# *TRANSFUSION SAFETY STUDY*

# *PROTOCOL*

# *STUDY OF RECIPIENTS OF BLOOD AND BLOOD COMPONENTS FROM DONORS WITH ANTIBODY TO HIV*

## *BACKGROUND AND RATIONALE*

One of the populations in which acquired immune deficiency syndrome (AIDS) was seen early in the present epidemic was hemophiliacs treated with commercially prepared clotting Factor concentrates. This occurrence was recognized as consistent with the hypothesis that the disease is caused by a transmissible agent which is capable of sustaining itself as a prolonged or chronic infection, and which is present in the blood of at least some infected persons. Commercial preparations of Factor VIII and IX are almost certain to expose persons with congenital clotting disorders to any virus that may be present in the blood of any of the tens of thousands of purchased plasmapheresis donations pooled to make each lot of these biologically active proteins. It is not surprising, therefore, that a newly introduced agent with the indicated characteristics would soon appear among those with congenital clotting disorders.

From the occurrence of AIDS among hemophiliacs, it seemed predictable that as the etiologic agent became increasingly prevalent in the United States, transfusion of whole blood and unpooled components would also become a mechanism of transmission. The first case in which transfusion was suspected as the source of AIDS was reported in 1982, and the number of transfusion-associated incidents is now sufficiently large that there is no doubt that the disease is a potential consequence of administration of blood and blood components.

Publicity about transfusion-associated AIDS resulted in great concern in the medical community, especially among blood collection services. With no specific etiologic agent identified, however, there were few measures that could be effectively applied. One approach was to ask persons aware of epidemiologic circumstances that could have exposed them to the risk of infection to refrain from giving blood. In addition, serious consideration was given to use of laboratory screening procedures known to be non-specific but that nonetheless might identify enough infections to improve the safety of the blood supply.

A major change in the situation was brought about by the identification of the infectious agent underlying the immunologic defects responsible for AIDS. Since 1983, materials from patients with persistent generalized lymphadenopathy or with AIDS have yielded isolates of retroviral strains with essentially identical characteristics. In addition to the fact that the same virus has been isolated from appropriate patients by several laboratories, the frequency of antibodies to components of the virus among persons in various groups with an increased risk of clinical disease has been approximately proportional to the extent of that risk. On the basis of this evidence, this virus is now generally accepted as the etiologic basis of AIDS. The virus has been variously termed human T-lymphotropic virus type III (HTLV-III), lymphadenopathy-associated virus (LAV), AIDS-associated virus (ARV), or human immunodeficiency virus (HIV). The latter designation has been proposed by a subcommittee of the International Committee for the Taxonomy of Viruses as the appropriate name for the retrovirus that has been implicated as the causative agent of AIDS. Whether other factors contribute to the frequency with which HIV infection leads to clinical disease is not yet established.

In view of the high level of public apprehension about the safety of transfusion, a particularly important finding was the identification by Gallo and his colleagues at the National Cancer Institute of a cell line that supports replication of HIV to very high titers without cellular destruction. This virus-cell system was immediately recognized as opening the way for large-scale production of virus-specific antigens. With the latter, assays for antibody as an index of experience with HIV could be rapidly developed and marketed for use in screening of blood donors. This goal was given very high priority by the Department of Health and Human Services, and contract awards were made on a competitive basis to five companies judged to have the best capabilities of achieving it. Licensure of an enzyme-linked immunoassay (EIA) occurred in March, 1985, and the test is rapidly being introduced into blood services throughout the country.

An EIA test was chosen because EIA is already a familiar procedure to blood services through its use (in preference to radioimmunoassay) for hepatitis B virus screening. Reliable results with manufacturers' kits will require only two to three hours of incubation, so that it will be applicable in instances in which blood is immediately needed for transfusion. Finally, with respect to the method, it is easily automated, which is obviously important to agencies that collect several hundred to one or two thousand donations each day.

Testing for antibodies to HIV is an appropriate approach because, in contrast to the situation in many other viral infections, the presence of antibody in serum is compatible with continued replication of the agent in circulating lymphocytes. In fact, from experience with other retroviruses as well as initial studies of HIV antibody to one or more antigenic components is often detectable at the same time that virus is isolated. Testing for anti-HIV, therefore, should identify many donors who are potentially infectious. Methods for demonstrating virus or viral components will require appreciably more time before they will be practical outside of research laboratories.

Nevertheless, it seems unlikely that the initial versions of an EIA procedure will be anything more than an interim measure for reliably identifying as many donors as possible who may have the potential for transmitting the agent. Three problems are easily identified:

(1) Any EIA may give a false-positive reaction because of non-specific adherence of immunoglobulins and other proteins in the test system. Under most circumstances, corroborative tests are available to define the specificity of the result. It is not yet clear, however, what procedures are fully corroborative with respect to anti-HIV positivity. This is particularly true for persons in the general population who may be at an early stage of infection or who have only limited expression of the agent. This present lack of complete confidence in other methods of evaluation poses unusual problems with respect to donors whom one may feel should be informed of a positive reaction. The climate of emotionality about AIDS makes reliability of any information given of extraordinary importance.

(2) The presence of detectable and confirmably specific antibody in the serum of an individual does not mean that he will necessarily transmit HIV to any or all persons exposed or under all circumstances. The infection may have been resolved, or it may be latent and fail to express itself in another host. Informing the person of anti-HIV positivity may induce emotional or social problems that are unnecessary unless other procedures now available only at a research level are applied to evaluation of his status.

(3) The absence of detectable antibody by EIA does not assure that the donor is non-infective. Instances of negativity or variable positivity in infected persons are known apart from the late stages of the disease when blood donation would be unlikely. Efforts are already being made to develop screening procedures that will detect viral components, and it is possible that these will be more predictive of risk to the recipient of any blood donation.

These limitations do not mean that use of EIA tests for donor screening should not be implemented as quickly as possible, but they do pose questions. Because the EIA procedure will identify at least some persons whose blood or blood derivatives would transmit HIV infection if administered, routine use has begun without those questions being answered. At the same time, however, plans should be made to obtain pertinent information as quickly as possible.

The present study was proposed by the National Heart, Lung, and Blood Institute (NHLBI) at the time it became technically conceivable that within six to 12 months enough anti-HIV assay kits could be produced to screen all blood and plasmapheresis donors in the United States. It was obvious that use of such a test would result in the discarding of all units from donors with a reaction interpreted as positive. As a consequence, the range and frequency of possible outcomes among recipients of EIA-positive blood and components would remain conjectural.

Accordingly, NHLBI issued a contract (NO1-HB-4-7002) under emergency powers for the collection of serum samples from donors prior to the time that screening would make rejection of anti-HIV-positive units mandatory. This work was undertaken as a preliminary but obviously essential prerequisite to the study proposed in the present protocol to determine the extent to which anti-HIV positivity is associated with transmission of the infectious agent.

The primary use envisioned for the donor repository was to identify potentially infectious donors and the recipients of their blood; it was not to determine prevalence of HIV infection in the general donor population. The project, therefore, was focused in four locations with the highest incidence of AIDS, as judged by information provided by the surveillance program of the Centers for Disease Control. These were: New York City and Northern New Jersey, both of which are served by the Greater New York Blood Program; the Miami area, served by the South Florida Blood Service; San Francisco and Bay area counties to the north of that city, served by the Irwin Memorial Blood Bank; and Los Angeles County, served by the Los Angeles-Orange Counties Region of American Red Cross Regional Blood Services.

The number of specimens needed to have an adequate sample of recipients was (and remains) unknown. To answer questions about donor and recipient characteristics possibly influencing transmission and its outcome, it was decided that it was prudent to try to identify 200 recipients who are likely to survive the disease or condition for which they were transfused, and who would give permission to be observed. A goal of 200,000 stored donor samples, therefore, was set. That number would provide 200 recipients as subjects if the rate of corroborated positivity is as high as 0.5 percent and the proportion of recipients available for follow-up is as low as 10 percent (assuming each unit donated will be used as components and be associated with two recipients). The 200,000 samples would also provide 200 recipient subjects if the rate of corroborated donor positivity is as low as 0.2 percent and the proportion of recipients available for follow-up is as high as 25 percent.

Beginning in early September, 1984, donors at the participating blood services were asked to permit the collection of an additional 10 ml of clotted blood for HIV antibody testing. They were also asked for permission to contact them later if the results of such tests indicated that further observations would yield useful scientific information. The level of participation varied from approximately 55 to 85 percent in the different geographic areas, but has not appeared to vary significantly with sex or age.

As of the close of repository collections in early 1985, the number of donor serum specimens in storage totaled 200,000, distributed as follows: New York Blood Center, 77,760; South Florida Blood Service, 31,104; Irwin Memorial Blood Bank, 27,500; and American Red Cross in Los Angeles, 67,418. These

donor repositories, and the donors' informed consent to be tested and contacted for further observations, form the basis for the present proposal.

Some comment on the ethical and legal aspects of the proposed approach seems necessary because of the potential for adverse emotional reactions among subjects told that they may be at increased risk of developing AIDS. In addition, it must be stressed that the investigators recognize the social consequences for the individual, whether donor or recipient, if the strictest confidentiality of information about HTLV-III exposure or infection is not maintained.

The donor participating in this study will have an advantage over routine donors in that initial positivity by the EIA screening procedure will be corroborated by additional standardized procedures not routinely available. Furthermore, participation in the project provides continuing evaluation of the individual's health that would otherwise be very expensive to obtain or entirely unavailable.

A policy for advising recipients of potential HIV exposure through transfusion has been adopted both in research studies and by some blood bank organizations in situations in which individuals received blood collected from persons who were clinically well at the time of blood donation but subsequently developed AIDS. In the present proposal for follow-up of recipients of blood components from persons who are anti-HIV-positive, the same general principles of recipient notification will be applied. The implications of the data to be gathered seem to the investigators to be sufficient justification for notifying the recipient. It may also be of benefit for the recipient to be aware of this information in the event of future medical problems.

## *SCIENTIFIC QUESTIONS TO BE ASKED*

1. With what frequency do donors with HIV antibody and other markers of HIV infection transmit their infection to recipients of their blood and blood components. Can the impact of using the anti-HIV EIA in blood banks be estimated?

2. What characteristics of *donors* influence whether transmission of infection takes place -- sex; age; extent of HTLV-III replication as evidenced by virologic and immunologic indices; the presence of other infectious agents transmissible to the recipient? Study of donors may demonstrate parameters that will be helpful in advising all anti-HTLV-III-positive persons about their infectivity.

3. Are there any differences in relative infectivity of whole blood and components (packed cells, platelets, etc.)? The information may permit inferences about relative amounts of intracellular and extracellular virus in peripheral blood.

4. What characteristics of *recipients* influence whether transmission of infection takes place -- sex, age, the underlying disease that resulted in a need for transfusion, the occurrence of immunologic stress by allogeneic exposures, the presence of immunodepression due to the underlying disease or therapeutic modalities used in its management, the acquisition of other transfusion-transmitted viral agents from the same or other donors?

5. When infection does occur, can host factors influencing its clinical expression be identified? Prospectively followed blood recipients will be identified sufficiently early in the course of infection and in relatively large numbers to answer this question.

6. What are the immunological consequences of blood transfusion in the absence of HIV exposure, and how may these affect the outcome when exposure does occur through transfusion? The alteration of T-cell subpopulations and other aspects of immune function by transfusion need further definition.

7. To what extent are sexual contacts of persons who receive a unit of anti-HIV-positive blood at risk of developing HIV infection?

8. To what extent are infants born to mothers after they received a unit of anti-HIV-positive blood at risk of developing HIV infection?

9. To what extent are mothers of infants who receive a unit of anti-HIV-positive blood at risk of developing HIV infection?

*Donor-recipient protocol*

*OBJECTIVES*

1. To identify and observe recipients of blood and blood components donated by persons whose sera give positive results during anti-HIV testing to determine if HIV infection is transmitted by the transfusion.

2. To identify and observe as controls, recipients of blood donated by persons whose sera are anti-HIV-negative by all applicable tests. It is not practical to apply case-control matching techniques to select this group. The goal, therefore, should be to recruit a large enough number to maximize the probability that their characteristics *as a group* will be similar to those of study recipients.

3. To utilize observations of donors, follow-up of recipients, and new laboratory procedures to determine the meaning of discrepancies among tests used in screening of the donor repository.

4. To assess the effects of transfusion upon the immune system of the transfusion recipient in the presence and absence of HIV exposure.

5. To assess the frequency of other transfusion-transmitted diseases in the presence and absence of HIV exposure.

6. To assess the frequency of transmission of HIV from recipients of anti-HIV blood to their sexual contacts.

7. To assess the frequency of transmission of HIV from women who received anti-HIV positive blood to infants born subsequently.

8. To assess the frequency of transmission of HIV from infants who received anti-HIV positive blood to their mothers.

9. To collect for long-term storage in a TSS-NHLBI repository plasma and cells at the time of each observation (six-month intervals) of each person entered into the Study.

## *STUDY POPULATIONS*

1. Recipients of blood or blood components donated by persons in the geographic areas served by one of the four participating blood collection agencies (listed below) who gave their permission at the time of blood donation to have serum tested for anti-HIV.

a. Greater New York Blood Program's facilities in all four of its divisions (New York City, Northern New Jersey, the Hudson Valley, and Long Island).

b. South Florida Blood Service.

c. Irwin Memorial Blood Bank at its facilities in San Francisco and adjacent counties to the north.

d. American Red Cross Regional Blood Services' facilities located in Los Angeles County.

This population will consist of: (A) Recipients of blood or blood components from donors whose positivity on EIA screening is corroborated by two additional tests (positive donors); (B) recipients of blood or blood components from donors whose negativity of EIA screening is corroborated by two other tests (negative donors); (C) recipients of blood or blood components from donors whose positivity on screening is not corroborated by two additional tests (probably negative EIA reactions); and (D) recipients of blood or blood components from donors whose sera give findings of unknown meaning.

The second population will consist of: (A) Recipients known to have received blood or components from identified positive donors, (B) recipient controls known to have received blood or components from the matched donor controls, (C) recipients of blood from donors with probably negative EIA reactions, and (D) recipients of blood from donors with presently uninterpretable findings.

In order to evaluate immunologic and serologic findings in donors with corroborated positivity for anti-HTLV-III (donor group A), it is necessary to have an anti-HTLV-III-negative control group matched for sex, age, and geographic area (donor group B). Even though measurement of T-cell subpopulations in the study will be as standardized as possible, the influence of sex, age, handling of specimens during field collections, and day-to-day variations in laboratory techniques make a relatively large comparison group necessary. In addition, HTLV-III infection should be correlated with information obtained from the history, physical examination, and other laboratory findings. In particular, the prevalence of serologic markers of other viruses (HBV, EBV, CMV) will provide indices of intensity of exposure. Evaluation of all of these factors requires a comparison group.

Characteristics of recipients in group A, who enter the study because of anti-HTLV-III positivity of one of their donors, are obviously dependent on chance factors influencing the need for transfusion and the distribution of units by the collection agency. They are likely in many instances to be persons with high transfusion requirements and extensive exposure to all transfusion-transmitted infections. In addition, group A recipients may be expected to include a relatively large proportion of persons with leukemia, lymphoma, and other malignancies for which cytotoxic and immunosuppressive drugs are given. To follow the usual practice of excluding such persons could reduce drastically the size of the unique population covered by the present study. Finally, it is important to learn if immunocompromise from any of the factors mentioned modifies the outcome of HTLV-III exposure.

Because the above-mentioned factors will make recipient group (A) very much more heterogeneous than any population of recipients usually studied, recipient group (B) is essential. Because of that heterogeneity, the latter would ideally be chosen as an individually matched set of subjects hospitalized for the same diagnosis or procedure at the same facility as the study recipients. That approach, however, is out of the question because of the multiple hospitals that will be involved. The most practical compromise is to utilize the recipients of blood and components from control donor group (B), and depend upon chance to result in sufficient similarity of the two groups. For this reason, donor groups (A) and (B) should be of at least equal size, and a ratio of 1:1 is to be sought.

Study of donor and recipient groups (C) is intended to evaluate the relative sensitivities of EIA screening at a blood service and corroborative procedures presently still in a research phase of development. Although it seems appropriate to classify sera from donors in this category as probably negative, the ability to identify the recipient and determine whether infection occurs represents a significant opportunity.

Finally, donor and recipient groups (D), which cannot be defined until there has been sufficient experience with immunoblot (Western blot), radioimmunoprecipitation (RIP), and other procedures now being introduced, offer the opportunity to evaluate differences that may be found among their results.

*PROCEDURES*

## Testing of Donor Specimens

At each blood service, testing of serum specimens in the TSS Donor Repository will be performed by a specifically designated staff and handled separately from testing of routine blood donors. All test sites will use the same commercially manufactured EIA test. This will allow prevalence determinations to be made and will aid in standardizing quality control approaches and data processing.

To minimize the time from donation to follow-up for some of the donor-recipient pairs, the repository samples will be tested in a last collected-first tested fashion. However, during the start-up of testing (the first five working days) the first samples to be tested will be the oldest samples so that in the event that problems occur, the potentially more valuable recent samples will not be lost.

The repository specimens will be tested as rapidly as possible in order to minimize the amount of time from donation or transfusion to follow-up. Rapid testing also has the advantage of enhancing the potential for efficiency in follow-up -- several donors to be recruited may live in the same area, and several units to be investigated may have been sent to the same hospital.

On each working day, the required number of specimens will be removed from the donor repository, thawed, and stored in a refrigerator at $4^{O}C$ until the anti-HTLV-III result is known. Initially positive samples and initially negative samples from potential control donors will be kept in the refrigerator for retesting in duplicate. All other initially negative samples will be returned to their original positions in the freezer.

Initially positive samples will be retested in duplicate on both HTLV-III and H9 plates on the same day or in an early run on the following day. If these samples are "positive" by the manufacturer's criteria, two aliquots will be removed, and the remaining serum in the original vial returned to its original position in the donor repository. One aliquot will be transferred to the subject repository at the clinical center for that city. The other aliquot will be immediately shipped frozen to the coordinating center's Central Processing Laboratory (CPL) for coding prior to corroborative testing.

When a positive sample is identified, the data clerk will use the unit number to determine whether any component of that donation was sent to a hospital. If any component still remains in the agency's inventory as a frozen product, the blood center director will be notified. If no component from the unit has been utilized, the Central Processing Laboratory will give lower priority to confirming the EIA result.

For each positive sample identified, an initially negative sample from a matched donor will be sought from among the samples tested on that day or as soon as possible thereafter. The criteria for matching the control with the anti-HTLV-III-positive (index) donor are: (1) A date of donation within one week of that of the index donor; (2) same sex as the index donor; (3) same age group as the index donor; (4) same geographic classification (with respect to prevalence of AIDS). To accomplish this, the blood center will determine the characteristics of the index donor using a computer-generated "master list" containing the necessary information, ordered by blood unit number. The control donor will be identified from a series of "matching lists" grouping all donors according to by sex, age group, and geographic classification.

The samples from potential control donors (*i.e.*, anti-HTLV-III negative donors on initial screening) will be retested in duplicate on both HTLV-III and H9 plates at the time their sera are thawed. If the result of

duplicate retesting is positive instead of negative, the above-described procedure for positive sera will be followed and another potential control evaluated to replace the index. The initial screen-negative donor will now be regarded as a study donor rather than a control. If the sample is negative by the manufacturer's criteria, two aliquots will be removed, and the remaining serum in the original vial returned to its original position in the donor repository. The aliquots will be handled in the same way as described for sera from study donors.

For all samples retested in duplicate on both the HTLV-III and H-9 plates which are false-positive (*i.e.*, the mean HTLV-III or HTLV-III:H9 ratio is less than the respective cut-offs, or only one of three HTLV-III results is above the cut-off) one aliquot will be removed, and the remaining serum returned in its original vial to its original position in the Donor Repository. The single aliquot will be immediately shipped frozen to the coordinating center's CPL for coding prior to further testing. These specimens will be tested on a lower priority basis than the positive or negative samples. If corroborative testing is positive, consideration may be given to enrolling the donor in the study.

### Additional Testing of Donor Specimens and Quality Control

Due to the probability of false positive screening results in a low prevalence population, it will be necessary to attempt to verify the specificity of EIA positivity. The procedures that presently seem appropriate for verification of anti-HTLV-III positivity include: repetition by a different EIA, and the identification of antibodies to specific viral antigens by immunoblot and RIP.

Use of an EIA procedure by another manufacturer will give some additional information and will provide concordance data but will not be considered satisfactory for confirmation of positive antibody status. RIP will be helpful because of its ability to demonstrate antibody to high molecular weight antigens, which may be the only antibodies present. Multiple corroborative procedures are necessary in order to obtain maximal information and because no single test is currently accepted as sufficiently sensitive and specific. In addition, the use of immunoblot and RIP procedures will allow us to identify antibodies reactive to specific viral proteins and to assess the significance of particular antibodies in the transmissibility and natural history of HTLV-III infection. A positive result on both immunoblot and RIP testing will be interpreted as verifying the presence of HTLV-III antibody.

Procedures based on evidence of viral latency or replication may be utilized at a later date in order to gain additional information about the initial donor sample. These procedures will not be used as a basis for selecting study subjects.

Positivity or negativity by EIA at the collection agency will be cause for priority in additional testing by the central laboratories using other procedures.

All samples sent for additional testing will be recoded at the CPL prior to such testing so that those performing the tests will not have any knowledge of EIA test results.

If the number of positive or negative specimens yields a sample size beyond the capacity for additional testing by the procedures adopted, then priority will be given to specimens from donors whose units or components are sent to medical centers representing the largest users of blood. If the number of donors for whom positivity or negativity is corroborated by the central laboratory exceeds personnel resources available to carry out follow-up, then, similarly, priority for additional testing will be given to specimens

from donors whose units or components are sent to medical centers representing the largest users of blood. These provisions are intended to ensure efficiency in follow-up.

Probable negativity of the EIA result will be defined as: (1) Repeatable EIA positivity supported by the H9 result at the collection agency, *and* (2) negativity of both the immunoblot and RIP findings.

Non-repeatability or H9 non-specificity by EIA at the collection agency will be cause for further investigation by the central laboratories using RIP and Western blot procedures in order to further assess if antibodies are present. These additional evaluations will be on a non-priority basis so that donors and recipients of positive and negative units can be reached as soon as possible. If the other tests are negative, no further participation of the donors will be requested; if one or more are positive, observation of the donors and corresponding recipients may be planned.

*Quality control* of the initial screening will be based primarily upon a proficiency panel to be used throughout testing. This panel will be prepared from anti-HTLV-III positive, negative, and borderline plasma samples obtained by the collection agencies during routine donor screening. The CPL will supply, in a random order, coded samples which have been thoroughly evaluated. An appropriate number will be assayed during the course of each day's work at each facility. This approach will permit comparison of test performance across different testing sites and, within each site, across different test runs.

A second method of quality control in screening will be re-evaluation by the central laboratory of a sample of donor specimens that are negative. A control group of donors will be selected (see below); these control donors' specimens will provide a set of negative sera equal in size to the positive set. Corroboration of negativity will be performed prior to control donor follow-up. If one of the corroborative tests is positive, then this donor will no longer be a potential control, but consideration will be given to enter such donors into the study.

### Later Retesting of Donor Specimens That Are Negative by the Initially Used Assay Procedure

As more sensitive procedures for detecting persons with the potential for HTLV-III transmission are developed, it may be desirable to retest specimens that were initially negative by EIA. It is anticipated that an appropriate sampling fraction of these negative samples will be tested. Any proposals concerning retesting must be formulated later and presented to NHLBI and its Advisory Committee at the appropriate time.

All specimens collected under contract NO1-HB-4-7002 are property of the Federal Government, and may not be used without specific approval of NHLBI, however valid the scientific purpose.

### Donor Follow-up

A serum sample has been stored for each donor who agreed to participate in the pre-screening part of the study. This sample indicates the donor's anti-HTLV-III status at the time of donation. However, follow-up observations are necessary to obtain serum, plasma and cells for determining the presence of viremia and immune function changes, for other clinical center and central laboratory tests, and for a subject repository. Some of these parameters may be associated with the potential of a donor to transmit HTLV-III infection at the time of the donation. Donors selected for further study will be followed for a

three-year period at specified intervals in order to learn the significance of these findings for their well-being.

Donors to be followed will be placed into four categories. Donors who are positive by EIA at the collection agency and by both additional tests (immunoblot or RIP) will be classified as anti-HTLV-III positive (donor group A). Donors who are negative by EIA at the collection agency and by both additional tests will be classified as anti-HTLV-III-negative (donor group B). Donors whose positivity by EIA at the collection agency is corroborated by neither of the additional tests will be classified as having probably negative screening reactions (donor group C). Donors positive on screening whose sera give results of unknown meaning when evaluated by other procedures will be the final group (donor group D).

Donors in donor groups (A) and (B) will be identified and contacted as soon as possible. Donors in donor groups (C) and (D) will be the subject of a later subprotocol.

The central laboratories responsible for corroborative testing will report their results to the Central Processing Laboratory for a final evaluation. If all findings are unambigious and the person lives in a geographic area feasible for follow-up, then the Director of the Central Processing Laboratory will provide the donor's Study identification number to the person at the Clinical Center responsible for donor recruitment.

Throughout the identification and follow-up of donors, all specifically identifying information will be available only to those persons for whom it is absolutely necessary for making contact. Each collection agency will develop its own plan for accomplishing this purpose, which must be based on its system of keeping records. In addition to this general confidentiality, attention must be given to keeping the patient manager responsible for follow-up blinded to the anti-HTLV-III test results until the end of the initial visit. No donor will be contacted until the patient manager or TSS-blood bank clerk has located the informed consent signed by the donor. Both will make all possible efforts to protect the identity of these persons.

From the blood unit number, the patient manager or data clerk will obtain the donor's name and other information necessary to make contact. When the donor is first contacted, he will be reminded of his previously expressed willingness to be contacted if further participation may be helpful in evaluating new tests related to transfusion safety. The donor will be asked to present himself at a convenient facility available to the blood service to discuss further participation. The site and time will be one of mutual convenience. If the donor states that he is not interested in further participation but wants to know his test result, he will be told that, to ensure privacy, test results cannot be given over the phone or by mail. To ensure confidentiality, it is essential that the person satisfactorily be identified as the person who made the donation. He must, therefore, provide adequate identification in person at a facility available to the blood service in order to learn his result.

When the donor presents himself to the blood service, he will be reminded again that he agreed to participate in a study of transfusion safety during a recent blood donation and will be asked to participate in the second phase of the study. A copy of his signed consent form for the pre-screening phase will be available. The donor will be asked to sign an informed consent agreeing to be interviewed, to have a simple physical examination, and to have a blood sample taken for HTLV-III testing (antibody and possible virus isolation) and tests of immune function (T-cell phenotypes, immunoglobulin levels, and possible special tests) at specified intervals for a period of three years. The donor will be given the opportunity to ask the patient manager any questions about the study and the meaning of anti-HTLV-III tests.

In order to minimize the effect of the donor's knowledge of his anti-HTLV-III test result, at least on baseline data, the donor will be asked to sign the informed consent and to complete the interview, physical exam, and blood drawing before receiving the test result. However, he will be informed that he has the option of receiving the result prior to participating in the second phase of the study. If the donor does not want to participate in the study, he will be given his test result at this time. If the donor agrees to participate, the interviewer will record whether the interview, physical exam and blood drawing were completed before or after the donor received his test result. When the donor requests the result, the patient manager will either call a physician member of the study team or will obtain the test result from a confidential file. The result will be communicated to the donor and its meaning discussed.

If a donor is positive for anti-HTLV-III, he will be given printed material concerning the possible significance of the findings and medical and mental health referral services in his community. The donor will be given a release form which would permit the blood center to release the test results to his personal physician in a fashion which will protect the donor's confidentiality. A brief description of the research study will also be provided to the donor's physician.

After obtaining informed consent, the patient manager will conduct the entry interview, which will contain questions about medical history, present health, and patterns of behavior, including sexual practices (specifically homosexual contacts) and recreational intravenous drug use. The patient manager will then conduct a partial physical examination, including height, weight, temperature, and examination of the mouth, exposed skin, and lymph nodes in the head and neck. If any abnormalities are found, a physician on the study staff will conduct a more complete physical examination, which will include checking for hepatosplenomegaly and a more extensive examination of the skin and lymph nodes, including axillary and inguinal nodes. The patient manager will also draw a sample of blood. The blood collection procedure, including the amount of blood to be drawn, is specified in the manual of operations.

The procedure for handling the completed questionnaires, physical exam records, and blood specimens is described in the manual of operations.

All subjects entered into the study will be placed into one of three categories based on symptoms, signs on physical exam, and laboratory findings: asymptomatic, AIDS-related findings, or AIDS. These categories are defined in the manual of operations and will be consistent with CDC criteria. Subjects who are asymptomatic will be followed at six-month intervals. Subjects with AIDS-related findings or AIDS at entry into the study will be followed every three months. Subjects who are asymptomatic at entry but who subsequently present with symptoms will be reclassified and followed at three-month intervals. Symptoms and/or findings related to conditions other than AIDS may also make subjects eligible for special follow-up.

For each donor participating in the study, a schedule of dates for follow-up visits will be kept in the central record. One month before the scheduled visit the patient manager will contact the donor to arrange the time and site for the visit. The follow-up visit should occur within one month of the date scheduled in the record.

Each follow-up visit will include a shorter version of the entry interview, a physical examination, and drawing a sample of blood, as described above.

## Recipient Follow-up

The four recipient groups will consist of the following: (A) Recipients known to have received blood or components from anti-HTLV-III positive donors; (B) recipient controls known to have received blood or components from matched donor controls; (C) recipients of blood or components from donors with probable false-positive screening results; and, (D) recipients of blood or components from donor group (D).

*Recipients in Group* (*A*). Adult recipients in this group will be identified by reviewing blood center records and then contacting the blood bank director of the hospital that received the blood component. Information will be obtained from the blood bank director concerning the patient who received the unit. The physician who ordered the blood component will also be identified from blood bank records and will be contacted by a physician on the study staff or the patient manager assigned to recipient follow-up (depending upon the written procedures of each clinical center). If he is not the physician currently responsible for the recipient, he will be asked to identify the physician who is responsible.

The responsible physician will be told about his patient's exposure to blood in which antibodies to HTLV-III were detected and about the study. The physician will be asked to give permission for his patient to be informed about the exposure and to be recruited for the study. If the physician agrees, the recipient will be contacted according to the procedures established by each clinical center. They include the following options:

1. The physician will be asked to contact his patient, inform the patient of the exposure, and recommend that the patient participate in the study. If the recipient is willing to participate in the study, the physician will inform the patient manager, who will then contact the recipient.

2. The patient manager will make the initial contact by telephone. Using a standardized script, an attempt will be made to be certain that the person on the telephone is actually the recipient. The patient manager will explain that a national study of transfusion safety is being conducted and that the recipient was selected to participate because he recently received blood or blood components.

3. The recipient will be informed about the exposure and the study jointly by the physician and patient manager in the physician's office.

If the physician refuses permission to contact his patient, the recipient will not be contacted. However, this refusal will not be accepted until an attempt is made to inform the physician that he should be careful not to suppress information relating to his patient's health unless he has a very good reason why his patient should not be given the information, and that he should seriously consider cooperating with a study as potentially significant as this one.

When the recipient presents himself to the study staff member, he will be told by the appropriate person (the personal physician, a physician member of the study team, or patient manager) about his exposure to blood in which HTLV-III antibody was detected and about the study. He will be given an opportunity to ask the staff member about the meaning of the exposure and the study, and will be asked to sign an informed consent to be interviewed, to have a simple physical examination and to have a blood sample taken for HTLV-III testing (antibody and possible virus isolation) and immune function testing. The patient will be told that these studies will be repeated at specified intervals for a period of three years.

*Recipients in Group* (*B*). Adult recipients of blood or components from negative donors and the physician who ordered the transfusion will be identified following the same procedure described above for recipients of positive blood.

The responsible physician will be contacted and told about the study. He will also be told that, although there is no evidence that his patient received anti-HTLV-III positive blood, we would like to evaluate the patient as part of the study. He will be asked for permission to contact the patient to enroll him in the study. The patient will not be contacted if the personal physician refuses permission.

If the physician agrees, procedures specified by each clinical center will be followed to contact the potential control recipient. They include the following options:

1. The physician will be asked to contact his patient, inform the patient of the study, and recommend that the patient participate in the study. If the recipient is willing to participate in the study, the physician will inform the patient manager, who will then contact the recipient.

2. The patient manager will make the initial contact by telephone and provide immediate assurance that the recipient is not being called because of known problems with any blood transfusions he has received. Nonetheless, the person's participation is asked because it is necessary to compare persons with no known risk to those with possible risk.

The potential recipient control will be told that this is a study of blood transfusion safety conducted to understand better the immunologic changes and the occurrence of infection, including HTLV-III, associated with transfusion. He will also be told that a sample of the blood that he received was stored and tested for antibodies to HTLV-III when such a test became available and that no antibodies were detected. However, certain aspects of his health need to be compared with those of a blood recipient who received blood later found to contain HTLV-III antibodies.

The potential control recipient will be given an opportunity to ask any questions he may have about the study and will be asked to sign an informed consent agreeing to be interviewed, to have a simple physical examination and to have a blood sample taken for HTLV-III testing (antibody and possible virus isolation) and tests of immune function. The recipient will be told that these studies will be at specified intervals for a period of three years.

If the recipient does not want to participate, there will be no attempts at further contact by any member of the study team. If the recipient signs the consent form, the patient manager responsible for recipient follow-up will conduct the interview and physical examination and will draw the blood sample as described above for donors.

*Recipients in Groups* (*C*) *and* (*D*). Adult recipients in groups C and D will be the subject of a subsequent subprotocol, for which consent forms and operational guidelines will be developed.

*Minors*. The recruitment procedures described for adults will be followed, with the legal guardian(s) acting as the recipient's representative(s). The legal guardian(s) will be asked to sign the informed consent form specifically designed for minor recipients. In addition, children over age 12 will be asked to sign an assent form. If the appropriate form(s) are signed, the patient manager will conduct the interview and physical examination, and will draw the blood sample. The parent/legal guardian will be interviewed. Minors over a certain age, specified by each clinical center, will also be interviewed.

Adult recipients for whom there is a legally appointed guardian will be approached in the same way as minors.

*Follow-up visits* for recipients will be handled in the same way as described above for donors.

### Household Sexual Contacts

At the time of the initial interview, a household roster will be obtained for each subject in recipient group (A). From this information and the personal history, appropriate subjects can be identified. A subprotocol and appropriate consent forms for their recruitment will be developed. In the interval, the following operational definitions will be used to determine potential eligibility.

With appropriate regard to the sensitivity of the questions, continuing primary sexual contacts will be defined. A primary sexual contact is defined as an individual who is the spouse or spouse equivalent and meets the following criteria: 1) Has been the single continuing partner of the patient during the prior six months, and 2) is expected to be available for further observation for the next two years. Observations will continue regardless of continuing sexual exposure.