR and thus would be added to the manufacturing pool.

There are at least four potential ways in which viral agents go undetected during donation and may thus be transmitted through blood products.[8] First, there exists a very rare chronic carrier state in which a clinically asymptomatic, yet infectious, donor will persistently test negative on a donation screening test. Second, a viral agent may have a large degree of genetic diversity so that laboratory screening tests fail to identify some infectious donors who harbor an atypical genetic variant. Third, laboratory error in performing screening tests may occur, allowing positive units to be made available for transfusion. Finally, the donor may have a negative laboratory test during the window period before the virus is detected by currently licensed screening tests. The majority of cases in which an infectious donation will be included in a plasma pool is a result of this last circumstance. As a result, modeling techniques have been developed to determine the risk estimates of incorporating these infectious window period units into the blood supply.

To determine the marker rate for HIV in plasma donations, we obtained data from California's Department of Health Services (DHS), which collects information on these rates for volunteer blood donors and paid donors at plasma collection facilities.[9] We obtained information on HIV, HBV, and HCV viral marker rates from the American Red Cross for donors who donate at their centers. The American Blood Resources Association provided us with data on repeatedly reactive test results for paid donors who donate at their centers. We adjusted these data to obtain the viral marker rates.

In addition, we obtained information on incidence rates among American Red Cross and American Blood Resources Association donors to adjust for the effect of such variables as first-time donor versus repeat donors, the length of the interdonation interval (the time period between donations), and the number of seroconverters found among plasma donors.[10] We also compared the residual risk of a potentially infectious plasma donation by a volunteer versus paid plasma donor actually entering a plasma pool by examining the effect of the length of the window period as well as the use of only "qualified donors" and the 60-day

[8]Stephan Kleinman and others, "The Incidence/Window Period Model and Its Use to Assess the Risk of Transfusion-Transmitted Human Immunodeficiency Virus and Hepatitis C Virus Infection," Transfusion Medicine Reviews, Vol. 11 (1997), pp. 155-72.

[9]The term "viral marker rates" refers to the rate at which a particular group has confirmed-positive tests for particular viruses, in this case for HIV, HBV, and HCV.

[10]Seroconverting donors are those donors who are recently infected and test negative on a currently licensed test. Donors who have seroconverted will test positive.

inventory hold program instituted by the paid plasma industry. (See app. I for the calculations we used to derive our risk estimates).

Overall Marker Rates of Infection Are Higher Among Paid Donors

Although it has been difficult to obtain data on viral marker rates among paid plasma donors, data collected by California highlight differences between paid plasma and volunteer whole blood donors.[11] As shown in table 2, among the 833,178 units tested, 89 units (.0107 percent) tested positive for HIV-1.[12] Donations at plasma centers showed a higher rate of testing positive for HIV-1 than did donations at blood banks. Plasma centers had an HIV-1 rate of .0266 percent (26.6 per 100,000 units tested), while units collected at blood banks had a rate of .0032 percent (3.2 per 100,000 units tested). Thus, California plasma centers had over an eight-fold higher rate of HIV-1 positive donations than blood banks had among their volunteer donors.

Table 2: HIV-1 Antibody Test Results From California Blood Banks and Plasma Centers, July to December 1996

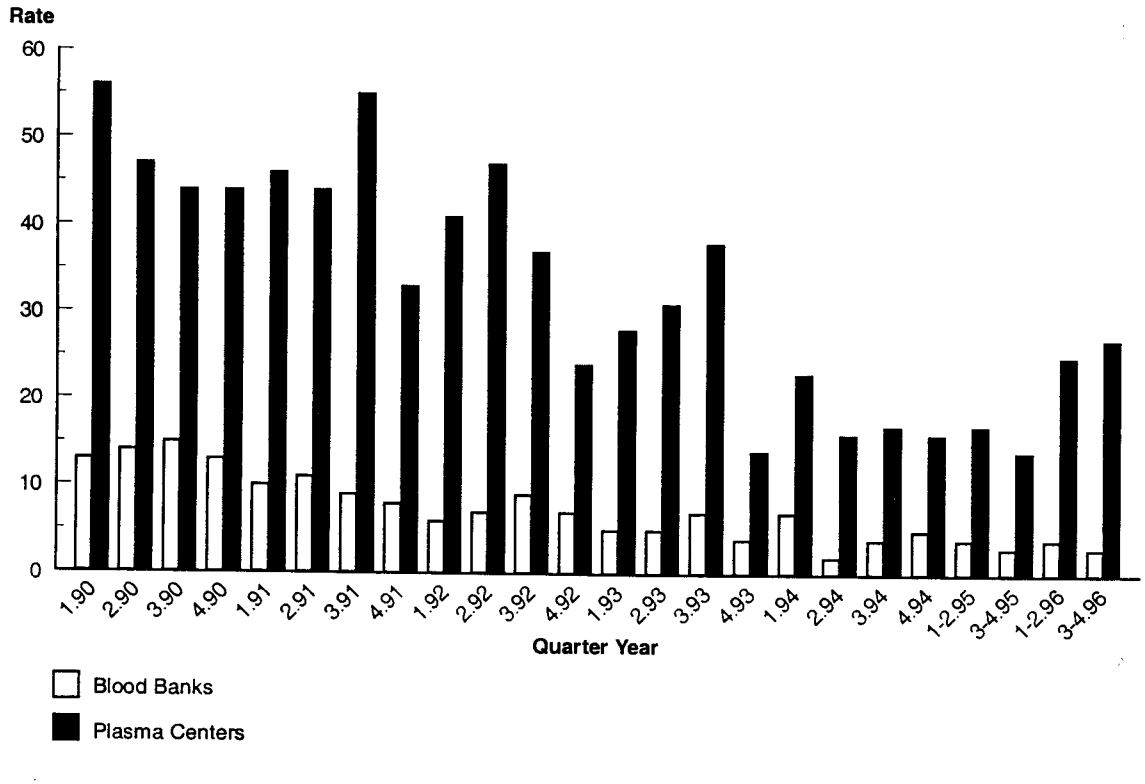
Facility type	Units tested	Number of confirmed HIV-1 positive units	Number of positive donations per 100,000
Blood banks	566,677	18	3.17
Plasma centers	266,501	71	26.64
Total	**833,178**	**89**	**10.68**

For both blood banks and plasma centers in California, the seroprevalence rates for HIV have decreased significantly over time. More than 7 million units were tested at California blood banks between 1990 and 1996. Over this period, HIV-1 seroprevalence among donors declined from .015 percent to .003 percent. Over 4.5 million units were tested at California plasma centers during this same time frame. The HIV-1 seroprevalence among plasma donors declined during this period from .056 percent in 1990 to .027 percent in the second half of 1996. However, while the rates of HIV are dropping in both groups, there is a consistent pattern of higher marker rates among paid donors than among volunteer donors. (See fig. 1.)

[11]DHS received results for HIV-1 and HIV-2 antibody testing from blood banks and plasma centers. For the last 6 months of 1996 they received results from 49 blood banks and 15 plasma centers. This information represents about 74.4 percent of the overall California facilities required to report HIV-1 and HIV-2 antibody test results. A recent updating of the facilities that are required to respond indicates that the response rate was actually higher than 74.4 percent.

[12]Two units also tested positive for HIV-2 with supplemental unlicensed testing. To date, FDA has not licensed a confirmatory test for HIV-2 infection. However, cross-reactivity between HIV-1 and HIV-2 is a strong possibility in instances where HIV-2 is confirmed by existing unlicensed testing.

Figure 1: Reported Confirmed HIV Rates Among Donations in California, 1990 to 1996



Note: Rates are per 100,000 commercial plasma donations and volunteer whole blood donations.

Source: DHS, Office of AIDS, Dec. 1997.

Although the California data were based on similar reporting requirements and time frames for paid and volunteer donors, they only examined HIV marker rates. There was also some question as to whether multiple counting of donors may have skewed the results of the reporting. Because of these concerns, we also obtained information on marker rates from the American Red Cross and American Blood Resources Association for donors who presented themselves to donate at their respective collection centers.

We obtained data from the American Red Cross on 2,954,773 volunteer whole blood donations from donors less than 60 years old (those used for

plasma products) between January 1, 1996, and June 30, 1997.[13] This includes donations that have occurred since the introduction of the HIV-1 antigen screening test, implemented on March 15, 1996. As shown in table 3, these data showed that 6.9 out of every 100,000 donations were found to test positive for HIV, while the rates were 33.4 per hundred thousand for HBsAg, and 112.4 per hundred thousand for HCV (results from confirmatory testing).[14] Assuming that no donation is positive for more than one virus, then 1 of every 6,549 volunteer donations is potentially infectious for HIV, HBV, or HCV.

Table 3: Marker Rates Among Volunteer Donations, January 1, 1996, to June 30, 1997

Marker	Number of confirmed positive units	Number of positive donations per 100,000
Anti-HIV	205	6.94
HBsAg	987	33.40
Anti-HCV	3,320	112.36
Total	**4,512**	**152.70**

We obtained data from the American Blood Resources Association on 4.6 million paid plasma donations in the second half of 1994.[15] The donations contained in these data only included repeatedly reactive test results—confirmatory testing was not performed. We have therefore adjusted these data by the rate at which repeatedly reactive donations confirm positive based on the rates seen in American Red Cross whole blood donations. As shown in table 4, these data showed that approximately 3.7 out of every 100,000 donations were positive for HIV, while the rates were 30.9 per hundred thousand for HBsAg and 226.2 per hundred thousand for HCV. Assuming that no donation is positive for more than one virus, then 1 of every 3,834 paid donations is potentially infectious for HIV, HBV, or HCV.

[13]These data represent 33 percent of the total collections by the American Red Cross during the time period noted above. Data were collected from 19 regions from an ongoing data collection and analysis effort by the American Red Cross. The regions represented in this data set make up the Infectious Diseases Data Center.

[14]The data presented for HBV include only HBsAg screening and not anti-HBc (hepatitis B core) screening. Because there are few contemporary studies determining the infectivity of reactive anti-HBc donations that are HBsAg nonreactive (and therefore are believed to be derived almost entirely from either immune individuals or individuals with false reactivity to the anti-HBc screening test), and because plasma donations from the commercial sector are not screened for anti-HBc, the contribution of anti-HBc is not addressed.

[15]Information on HCV is based on a smaller data set of 2.5 million donations. All data were collected from approximately 340 source plasma collection centers that were certified under the Quality Plasma Program at the time.

Table 4: Marker Rates Among Paid Plasma Donations, July to December 1994

Marker	Number of confirmed positive units[a]	Number of positive donations per 100,000
Anti-HIV	169	3.67
HBsAg	1,423	30.93
Anti-HCV	5,655	226.20
Total	**7,247**	**260.80**

[a]Adjusted by the rates at which whole blood donations that are repeatedly reactive are confirmed positive.

These three data sets show differing viral marker rates for volunteer and paid plasma donors. The California data show much higher HIV-1 marker rates for paid plasma donors than volunteer donors. However, the data from the American Blood Resources Association show lower rates for HIV, similar rates for HBV, and higher rates for HCV than data obtained from the American Red Cross. Overall, the rates for paid donors are 1.7 times higher than the rates for volunteer donors, which is due to the higher rates among paid donors for HCV.

Voluntary Standards Introduced by the Source Plasma Industry to Reduce Transmission of Viruses

The source plasma industry has recently introduced voluntary standards aimed at reducing the viral risks posed by two categories of paid plasma donations: donations from one-time donors and donations from donors who may be in the window period. One-time donors are a concern because some data show that the rates of viral infection are much higher among such donors. The individuals may either not be aware that they are infected or may be test-seeking. Donors in the window period are a concern because they may not be aware of their infection and the screening tests will not detect the infection.

The first voluntary initiative, implemented in July 1997, eliminates the use of plasma from one-time donors. This standard requires that no units of plasma can be accepted for further processing unless the donor has successfully passed at least two health history interviews and two panels of all required screening tests within a 6-month period. Qualified donors are those who have passed through these criteria. Applicant donors, on the other hand, are individuals presenting themselves who have not been previously qualified as a donor in the past 6 months.

This standard on first-time donors does not apply to volunteer donors. Neither the American Red Cross nor America's Blood Centers imposes

such a requirement for the use of plasma recovered from whole blood donations. Because the patterns of donation are very different for volunteer whole blood donors (who can donate no more frequently than once every 8 weeks) compared with paid plasma donors (who can donate as often as twice a week), the volunteer sector does not view a restriction that would require holding plasma until a donor returns to be a practical requirement. In fact, the average interval between donations for an American Red Cross donor is about 5 months.

A second industry initiative is an inventory hold program that holds source plasma donations for 60 days. During this time, if a donor seroconverts and subsequently tests positive—or is otherwise disqualified—the earlier donation can be retrieved from inventory and destroyed. This standard, however, does not establish a true quarantine program that would exclude units from donors in the window period of infection, when viral infection cannot be determined. A donor who was within the window period could return 2 days after the initial donation, pass both health history interviews and screening tests, and contribute infected units that would be used after 60 days, if the donor were not tested again at a time outside the window period. The data provided to us for estimating risks for source plasma donations take this possibility into account.

Furthermore, the 60-day inventory hold period does not appear to be adequate for all viruses under consideration, based on published data. The window period for HIV, HBV, and HCV, using detection of seroconversion as an end point, are approximately 22 days, 59 days, and 82 days, respectively.[16] Thus, the 60-day hold period does not encompass the window period for HCV and is barely within the limit for HBV. However, the majority of window period units would be interdicted as most of these would fall within the 60-day hold period. PCR testing would close the window period for these viruses to approximately 11 days for HIV, 34 days for HBV, and 23 days for HCV.[17] As a result, if such testing becomes available for mass screening, the 60-day inventory hold would cover the window period for these three viruses.

Incidence Rate for Paid Donors Is Higher Than for Volunteer Donors

We found the incidence rates of HIV, HBV, and HCV infection to be much higher for paid donors than for volunteer donors. These rates include donors who pass the initial screening and donate but subsequently

[16]George Schreiber and others, "The Risk of Transfusion-Transmitted Viral Infections," New England Journal of Medicine, Vol. 334 (June 27, 1996), pp. 1685-90.

[17]George Schreiber and others, "The Risk of Transfusion-Transmitted Viral Infections."

seroconvert and are detected at a later donation. As a result, potentially infectious units from these donors may have been incorporated into a plasma pool for manufacturing.

Since prevalence rates of viral markers merely indicate the proportion of infected persons in the population at a given time, independent of when infection occurred, they do not accurately portray the chances of incorporating an infectious window period unit into a plasma pool. Thus, to calculate the risk of collecting potentially infectious units—the incidence rate—the number of individuals who are seroconverting and the time between donations for such individuals (the interdonation interval) need to be taken into account.

Incidence Rate for Volunteer Donors

The data used to calculate incidence rates among volunteer donors are based on approximately 1 million donations from repeat donors under the age of 60 for the American Red Cross between July 1, 1996, and June 30, 1997. The interdonation interval for these donors averaged 154 days. However, repeat donors account for only 80 percent of volunteer blood donations. Thus, incidence calculations from first-time donors also need to be taken into account to obtain an overall risk estimate of collecting an infectious window period unit. A modified screening test was used to determine incidence rates among first-time donors, which showed that first-time whole blood donors have a 2.4 times higher HIV rate of prevalent infections than repeat donors.[18] This information is combined to estimate the total incidence among volunteer blood donors. (See table 5.)

Table 5: Estimated Incidence Rates for Volunteer Donations, July 1, 1996, to June 30, 1997

Marker	Repeat donors (80%)	First-time donors (20%)	Total (per 100,000 person-years)[a]
Anti-HIV and Ag	2.59	6.22	3.31
HBsAg	6.25	15.02	8.01
Anti-HCV	11.65	28.96	14.91

[a]Person years is the number of donations multiplied by the mean time between donations divided by 365 days.

Incidence Rate for Paid Donors

We also obtained data from the American Blood Resources Association that were based on all of the approximately 4 million donations at the American Blood Resources Association-member centers over a 4-month

[18]M. P. Busch and others, "Estimation of HIV Incidence in U.S. Blood Donors Using a Novel Detuned Anti-HIV EIA Test Strategy," Fifth Conference on Retroviruses and Opportunistic Infections (Chicago, Ill.: Feb. 1-5, 1998). We used a similar calculation to estimate incidence rates for HBV and HCV among volunteer donors.

period in the second half of 1997. The average interdonation interval among these donors was 5.3 days. The American Blood Resources Association's qualified donor program does not collect plasma from first-time donors; therefore, no adjustment is needed for first-time donors. Table 6 shows the incidence rates among qualified source plasma donors for this period.[19]

Table 6: Estimated Incidence Rates for Paid Donations, July 1, 1997, to October 31, 1997

Marker	Total (per 100,000 person-years)[a]
Anti-HIV	61.80
HBsAg	245.50
Anti-HCV	63.52

[a]Person years is the number of donations multiplied by the mean time between donations divided by 365 days.

When comparing the incidence rates between paid and volunteer plasma donors, we found that the incidence rates for HIV, HBV, and HCV were much higher for paid donors. HIV incidence rates were 19 times higher among paid donors (61.8 versus 3.3 for volunteer donors), while HBV and HCV rates were 31 times (245.5 versus 8.0) and 4 times higher (63.5 versus 14.9), respectively.

Residual Risks for Incorporating an Infectious Unit Into a Plasma Pool Are Higher for Paid Donors Than for Volunteer Donors

Calculating the chances that an infectious unit will be made available for pooling includes factoring in the length of the window period expressed as a fraction of a year. Calculating this residual risk is a more statistically appropriate way to determine the true impact of window period donations.

Residual Risk for Volunteer Donors

We calculated the residual risk of a potentially infectious unit being made available for pooling for units collected from volunteer donors. These estimates are shown in table 7.

[19]The incidence rates for paid donors for HIV are based on antibody test results, whereas the rates for volunteer donors are based on antibody and antigen tests.

Table 7: Estimated Residual Risk for Volunteer Plasma Donations

Marker	Estimated residual risk per million donations	Point estimate
Anti-HIV and Ag	1.45	1:689,655
HBsAg	12.95	1:77,220
HBsAg (with adjustment)[a]	28.89	1:34,614
Anti-HCV	33.50	1:29,850
Total	**47.91**	**1:20,872**
Total (with adjustment)[a]	**63.85**	**1:15,662**

[a]Adjusted for the transient response on the HBsAg test.

The estimated adjusted risk per million donations—that is, the residual risk—represents the incidence rate multiplied by the window period for each virus. The resulting point estimate for the risk of pooling an HIV seronegative unit from a window period donation is 1 in 689,655.[20] For HBV and HCV, the corresponding estimates are 1 in 77,220 and 1 in 29,850, respectively. When combined, we calculated the risk of incorporating an infectious HIV, HBV, or HCV window period unit into a plasma pool from volunteer donors at 1 in every 20,872 units.[21]

Some researchers believe that an additional factor should be taken into account when determining the risks associated with HBV.[22] This is because individuals who become infected with HBV show different patterns of response over time on the HBsAg test. (See app. I for a more complete discussion.) If such an adjustment is taken into account, the estimated total incidence per 100,000 person years for HBsAg would be 17.9, with an estimated adjusted risk per million donations of 28.9 and a point estimate of 1 in 34,614. This would yield an overall risk of incorporating an

[20]Estimates that are commonly quoted are point estimates. However, confidence intervals give a better sense of possible risk. Point estimates are necessary for calculating purposes but should not be construed as definitive. Scientists know that statistical measurement is not precise. Thus, they calculate a range, or confidence interval, of estimates that is wide enough that they are confident in believing that the real number is somewhere between the two endpoints of the range.

[21]This calculation assumes that the risks of incorporating a potentially infectious HIV, HBV, or HCV window period unit are independent. In fact, some units might be infectious with more than one virus. However, data on the over 4 million source plasma donations used in these analyses show that among the 215 confirmed positive donors, only 1 was positive for more than one virus.

[22]George Schreiber and others, "The Risk of Transfusion-Transmitted Viral Infections."

infectious window period HIV, HBV, or HCV unit into a plasma pool of 1 in every 15,622 units (instead of 1 in 20,872 without the adjustment).[23]

Residual Risk for Paid Donors

We also calculated the estimated residual risk for paid plasma donors, with and without a 60-day inventory hold. (See table 8.)

Table 8: Estimated Residual Risk for Paid Plasma Donations

	Without 60-day hold		With 60-day hold	
Marker	Risk per million donations	Point estimate	Risk per million donations	Point estimate
Anti-HIV	37.25	1:26,846	1.47	1:680,272
HBsAg	396.84	1:2,520	53.84	1:18,574
Anti-HCV	142.70	1:7,008	35.94	1:27,824
Total	**576.79**	**1:1,765**	**91.25**	**1:10,959**

The point estimate for the risk of collecting an HIV window period unit at a paid plasma donation center is 1 in 26,846. For HBV and HCV, the corresponding estimates are 1 in 2,520 and 1 in 7,008, respectively. Overall, the risk of incorporating an infectious HIV, HBV, or HCV window period unit into a plasma pool without taking into account the 60-day inventory hold program was 1 in 1,765 for paid plasma donors—12 times the risk for volunteer donors.

To obtain an overall residual risk of incorporating a potentially infectious window period unit into a plasma pool from paid donors, the American Blood Resources Association data also took into account the effect of the 60-day inventory hold program for source plasma. This resulted in an overall risk estimate that would allow for the interdiction of numerous infectious window period units captured by the 60-day hold program. The resulting point estimate for the risk of pooling an HIV seronegative unit that is from a window period donation is 1 in 680,272. For HBV and HCV, the corresponding estimates are 1 in 18,574 and 1 in 27,824, respectively.

[23]The data from which the adjustment factor is calculated are based on tests in use in the late 1970s and early 1980s. Current HBsAg tests are more sensitive, extending the time period of HBsAg detection. However, there have been no contemporary studies using current tests defining the duration of HBsAg reactivity in individuals with acute infection. Data from one recently presented paper suggest that an adjustment based on current tests might result in a rate of 1 in 46,156 for HBV, with an adjusted total risk of 1 in 17,670.

Thus, the overall residual risk for paid plasma for HIV, HBV, and HCV is 1 in 10,959, compared with 1 in 20,872 for volunteer donors.[24] This would mean that approximately 5.5 infectious units would be included in every 60,000 paid donations, whereas about 2.9 infectious units would be included in every 60,000 volunteer donations. Using the estimates based on the adjustment for HBV among volunteer donors (1 in 15,662) would mean that 3.8 infectious units would be included in every 60,000 volunteer donations.

Overall Comparison of Risks of Pooling Infectious Units

When comparing the overall residual risk of incorporating an infectious window period unit into a plasma pool for each of the three viruses examined in this study, the rates for HIV for volunteer and paid plasma donors are virtually identical (1 in 689,655 and 1 in 680,272, respectively); the rates for HCV are also similar (1 in 29,850 to 1 in 27,824). The major difference can be found for donors infected with HBV, where the residual risk for volunteer plasma donors is 1 in 77,220 compared with 1 in 18,574 for paid plasma donors. But taking into account the adjustment factor for HBV in volunteer plasma donors, the adjusted HBV estimate for volunteer donors becomes 1 in 34,614. Thus, while the risk for HBV transmission is greater for paid donors, the overall residual risks for the three viruses are closer once the 60-day hold is taken into account (1 in 15,662 for volunteer plasma donors versus 1 in 10,959 for paid plasma donors). This difference in the overall residual risk is statistically significant. Thus, the data suggest that the current risks of incorporating an infectious unit into a plasma pool remain somewhat higher for paid donors.[25] (See table 9.)

[24]The window period calculation for anti-HIV for volunteer donors is based on the antigen test. However, data for paid plasma donations are based on the HIV antibody test. Thus, we used a 22-day window period to calculate the incidence rate in paid donors because this is the length of the window period using the HIV antibody test. If the antigen test were used to calculate a risk estimate for paid plasma donors, the risk would be reduced to 1 in 1,000,000 (instead of 1 in 680,272). However, this does not change the overall risk of incorporating an infectious window period unit into a plasma pool to any degree for the three viruses studied in this report. If the antigen window period calculation were used to calculate the overall risk from paid donors of incorporating an infectious HIV, HBV, or HCV unit into a plasma pool, the risk would change only slightly to 1 in 11,016.

[25]The calculations of residual risk for the two groups are based on slightly different models, but both take into account the possibility of window period donations from donors whose last donation is nonreactive on the currently licensed screening tests.

Table 9: Residual Risks for Volunteer and Paid Plasma Donations

	Residual risk (1,000,000 donations)		Point estimate	
Marker	**Volunteer**	**Paid**	**Volunteer**	**Paid**
Anti-HIV	1.45	1.47	1:689,655	1:680,272
HBsAg	28.89	53.84	1:34,614	1:18,574
Anti-HCV	33.50	35.94	1:29,850	1:27,824
Total	**63.85**	**91.25**	**1:15,662**	**1:10,959**

Manufacturer Reductions in Plasma Pool Sizes Tend to Not Benefit Frequent Users

Concerns have been raised about the size of plasma pools because larger pools mean that a recipient of a product is exposed to more donors, raising the risks of infection because larger pools have more potentially infectious units included. In response to these concerns, manufacturers have recently taken steps to reduce the size of the plasma pools they use for producing plasma derivatives. Modeling techniques indicate that this effort can have an impact on infrequent users by minimizing their exposure to a certain number of donors. However, for frequent users of plasma products, such as hemophilia patients, this limit has a negligible impact due to the large number of different pools to which they are exposed throughout their lifetime.

The different proteins that make up the various components of plasma are present in only minute quantities in a single donation of plasma. Therefore, most plasma product manufacturing facilities have been designed to work at large scales, using large plasma pools made up of donations from numerous donors, in order to permit manufacturing of sufficient quantities of products. The number of units combined into a common mixture for processing is known as the pool size.[26]

There has been discussion by the plasma industry, FDA, consumer groups, and some Members of Congress regarding the potential benefits of reducing the sizes of pools used by manufacturers to produce finished plasma products. While no units of plasma known to be positive for viruses are combined in plasma pools for production, infectious units may escape detection. A single unit has the potential to contaminate an entire pool. The larger the number of donors who contribute plasma to a pool, the greater the possibility that there will be at least one infectious unit

[26]Here we use the term "pool size" to include the total number of donors whose units are used in the production of a particular product and any material taken from other donations that were not in the initial manufacturing pool. Thus, the pool sizes to which we refer include the total number of donors to whom a recipient of a product is exposed.

included. Based on the estimates we calculated above, a pool of as few as 11,000 donations will still include one infectious unit.

Manufacturers Have Reduced Plasma Pool Sizes

As recently as a year ago, FDA believed that initial fractionation pools contained 1,000 to 10,000 source plasma units or as many as 60,000 recovered plasma units. However, in response to congressional inquiry, the FDA obtained information from plasma manufacturers showing that, after adjusting for the combination of intermediates, pooling of material from several hundred thousand donors for single lots of some products sometimes occurred. For example, albumin can be added during intermediate processing steps or to a final product, such as factor VIII, for use as an excipient or stabilizer.[27] This albumin often has been derived from another plasma pool that contains donations from others that are not part of the original pool.

As a result of the concerns raised about pool size, the four major plasma fractionators have voluntarily committed to reducing the size of plasma pools (measured by total number of donors) to 60,000 for all currently licensed U.S. plasma products, including factor VIII, factor IX, albumin, and IGIV. This measurement takes into account the composition of starting pools, the combining of intermediates from multiple pools, and the use of plasma derivatives as additives or stabilizers in the manufacturing process. However, prior production streams are still being processed and distributed; as a result, products distributed through the end of 1998 may have been produced from pools that exceeded the 60,000-donor limit.

The American Red Cross has also chosen to voluntarily reduce the size of the plasma pools from which its products are manufactured. As a policy, the American Red Cross has a 60,000-donor limit for plasma products that are further manufactured by Baxter Healthcare. Seventy-five percent of all American Red Cross plasma manufactured by the Swiss Red Cross is presently at the 60,000 limit, with plans to have all production at that level in the near future.

[27]Excipients are additives, other than the active ingredient of a drug, that confer a desired property on the final dosage form. This may include a preservative to prevent microbial growth or a stabilizer that maintains potency. A stabilizer maintains the integrity of the active ingredient against chemical degradation or physical denaturation.

Modeling Techniques Show Potential Benefits of Reductions in Pool Size for Infrequent Users

Modeling techniques have been used to determine the degree of infectivity present in plasma pools of varying sizes. One major study using such a technique found that limiting the number of donors in a pool may only be beneficial for infrequent recipients.[28] For example, the researchers calculated that for an infectious agent with a prevalence of 1 in 500,000 (such as a rare or emerging virus), a pool made up of 10,000 donations would yield a 2 in 100 chance of exposure to that agent for a one-time recipient. For frequent users of plasma products (100 infusions), this same pool size of 10,000 would yield an 86 in 100 chance of exposure to that agent, based on an assumption that the products would come from different pools. This effect is not significantly decreased by reducing the number of donors in a pool. Table 10 shows the effect of manufacturing scale on risk of exposure.

Table 10: Effect of Manufacturing Scale on Risk of Exposure

	Chance of exposure (percent)		
Scale of manufacturing	Infrequent users (1 infusion)	Moderate users (10 infusions)	Frequent users (100 infusions)
Prevalence of agent = 1 in 500,000			
60,000	11	70	100
25,000	5	39	99
10,000	2	18	86
6,000	1	11	70
2,500	0.5	5	39
Prevalence of agent = 1 in 50,000			
60,000	70	100	100
25,000	39	99	100
10,000	18	86	100
6,000	11	70	100
2,500	5	39	99
Prevalence of agent = 1 in 5,000			
60,000	100	100	100
25,000	99	100	100
10,000	86	100	100
6,000	70	100	100
2,500	39	99	100

Source: Thomas Lynch and others, "Considerations of Pool Size in the Manufacture of Plasma Derivatives."

[28]Thomas Lynch and others, "Considerations of Pool Size in the Manufacture of Plasma Derivatives," Transfusion, Vol. 36 (1996), pp. 770-75.

These modeling data suggest that smaller plasma pool sizes will reduce the likelihood of transmission of viral agents to infrequent users of plasma products but will not have a major effect on those who are frequent recipients of such products.

It is also important to note that risk of exposure does not always equate with risk of infection. In fact, risk of exposure is always greater than or equal to risk of infection. For example, the recent transmission of HCV by a plasma derivative that had not undergone viral inactivation procedures showed that the risk of seroconversion of recipients of this product increased with the number of positive HCV lots infused and the quantity of HCV viral material infused. However, not all recipients were infected; the highest percentage of seroconversions seen with the highest levels of HCV virus infused did not exceed 30 percent.[29]

The reasons for not observing seroconversions in 100 percent of the recipients may be due to two factors: (1) the recipient's dose and (2) the reduction of infectiousness related to steps in the manufacture of the product in addition to viral removal and inactivation, such as duration of storage.

Risk of Infection Reduced Through Viral Inactivation and Removal Techniques

Since it is possible that certain infectious units could make it through the donor screening, deferral, and testing process, manufacturers have introduced additional steps in the fractionation process to inactivate or remove viruses and bacteria that may have made their way into plasma pools. These techniques virtually eliminate enveloped viruses, such as HIV, HBV, and HCV. However, they are only partially effective against nonenveloped viruses, such as HAV and human parvovirus.[30]

All plasma components listed in table 1 undergo viral inactivation or removal steps during the manufacturing process.[31] To be effective, inactivation techniques must disrupt the virus, rendering it noninfectious. The two main inactivation techniques are heat treatment and

[29]Joseph Bresee and others, "Hepatitis C Virus Infection Associated With Administration of Intravenous Immune Globulin: A Cohort Study," Journal of the American Medical Association, Vol. 276 (Nov. 20, 1996), pp. 1563-7.

[30]Parvovirus is the cause of Fifth disease, a common childhood illness, which is usually mild and of brief duration. Approximately 50 percent of the population has been infected by parvovirus at some time.

[31]FDA has encouraged manufacturers to incorporate viral inactivation or removal procedures for enveloped viruses. Currently, only two IGIM products are manufactured without the use of viral inactivation procedures.

solvent-detergent treatment. Heat treatment is accomplished either by exposing the freeze-dried product to dry heat or suspending it in a solution. Another technique heats the completely soluble liquid product with the addition of various stabilizers, such as sucrose and glycine.[32] The second technique, solvent-detergent washing, exposes the product to an organic solvent to dissolve the lipid coat of viruses, rendering them inactive without destroying the plasma-derived products. The lipid membrane contains critical viral proteins needed for infection of host cells. Disrupting the viral lipid envelope renders the virus noninfectious. However, solvent-detergent inactivation is only partially effective in eliminating non-lipid-coated viruses, such as HAV or human parvovirus.

To disable the virus without inactivating plasma derivatives, a delicate balance in these procedures must be maintained. Heat and chemicals are particularly damaging to plasma proteins. A number of potentially safer methods are in use or under investigation. These include the use of filters to remove virus particles on the basis of the size of the virus; antibodies to capture the desired protein, while the viruses and unwanted components are washed away;[33] and irradiation to inactivate viruses. Virucidal agents that can be removed during further manufacturing and exposure to ultraviolet light may also be safer methods for disabling viruses. Genetic engineering techniques are also being used to produce recombinant factors VIII and IX—that is, the genes to produce the proteins have been cloned and harvested in the laboratory. These products have, so far, been found free of human viruses. However, manufacturing of these recombinant products may include the use of human-derived products during production or as excipients in the final container. FDA has approved recombinant factor VIII and IX.

Determining the effectiveness of these different procedures is accomplished by assessing the amount of viral clearance obtained through a particular inactivation or removal process. It is based on the amount of virus that is killed or removed and, therefore, the extent to which these processes eliminate viruses through manufacturing. Individual

[32]Extensive research has carefully calculated specified temperatures and times for different heat treatment processes. For example, FDA regulations require that albumin (human) and plasma protein fraction be heated for 10 to 11 hours at 60 degrees centigrade in the final container and in the presence of defined stabilizers to ensure viral inactivation (21 C.F.R. 640.91(e), 640.81).

[33]One example of this process is the use of a monoclonal antibody column to purify a plasma derivative such as factor VIII. In this case, antibodies to the factor VIII are generated in large amounts in tissue culture. The antibodies are attached to a support within the column. The plasma pool or intermediate product is passed through the column. The factor VIII binds to the specific antibody while the fluid containing other plasma-derived products, and possible contaminating viruses and other agents, flows through the column. The factor VIII can later be separated from the antibody column.

manufacturing steps can be specifically designed for viral clearance or they may be intended primarily as a purification process that will also assist in killing or removing viral agents. To meet FDA approval of their particular inactivation or removal technique, manufacturers must separately validate each clearance step.

The viral inactivation and removal steps currently in use have all been demonstrated to reduce the levels of virus and, in many cases, likely eliminate them. (See app. III for a more complete discussion of viral clearance.) Even when the virus is not completely eliminated, a significant reduction in viral load is of value. While theoretically even a single virus is capable of causing infection, research has shown that infection is much more likely to occur with higher concentrations of virus.[34] As a result of these techniques, there have been no documented cases of HIV, HCV, or HBV transmission since 1988 for plasma products that were properly inactivated.

Recent Noncompliance With Current Good Manufacturing Practices Could Jeopardize the Safety of Plasma Products

Although viral inactivation and removal techniques have been shown to be highly effective, they are only useful if the steps in the manufacturing process are carried out properly. Recent FDA inspections of plasma fractionation facilities have found numerous violations of current good manufacturing practices. Without strict adherence to these practices, the safety of plasma products could be compromised.

The objective of good manufacturing practices is to ensure that plasma products are safe, effective, adequately labeled, and possess the quality purported. To achieve this goal, plasma manufacturers should operate in compliance with applicable regulations and principles of quality assurance.

To ensure that manufacturing processes, including inactivation procedures, follow current good manufacturing procedures, FDA is authorized to inspect plasma fractionation establishments. If the manufacturer does not conform to the standards in its license or the regulations such that the safety and purity of the product is not ensured and this constitutes a danger to health, necessitating immediate corrective action, and the deficiencies are well documented, FDA may pursue an action to suspend the facility's license.

[34]Joseph Bresee and others, "Hepatitis C Infection Associated With Administration of Intravenous Immune Globulin."

When deficiencies are noted during an inspection, FDA may also issue a warning letter to the facility. A warning letter does not suspend operations but rather gives the facility an opportunity to correct deviations. A warning letter acts as notification to a firm that FDA considers its activities to be in violation of statutory or regulatory requirements and that failure to take appropriate and prompt corrective action may result in further action by FDA.

Recent inspections conducted at the four major fractionation companies found numerous deficiencies in each company's adherence to current good manufacturing practices and resulted in consent decrees with two of the companies. (See table 11.)

Table 11: Outcomes of Recent FDA Inspections

Company	Inspection year	Number of observations found	Outcome
Alpha Therapeutic	1997	139	Consent decree with FDA[a]
Baxter Healthcare	1997	96	No regulatory action
Bayer Corporation	1997	107[b]	No regulatory action
Centeon	1996	87	Consent decree with FDA

[a]A consent decree of permanent injunction is a court-ordered action against a firm or individual, which either mandates corrective actions that must be taken or which prohibits the operation of the firm unless and until such actions are taken.

[b]Thirty observations were found at Bayer's Berkeley, California, facility; 77 were found at its Clayton, North Carolina, facility.

Many of the facilities slowed production as the firms reallocated resources to work on their corrective actions. The consent decree with Centeon required the company to cease distribution of all but two of its products while it brought its manufacturing standards into compliance with FDA statutes and regulations. In May 1997, FDA authorized the distribution of Centeon's products from the facility. In a subsequent inspection, completed in July 1998, FDA found that Centeon had failed to fully comply with the consent decree and was notified to immediately cease manufacturing, processing, packing, holding, and distributing all biological and drug products manufactured at its facility. However, exceptions could be made for products deemed medically necessary.

Examples of observations found by FDA inspectors during inspections of various plasma fractionation facilities included the following:

- In-house developed software that had not been validated was being used for performance of finished product testing.
- Calibration and preventive maintenance records were incomplete and sometimes inaccurate.
- Reports of problems with plasma products after distribution were not being reviewed and investigated in a timely manner.
- Viral inactivation processes used on several lots of factor VIII had deviations that were undetected or not corrected.
- Albumin product lots that failed final container testing for sterility were reprocessed by repooling, and there was no validation for these reprocessing steps.
- The cleaning process and removal of cleaning agent residues from fractionation kettles, bulk tanks, buffer tanks, or centrifuge bowls were not validated.
- Albumin manufacturing processes were not validated, and final products did not consistently conform to release specifications. (In 1997, 54 percent of albumin lots for one company failed final container inspection due to visible evidence of proteinaceous material.)

To overcome these problems, the major fractionation companies have taken certain steps, such as increasing quality assurance and quality control and production staff and training, implementing capital investments at the fractionation facilities, and equipment process validation.

FDA has also taken several actions within the last year to better ensure manufacturer compliance with current good manufacturing practices. In a previous study examining the safety of the blood supply, we had found inconsistencies in FDA inspection practices.[35] As a result of this and another study examining FDA's regulatory role in the field of biologics, a new inspection program was adopted.[36] Under this program, FDA has designated two groups of investigators: one to focus on blood banks and plasmapheresis centers and another to focus on plasma fractionation and manufacturers of allergenic products, therapeutics, licensed in-vitro diagnostics, and vaccines. This approach is intended to ensure that all FDA current good manufacturing practice inspections are conducted by a single agency unit using a similar approach. If properly implemented, these actions by plasma manufacturers and FDA should help alleviate the

[35]Blood Supply: FDA Oversight and Remaining Issues of Safety (GAO/PEMD-97-1, Feb. 25, 1997).

[36]Office of Inspector General, Review of the Food and Drug Administration's Inspection Process of Plasma Fractionators, A-03-97-00350 (Washington, D.C.: Department of Health and Human Services, June 1997).

problems related to adherence to current good manufacturing practices and quality assurance.

Agency Comments

We provided copies of a draft of this report to FDA and the Centers for Disease Control and Prevention for their review. Both generally agreed with our findings. They provided technical comments, which we incorporated as appropriate. We also provided copies of the draft report to the American Red Cross, the American Blood Resources Association, and the International Plasma Products Industry Association. Each provided technical comments, which we incorporated as appropriate. The American Blood Resources Association provided additional data on viral marker rates, which we have included.

We will send copies of this report to the Secretary of Health and Human Services, the Lead Deputy Commissioner of FDA, and others who are interested. If you have any questions or would like additional information, please call me at (202) 512-7119 or Marcia Crosse, Assistant Director, at (202) 512-3407. Other contributors to this report were Kurt Kroemer, Project Manager, and Richard Weston, Senior Social Science Analyst.

Sincerely yours,

Bernice Steinhardt
Director, Health Services Quality
and Public Health Issues

Contents

Figure

Abbreviations

ABRA	American Blood Resources Association
ALT	alanine aminotransferase
DHS	Department of Health Services
FDA	Food and Drug Administration
HAV	hepatitis A virus
HBc	hepatitis B core
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
IGIM	immune globulin transmusular
IGIV	immune globulin intravenous
LRF	log reduction factor
PCR	polymerase chain reaction
PPF	plasma protein fraction

Calculations for Risk Estimates

Our analysis of viral risks from volunteer and paid plasma donors included calculations for the three major viruses known to be transmissible through plasma products—HIV, HBV, and HCV—and is based on a model that calculated similar estimates for whole blood donations.[37] We did not estimate risks associated with nonenveloped viruses, where current removal or inactivation techniques are only effective to a limited extent, because no screening tests are currently used for these viruses. The nonenveloped viruses currently known to be transmitted through plasma, primarily HAV and human parvovirus, are generally not life threatening.[38]

The window period outlined in our calculations is based on the "conventional" window period—defined as the interval between the time the donor acquired the infection and the development of a positive laboratory test. The conventional window period differs from the "infectious" window period

if there is a lag between the acquisition time of infection and the donor's ability to transmit the infection to others by blood transfusion. Theoretically, such a lag would exist if, on initial exposure to the virus, the donor were able to sequester the virus in the organs of the immune system before becoming infectious.[39]

Experimental animal evidence suggests that the difference between the conventional and infectious windows for retroviruses, such as HIV, may range from 2 to 14 days.[40]

Two ways of measuring risk of infection from blood transfusions are to examine prevalence and incidence of disease. Prevalence indicates the overall proportion of infected persons in the population at a given time, independent of when the infection occurred. Incidence is the proportion of persons newly infected in the population during the period of time under study, or the rate of new infections. As such, incidence is calculated as the number of seroconverters divided by the person-time of observation, where the person-time of observation equals the number of donations multiplied by the mean time between donations (interdonation interval).

[37]George Schreiber and others, "The Risk of Transfusion-Transmitted Viral Infections."

[38]Bernard Horowitz and others, "Viral Safety of Solvent-Detergent Treated Blood Products," in Virological Safety Aspects of Plasma Derivatives, F. Brown, ed., Vol. 81 (1993), pp. 147-61.

[39]Stephan Kleinman and others, "The Incidence/Window Period Model and Its Use to Assess the Risk of Transfusion-Transmitted Human Immunodeficiency Virus and Hepatitis C Virus Infection."

[40]M. T. Niu and others, "Primary Human Immunodeficiency Virus Type 1 Infection: Review of Pathogenesis and Early Treatment Intervention in Humans and Animal Retrovirus Infections," Journal of Infectious Diseases, Vol. 168 (1993), pp. 1490-1501.

To calculate the overall residual risk from window period donations, the incidence rate is multiplied by the length of the window period from seroconverting (repeat) donors. Adjustment factors can also be used to incorporate the effect of first-time donors and probability estimates for donors who do not return but may be in the infectious window period when they donate.

Viral Marker Rates

Information on viral marker rates among volunteer and paid plasma donors in California was obtained from California's Department of Health Services, Office of AIDS, HIV/AIDS Epidemiology Branch. Data illustrated in figure 1 are for HIV-1 confirmed positive test results. Starting in the second quarter of 1991, the totals do not include autologous donations. Information pertaining to data for the second half of 1996 were obtained from 49 blood banks and 15 plasma centers, representing approximately 75 percent of the overall California facilities required to report HIV antibody test results to DHS.[41]

Table I.1 outlines the calculations for the viral marker rates for volunteer plasma donors. This information was obtained from 19 American Red Cross regions and was based on 2,954,773 donations from donors under age 60. This is approximately 33 percent of the total number of donations made to the American Red Cross during the reporting period of this data collection effort (January 1, 1996, to June 30, 1997).

[41]California Health and Safety Code, Chap. 7, Sect. 120980(j).

Table I.1: Marker Rates Among Volunteer Donations

	Number	Percent	Calculation
Anti-HIV marker			
Repeat reactive	2,551	.09	2,551 ÷ 2,954,773
Confirmed negative	1,185	46.45	1,185 ÷ 2,551
Confirmed indeterminate	1,161	45.51	1,161 ÷ 2,551
Confirmed positive	205	8.04	205 ÷ 2,551
Positive donations per 100,000	6.94	NA	100,000 x (205 ÷ 2,954,773)
HBsAg marker			
Repeat reactive	1,404	.05	1,404 ÷ 2,954,773
Confirmed negative	417	29.70	417 ÷ 1,404
Confirmed indeterminate	NA	NA	NA
Confirmed positive	987	70.30	987 ÷ 1,404
Positive donations per 100,000	33.40	NA	100,000 x (987 ÷ 2,954,773)
Anti-HCV marker			
Repeat reactive	5,728	.19	5,728 ÷ 2,954,773
Confirmed negative	1,289	22.50	1,289 ÷ 5,728
Confirmed indeterminate	1,119	19.54	1,119 ÷ 5,728
Confirmed positive	3,320	57.96	3,320 ÷ 5,728
Positive donations per 100,000	112.36	NA	100,000 x (3,320 ÷ 2,954,773)

Note: NA = not applicable.

There were 205 confirmed positive HIV donations found among the 19 regions reporting for the Infectious Disease Data Center. The number of positive donations per 100,000 is derived by dividing these 205 cases by the number of total donations and then multiplying the resulting figure by 100,000. For HIV, this calculation yielded an estimated 7 positive donations per 100,000 given at American Red Cross centers. Similar calculations can be used to obtain estimates for HBV and HCV. To obtain our estimate of 1 in every 6,549 volunteer donations as potentially infectious for HIV, HBV, or HCV, we added the positive donations per 100,000 for each virus (6.93 + 33.40 + 112.36) and divided 1 million by this amount.

Table I.2 outlines the calculations for the viral marker rates for paid plasma donations. The calculations are based on 4,600,000 donations for HIV and HBV and 2,500,000 donations for HCV made in the second half of 1994 to 340 American Blood Resources Association collection centers. The

number of confirmed positive donations is obtained by multiplying the number of units found to be repeatedly reactive by the rate at which units are confirmed positive in volunteer whole blood donations for the specific virus in question. (See table I.1 for these confirmed positive rates for each virus.) The number of positive donations per 100,000 is derived by dividing the number of confirmed positive donations by the total number of donations and multiplying by 100,000. Similar calculations can be used to obtain estimates for HBV and HCV. To obtain our estimate of 1 in every 3,834 paid donations as potentially infectious for HIV, HBV, or HCV, we added the positive donations per 100,000 for each virus (3.67 + 30.93 + 226.20) and divided 1 million by this amount.

Table I.2: Marker Rates Among Paid Plasma Donations

Marker	Number of repeatedly reactive donations	Number of confirmed positive donations[a]	Number of positive donations per 100,000
Anti-HIV	2,116	169.28 x (2,116 x .080)	3.67 x [(169 ÷ 4,600,000) x 100,000]
HBsAg	2,024	1,422.87 x (2,024 x .703)	30.93 x [(1,423 ÷ 4,600,000) x 100,000]
Anti-HCV	9,750	5,655.00 x (9,750 x .580)	226.20 x [(5,655 ÷ 2,500,000) x 100,000]

[a]Based on rates found among volunteer whole blood donors.

Incidence Rates and Residual Risk

Table I.3 outlines the incidence rates among repeat volunteer plasma donors, while table I.4 outlines the corresponding overall incidence rates for volunteer donors, taking into account first-time donations. These calculations are drawn from 1 year of donations for which the American Red Cross had the most recently available data (1,098,942 donations from July 1, 1996, to June 30, 1997).

Table I.3: Incidence Rates Among Repeat Volunteer Plasma Donations

	Rate	Calculation
Anti-HIV marker: 12 seroconverters		
Seroconverters per 100,000 donations	1.09	100,000 x (12 ÷ 1,098,942)
Incidence per 100,000 person-years	2.59	(12 x 100,000) ÷ [1,098,942 x (154 ÷ 365)]
Risk per million donations	1.14	10 x (2.59 x 16[a] ÷ 365)
HBsAg marker: 29 seroconverters		
Seroconverters per 100,000 donations	2.63	100,000 x (29 ÷ 1,098,942)
Incidence per 100,000 person-years	6.26 18.59[b]	(29 x 100,000) ÷ [1,098,942 x (154 ÷ 365)] 6.26 x 2.97
Risk per million donations	10.12 30.05[b]	10 x (6.26 x 59[a] ÷ 365) 10 x (18.59 x 59[a] ÷ 365)
Anti-HCV marker: 54 seroconverters		
Seroconverters per 100,000 donations	4.91	100,000 x (54 ÷ 1,098,942)
Incidence per 100,000 person-years	11.65	(54 x 100,000) ÷ [1,098,942 x (154 ÷ 365)]
Risk per million donations	26.17	10 x (11.65 x 82[a] ÷ 365)

Note: Interdonation interval = 154 days.

[a]Window period (in days).

[b]The second number contains a calculation based on the transient antigenemia for HBV. The duration of this transient period has been estimated at 63 days. An interdonation interval of 154 days suggests that 41 percent of donors with transient antigenemia would be identified by the HBsAg test (63 ÷ 154 = .41). It is believed that 70 percent of HBV-infected donors show this transient effect, 25 percent have a primary antibody response but no detectable antigenemia, and 5 percent become long-term carriers. See J. H. Hoofnagle and others, "Serologic Responses in Hepatitis B," in Viral Hepatitis: A Contemporary Assessment of Etiology, Epidemiology, Pathogenesis, and Prevention, G. N. Vyas and others, eds. (Philadelphia, Pa.: Franklin Institute Press, 1978), pp. 219-42; and L. T. Mimms and others, "Effect of Concurrent Acute Infection With Hepatitis C Virus on Acute Hepatitis B Virus Infection," British Journal of Medicine, Vol. 307 (1993), pp. 1095-7. The following represents the adjustment factor incorporated into the calculation:

(.70 x 41) + (.25 x 0) + (.05 x 100) = 33.7 percent;

1 ÷ .337 = 2.97 (correction factor); because only 33.7 percent of donors seroconverting for HBV are likely identified with the HBsAg test, the observed incidence rate of HBsAg is multiplied by 1 ÷ 0.337 or 2.97; and

6.26 x 2.97 = 18.59, where 6.26 is the incidence rate per 100,000 person-years for HBsAg without the adjustment factor.

To obtain an incidence rate for repeat donors, we multiplied the number of seroconverters (12) by 100,000 and divided the resulting number by the total number of donations times the interdonation interval as a fraction of a year. We calculated an incidence rate per 100,000 person years for HIV at 2.59. Taking this rate and multiplying it by the window period (as a

fraction of a year) resulted in a risk per million of 1.1. Similar calculations can be used to obtain estimates for HBV and HCV.

Table I.4: Total Incidence Rates and Residual Risk Estimates Among Volunteer Plasma Donations

	Rate	Ratio	Calculation
Anti-HIV marker			
Repeat donors (80%)	2.59		(See table I.3.)
First-time donors (20%)	6.22		2.59 x 2.4
Estimated total incidence (per 100,000 person-years)	3.31		(2.59 x .8) + (6.22 x .2)
Estimated adjusted risk (per million donations)	1.45		10 x [3.31 x (16[a] ÷ 365)]
Point estimate		1:689,655	1,000,000 ÷ 1.45
HBsAg marker			
Repeat donors (80%)	6.26 18.59[b]		(See table I.3.)
First-time donors (20%)	15.02		6.26 x 2.4
Estimated total incidence (per 100,000 person-years)	8.01 17.87[b]		(6.26 x .8) + (15.02 x .2) (18.59 x .8) + (15.02 x .2)
Estimated adjusted risk (per million donations)	12.95 28.89[b]		10 x [8.01 x (59[a] ÷ 365)] 10 x [17.87 x (59[a] ÷ 365)]
Point estimate		1:77,220 1:34,614[b]	1,000,000 ÷ 12.95 1,000,000 ÷ 28.89
Anti-HCV marker			
Repeat donors (80%)	11.65		(See table I.3.)
First-time donors (20%)	27.96		11.65 x 2.4
Estimated total incidence (per 100,000 person-years)	14.91		(11.65 x .8) + (27.96 x .2)
Estimated adjusted risk (per million donations)	33.50		10 x [14.91 x (82[a] ÷ 365)]
Point estimate		1:29,850	1,000,000 ÷ 33.5
Total	**47.91[c]** **63.85[b,c]**	**1:20,872** **1:15,662[b]**	**1,000,000 ÷ 47.91** **1,000,000 ÷ 63.85**

Note: First-time donors are 2.4 times more likely to donate a positive unit than repeat donors.

[a]Window period (in days).

[b]Calculation based on the transient antigenemia for HBV.

[c]Total risk per million donations.

Since approximately 80 percent of whole blood donations are collected from repeat donors, a correction factor is made taking into account the weighted average of first-time donation to ascertain the estimated total residual risk (for HIV, this is 3.32 incident cases per 100,000 person-years). To determine the risk that a donor was already infected and in the

infectious, seronegative window period, the adjusted incidence rate for HIV of 3.32 was multiplied by .044 (the 16-day window period for antigen expressed as a fraction of a year), yielding a residual risk of 1.5 per million donations. Our point estimate was calculated by taking this residual risk and dividing by 1 million. Similar calculations can be used to obtain estimates for HBV and HCV.

Table I.5 highlights the corresponding incidence rates and residual risk for paid plasma donors without taking into account the 60-day hold program. This information was obtained from the American Blood Resources Association and was based on 4,011,449 donations from 370 collection centers from July 1997 through October 1997. The confirmed positive donations were analyzed to ensure that they were, in fact, from qualified donors. Additionally, donation histories were examined for approximately 16,000 nonreactive donors (representing 300,288 donations) to obtain probability estimates for the effect of donors who did not return but may have donated a seronegative, but infectious, window period unit at their last donation. Calculations made above for volunteer donors were done in a similar fashion for paid plasma donors to obtain incidence rates, risks per million donations, and a point estimate.

Table I.5: Incidence Rates and Residual Risk Estimates for Paid Plasma Donations, Without the 60-Day Inventory Hold Program

	Rate	Ratio	Calculation
Anti-HIV marker: 36 seroconverters			
Seroconverters per 100,000 donations	.89		100,000 x (36 ÷ 4,011,449)
Incidence per 100,000 person-years	61.80		(36 x 100,000) ÷ [4,011,449 x (5.3 ÷ 365)]
Risk per million donations	37.25 27.09[b]		10 x [61.80 x (22[a] ÷ 365)] 10 x [61.80 x (16[a] ÷ 365)]
Point estimate		1:26,800 1:36,900[b]	1,000,000 ÷ 37.25 1,000,000 ÷ 27.09
HBsAg marker: 143 seroconverters			
Seroconverters per 100,000 donations	3.56		100,000 x (143 ÷ 4,011,449)
Incidence per 100,000 person-years	245.50		(143 x 100,000) ÷ [4,011,449 x (5.3 ÷ 365)]
Risk per million donations	396.84		10 x [245.5 x (59[a] ÷ 365)]
Point estimate		1:2,520	1,000,000 ÷ 396.84
Anti-HCV marker: 37 seroconverters			
Seroconverters per 100,000 donations	.92		100,000 x (37 ÷ 4,011,449)
Incidence per 100,000 person-years	63.52		(37 x 100,000) ÷ [4,011,499 x (5.3 ÷ 365)]
Risk per million donations	142.70		10 x [63.52 x (82[a] ÷ 365)]
Point estimate		1:7,008	1,000,000 ÷ 142.7
Total	**576.79[c]** **566.63[b,c]**	**1:1,734** **1:1,765[b]**	**1,000,000 ÷ 576.79** **1,000,000 ÷ 566.63**

Note: Interdonation interval = 5.3 days.

[a]Window period (in days).

[b]Calculation based on the incorporation of the antigen window period to determine its effect on the overall calculations for paid plasma donors. This was done in order to compare similar window periods for volunteer and paid plasma donors.

[c]Total risk per million donations.

Table I.6 outlines the overall residual risk of incorporating an infectious window period unit from a paid plasma donor into a plasma pool. This table takes into account the effect of the 60-day inventory hold program to interdict window period units.

Table I.6: Incidence Rates and Residual Risk Estimates for Paid Plasma Donations, With the 60-Day Inventory Hold Program

Marker	Incidence per 100,000 person-years	Residual risk per million	Point estimate	Calculation
Anti-HIV		1.47	1:680,272	1,000,000 ÷ 1.47
	61.80	~1.00[a]	1:1,000,000[a]	1,000,000 ÷ 1.00
HBsAg	245.50	53.84	1:18,574	1,000,000 ÷ 53.84
Anti-HCV	63.52	35.94	1:27,824	1,000,000 ÷ 35.94
Total		**91.25**	**1:10,959**	**1,000,000 ÷ 91.25**
		90.78[a]	**1:11,016[a]**	**1,000,000 ÷ 90.78**

[a]Calculation based on the incorporation of the antigen window period to determine its effect on the overall calculations for paid plasma donors. This was done in order to compare similar window periods for volunteer and paid plasma donors.

The residual risk per million in table I.6 was obtained from the American Blood Resources Association and included several probability estimates for window period donations when the last donation was positive and for window period donations when the last donation was nonreactive. These latter probability estimates were performed for the approximately 300,000 nonreactive donations that made up the American Blood Resources Association's data set. The residual risk per million of approximately 1.0 for HIV is based on an antigen window period unit of 16 days. This was calculated from information obtained from the American Blood Resources Association, which indicated that PCR testing would reduce the residual risk to .49 per million donations (11-day window period). Thus, the 1.0 used in our calculations to estimate the 16-day antigen window period is simply the midpoint between 1.47 for anti-HIV (22-day window period) and .49 using PCR testing.

When final comparisons are made, the overall risk of incorporating an infectious HIV, HBV, or HCV window period unit into a plasma pool was 1 in 20,872 for volunteer plasma donors (or 1 in 15,662 taking into account the transient nature of HBV) and 1 in 10,957 for paid plasma donors.

Additional Information on Testing Procedures

FDA's protocols for viral testing stipulate that if the initial test for viruses is reactive, then a retest should be performed to verify the initial result. If the retest is also reactive, the blood facility should perform a second, more specific test to confirm the presence of the viral marker.[42] Deciding whether a donation is or is not positive is also affected by the sensitivity and specificity of the viral tests.[43] Initial tests are fast and usually automated and screen large numbers of samples. They are extremely sensitive in order to minimize the number of false-negative outcomes. Confirmatory tests are more time consuming and usually less sensitive than initial tests but are very specific. Table II.1 outlines the different types of viral test results and the consequent actions.

Table II.1: Results From and Actions After Viral Testing

Result	Definition	Action
Initial test results		
Initially reactive	Initial test is reactive.	A retest in duplicate is performed.
Repeatedly reactive	One or both duplicate tests are reactive.	A confirmatory test is performed (this test is not always required); the prospective donor is deferred, and the collected unit is discarded.
Negative	Initial test is negative; or if reactive, both duplicate tests are negative.	None; the donor is not deferred.
Confirmatory test results		
Indeterminate	Duplicate tests are repeatedly reactive and confirmatory test is neither positive nor negative.	The donor is deferred and the collected unit is discarded.
Positive	Duplicate tests are repeatedly reactive and confirmatory test is positive.	The donor is deferred and the collected unit is discarded.

[42]A false-negative test result fails to detect the viral marker in a sample that contains the viral marker. A false-positive test result incorrectly indicates that the viral marker is present in a sample that lacks the viral marker. Confirmatory tests can also yield "indeterminate" results, meaning that it is not possible to be certain whether the individual is infected with the virus. Some studies have suggested that—depending on the population—most indeterminate confirmatory tests are probably negative. The status of donors who have indeterminate test results is resolved over time by additional testing. Units that test repeatedly reactive for HIV, HBV, and HCV may not be used for transfusion or for further manufacturing regardless of the more specific or confirmatory test results, except in special circumstances.

[43]"Sensitivity" is the probability of a unit's testing positive if a viral marker is truly present. As sensitivity increases, the number of persons whose blood contains the virus but who are missed (false negatives) by being incorrectly classified decreases. In other words, sensitivity = true positives ÷ (true positives + false negatives). "Specificity" is the probability of a unit's testing negative if a virus is truly absent. A highly specific test is rarely positive when a virus is not present and therefore results in fewer persons without the virus being incorrectly classified (false positives). In other words, specificity = true negatives ÷ (true negatives + false positives).

Any unit that is repeatedly reactive is considered positive even if confirmatory tests determine that the testing procedure produced a false-positive result. Such results require that the donor be deferred. FDA recommends but does not require that donors who are repeatedly reactive but indeterminate or negative by a confirmatory test should be notified and placed on donor deferral registries.

As an added precaution against the inclusion of any plasma that may contain undetectable HIV virus, one company performs additional tests for the HIV antibody. Each donation is tested according to the standards noted above by supplementary testing using a different antibody test than that used in the initial screening procedure. The testing uses "minipools" derived from samples of 64 donations.[44] Units corresponding to test samples that are confirmed reactive for anti-HIV at individual sampling are then rejected and the donor is deferred. Only nonreactive donations are considered to be acceptable for further manufacture.

PCR testing—which is more sensitive than licensed antigen or antibody detection methods currently used to screen collected plasma—will be done on pools of plasma rather than single donations. This approach is being pursued because of the constant state of rapid evolution of nucleic acid diagnostics and increased cost-effectiveness of pool testing. FDA has noted that it considers pool testing an interim step, but the agency does believe that testing of plasma pools has public health benefits and should be implemented. Consistent with this position, tests for plasma pools are now under "investigational new drug" status and are planned to be used by all fractionators to test all units of donated plasma in minipools.

Some companies have also determined that every product lot that is to be released should be tested one more time to ensure that there were not errors during the testing of the plasma, testing of the pools, and the manufacturing of the product. Final testing of lots for some companies includes tests for HBsAg, while other companies test for HIV using antibody testing and for HIV, HBV, HCV, and HAV using PCR tests.

[44]Minipool testing is done by taking samples of individual donations and combining them. If the minipool is found to be reactive for anti-HIV, then the reactive sample is identified by individual sample testing.

Viral Clearance Through Inactivation and Removal

Heating and chemical inactivation are the two main methods in use today to inactivate viruses. Heating in solution, terminal dry heat, vapor heating, and dry heat under solvent are commonly used. For chemical inactivation, manufacturers typically use solvent-detergent techniques, ethanol (during fractionation), and low pH. Viral removal steps include partitioning and nanofiltration. Partitioning during purification includes ethanol fractionation and chromatography, whereas nanofiltration can be accomplished through adsorption or through filters that discriminate to 15 to 100 nanometers.

To be effective, viral inactivation techniques must destroy at least one of the essential elements of viral replication.[45] These techniques work in different ways to accomplish this task. Photosensitizing techniques use light-activated dyes that are irradiated, causing the dyes to convert to molecules that can destroy DNA or membrane lipoproteins. Heat treatment denatures viral proteins and nucleic acids, rendering them incapable of viral replication. Irradiation processes inhibit viral DNA by inducing breaks and linkages. Solvent-detergent techniques destroy the viral envelope in lipid-enveloped viruses. Viral removal methods, including chromatography and filtration, physically separate virus particles and other impurities from the desired plasma proteins.

Validation of viral clearance steps is accomplished through a scaled-down production method to a laboratory model. Material is spiked with a marker virus (such as bovine viral diarrhea virus for HCV or duck hepatitis B virus for HBV); titers are then compared in the starting and ending material after performing the operations dictated by the laboratory model. This scaled-down model must maintain the physical parameters that will replicate the production method, including time, temperature, pressure, concentration, flow rates, and pH. It must also maintain the physical dimensions of volume, load, and surface area and column dimensions. These validation models cannot demonstrate complete elimination of a virus, but they can highlight the difference in titers in the beginning and end of the production model. This modeling will highlight the actual viral kill that has been accomplished through inactivation, removal, or both. The effect of multiple clearance steps may be combined if each step is independently validated and each is based on a mechanism that is different from other clearance steps.

[45]Viral replication requires cell attachment by the virus to a cell receptor, penetration of the cell, replication and translation of viral nucleic acids, and exit from the cell with integrated viral particles.

Units that have been tested for HIV that were in the window period show a range of genome copies per milliliter of 10^3 to 10^7 (with occasional spikes to 10^8 range), while seropositive units are in the range of 10^3 to 10^6. For albumin, the viral log reduction factor (LRF) using pasteurization has been shown to be greater than 7, while partitioning during fractionation shows LRFs to be greater than 6.[46] Additionally, there have been no cases of HIV, HCV, or HBV transmission through albumin since initiation of heating (at 60 degrees Celsius for 10 hours) of the final containers. For IGIM, the cumulative LRF for HIV in one model was greater than 10.9 (6.2 using ethanol fractionation and 4.7 using solvent-detergent techniques). For IGIV, the cumulative LRF for one process was greater than 17.5 (5.9 using ethanol fractionation, 5.2 using solvent-detergent techniques, and 6.4 using pH 4). Processes for IGIV from another model show LRFs of 13.2 and 11.4 using ethanol fractionation and heat treating or ethanol fractionation and a pH of 4 using Pepsin, respectively). For antihemophilic factor, the cumulative LRF for one process was greater than 15.7 (5.2 using purification and 10.5 using heat treating at 60 degrees Celsius for 10 hours), while another company's procedure showed LRFs of greater than 12 (2 using affinity chromatography and greater than 10 using solvent-detergent techniques). Similar reductions are found for coagulation factor IX. Thus, these LRFs for HIV are well above the levels of genome copies per milliliter found in units that are from window period and seropositive donations.

For HCV, genome copies per milliliter found in window period units ranges from 10^3 to 10^8, while seropositive units range from 10^3 to 10^6. For albumin products, LRFs for HCV model viruses have been shown to be greater than 11 using pasteurization and processing techniques. For IGIM, the corresponding LRF for one process was greater than 10.1 (3.3 using ethanol fractionation, greater than 5 using solvent-detergent techniques, and 1.8 using filtration). For IGIV, one company's clearance profile was greater than 11.5 (3.2 using ethanol fractionation, greater than 4.2 using solvent-detergent techniques, and greater than 4.1 at pH 4), while another procedure showed an LRF greater than 10.1 (3.5 using ethanol fractionation and greater than 6.6 using heat treating at 60 degrees Celsius for 10 hours). For antihemophilic factor, one process had a cumulative LRF of greater than 17.0 (7.6 using affinity chromatography, greater than 4.5 using solvent-detergent techniques, and greater than 4.9 using dry heating). LRFs greater than 10.3 are found for coagulation factor IX. Again, these LRFs are

[46]LRF = log x [(V1 x T1) ÷ (V2 x T2)], where V1 = volume of starting material, T1 = concentration of virus in starting material, V2 = volume of material after process step, and T2 = concentration of virus after the step. LRFs less than 1 are not considered significant, whereas LRFs greater than 4 are clearly effective.

well above the levels of genome copies per milliliter found in units that are from window period and seropositive donations.

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